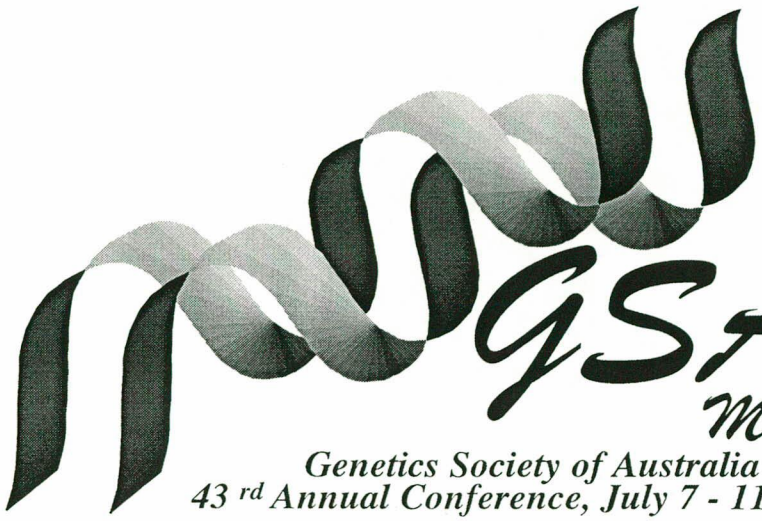
CHRIS MORAN
Animal Science
University of Sydney

Genetics Society of Australia
43 rd Annual Conference, July 7 - 11, 1996



Genetics Society of Australia
43 rd Annual Conference, July 7 - 11, 1996

ORGANISING COMMITTEE

Philip Batterham, Chairperson
Meryl Davis
Dawn Gleeson
Jenny Graves, GSA President
John McKenzie, Treasurer
Peter Papagiannopoulos, Postgraduate Student Representative
Cheryl Grant, Secretarial Assistance

CONTENTS

GENERAL INFORMATION

The 43rd Annual GSA Conference will be held at the University of Melbourne from Sunday July 7 to Thursday July 11. This meeting will incorporate the Australasian Genome Mapping Workshop.
The University of Melbourne is located a short tram ride from the city and Southbank and a few minutes walk from Lygon Street where there are numerous eating places and shops. Accommodation has been organised at Queen's College, one of the residential colleges of the University.

REGISTRATION

The cost of registration for GSA members is $120 (non-GSA members $150). Student registration is $40 unless a student is presenting a talk or poster in which case the registration is waived. Day registration is $40 per day or part thereof ($10 students).
Collection of the final program, badges and conference abstracts will take place at the Mixer on the evening of Sunday, July 7. A desk will be set up near The Junior Common Room of Queens College, College Crescent, Parkville, from 7.00 pm. A similar desk will be located outside the Public Lecture Theatre in the Old Arts Building at the University of Melbourne from Monday July 8 at 8.00 am. (See map enclosed)

THE MIXER

The mixer will be held in The Junior Common Room at Queen's College, College Crescent, Parkville, on Sunday evening between 7.00 pm - 10.00 pm. Drinks and savouries will be provided. (Cost is included in the registration.) *The mixer has been generously supported by **Bio-Rad Laboratories Pty.Ltd.***

MEALS

Breakfast is provided for those staying at Queen's College. The University Coffee Shop in the Union Building which serves tea, coffee, juices, pastries, sandwiches and rolls, opens at 7.30 am. The main cafeteria which serves a complete range of breakfast items opens at 8.00 am each morning.

Lunch will be provided at the Poster Session on Tuesday July 9 in Wilson Hall, at the University. The cost is included in the registration fee. Lunches on the other days of the conference are not included in the registration fee. All University food outlets will be open during the conference.
For those participating in the zoo excursion on Thursday July 11, lunch is included in the cost.

Annual Dinner

Our annual dinner will be held in Eakins Hall of Queen's College on Wednesday, July 10, from 7.30 pm.

ZOO EXCURSION

On Thursday July 11 at 12.30 pm there will be a picnic lunch at the Melbourne Zoological Gardens, Parkville, where you will have the opportunity to visit the world famous Butterfly House. The cost of a picnic lunch and entry to the Zoo is $20 per adult, $10 for children under 12 years.

CHILD CARE

Limited places are available at the University Creche. Places will be allocated in order of application. Other child care facilities are available in the Carlton -Parkville area. For more information about either option contact Cheryl on (03) 9344 6246

PARKING

For those staying at Queen's College parking is available in the grounds of the College.
There is all-day meter parking in College Crescent and neighbouring areas. Four hour and two hour meter parking is available in Royal Parade and Swanston Streets respectively. This is a non teaching period at the University so those arriving before 9.00 am should be able to find a park. There is a reasonably priced commercial car park in Elgin Street. After 5.00 pm parking is available within the University grounds for $2.

THE PUBLIC LECTURE

The lecture given by Dr. Tony Griffiths at 6.30 pm on Tuesday evening "Learning Genetics" will also form part of the University of Melbourne, Dean of Science's Lecture series and is therefore open to the general public free of charge. Registrants are most welcome to bring accompanying persons.

STUDENT AWARDS

As a result of the generous contribution of the Promega Corporation and Sigma Aldrich we will be offering student awards for the following:

$500 for the Best Poster Presentation
$500 for the Best Oral Presentation

GSA Overseas Visitors

Year	GSA AGM	Place	Visitors
1979	26th	University of Adelaide	Bryan Clarke
1980	27th	Newcastle University	Norman Giles
1981	28th	University of Sydney	Mike Bennet Dick Flavell Ken Frey
1982	29th	Melbourne University	Guy Bush Larry Sandler
1983	30th	University of New England	Bruce Wallace
1984	31st	Australian National University	David Hogness Bob Williamson
1985	32nd	Macquarie University	John Maynard Smith Stephen O'Brien
1986	33rd	Flinders University	Bikram Gill Bryan Clarke Jack Szostak David Wolstenholme
1987	34th	Australian National University	Dan Hartl Art Hilliker Tim Prout
1988	35th	La Trobe University	Robin Holliday Alan Templeton
1989	36th	University of New South Wales	Elizabeth Blackburn Mike Clegg Jeff Doyle
1990	37th	Queen Elizabeth Hospital Adelaide	John Avise Phil Ingham Sue Gibson Andrew Paterson Jim Womack
1991	38th	Monash University	Heinz Saedler John Thompson Ron Woodruff
1992	39th	University of Queensland	Neil Copeland Barry Hall Michael Golubovsky
1993	40th	University of Adelaide	Spyros Artavanis-Tsakonas Lorna Casselton
1994	41st	University of New England	Lief Andersson Chip Aquadro David Baillie
1995	42nd	Australian National University	Jacques Drouin Rob Nicholls John VandeBerg

Trade Representatives

The following companies have supported the Genetics Society of Australia Inc. by being Sustaining Members of the Society in 1996.

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PCR ACCESS!

The Access RT-PCR* System

***PCR ACCESS!* and Promega** introduce the Access RT-PCR System: a two enzyme, single tube system designed for PCR amplification using an RNA molecule as the starting template. Avian Myeloblastosis Virus reverse transcriptase (AMV-RT) is used for first strand cDNA synthesis. After generation of this DNA, amplification is performed using heat-stable *Tfl* DNA polymerase. No additions to the reaction mix are required between the reverse transcription and DNA amplification steps.

THE BEST ENZYME COMBINATION FOR THE BEST RESULTS

* AMV-RT is a very robust enzyme and processes even those RNA molecules with a high degree of secondary structure.
    * In the optimized buffer provided with the Access RT-PCR System, AMV-RT is active at 48°C. This high reaction temperature further enhances the ability of the enzyme to process even the most difficult RNA templates.
    * *Tfl* DNA polymerase ensures highly efficient amplification.

SUPERIOR DETECTION LIMITS

* The 540bp β-actin RT-PCR product can be detected in as little as 1pg of total RNA after 40 cycles.
* We obtain sensitivity with the Access RT-PCR System comparable to that obtained with the commercially available combination of MuLV RT and *AmpliTaq*® DNA Polymerase: with much greater convenience.
    * 1,000-fold better sensitivity is obtained with the Access RT-PCR System as compared with the one-enzyme *Tth* method.
    * Using an *in vitro*-synthesized IL-1α transcript, less than 100 molecules of starting material are required (Miller, K. *et al.* (1995) *Promega Notes* ***53***, 2).

CONVENIENT PROTOCOL

* Once the Master Mix is assembled and the template RNA added, no further additions to the sample reactions are required.
* Minimal hands-on time.
* Reduce risk of sample contamination.
* The optimized buffer system used in the Access RT-PCR System protocol circumvents problems which are often encountered with fidelity when manganese-containing buffers are used (Fromant, M. *et al.* (1995) *Anal. Biochem.* **224**, 347).

Order the Access RT-PCR System DIRECT from Promega!!!

ORDERING INFORMATION

PRODUCT	CAT. #	LIST PRICE
Access RT-PCR System	**A1250**	**$575**
Access RT-PCR Introductory System	**A1260**	**$150**

* PCR is a patented process. Purchase of this product does not include a license to perform PCR. Use of this product is recommended for persons that either have a license to perform PCR, or are not required to obtain a license.

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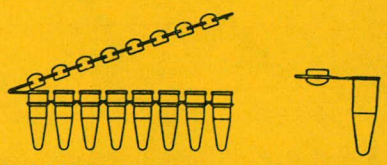

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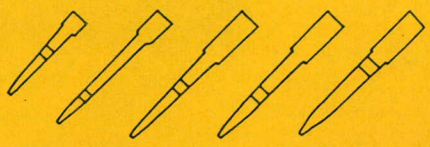

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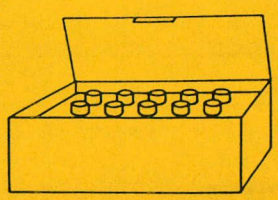

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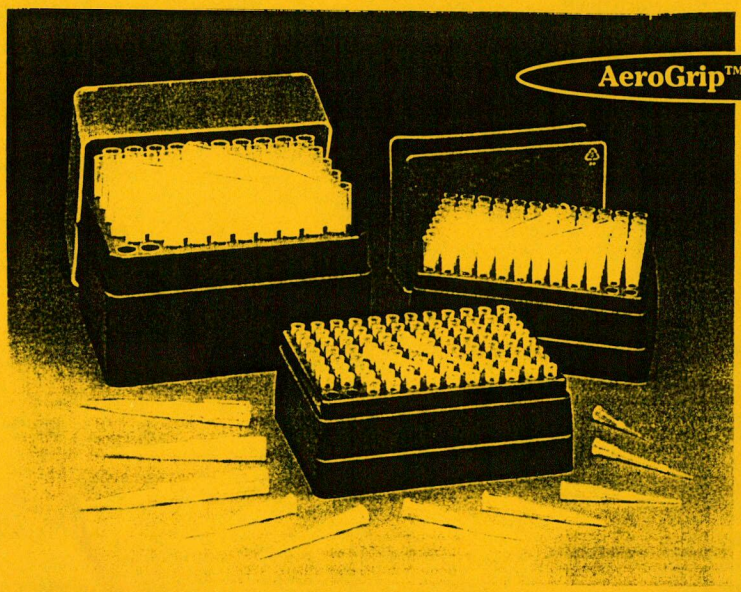

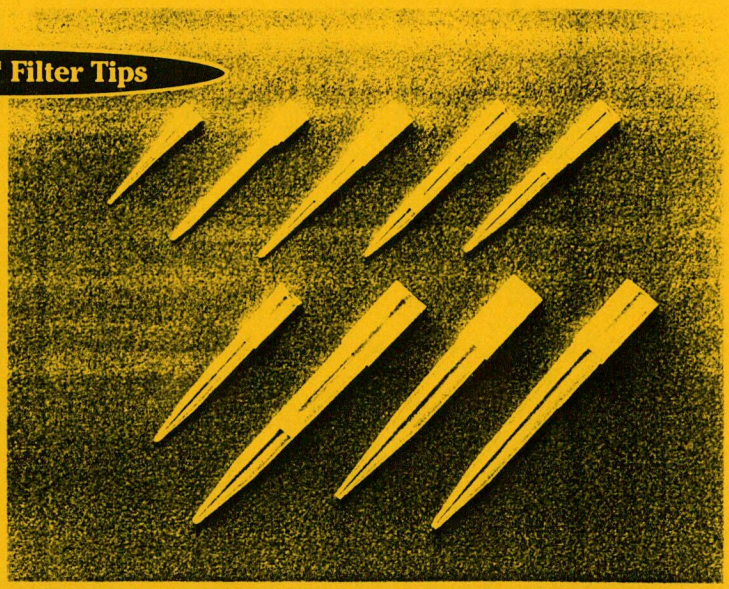

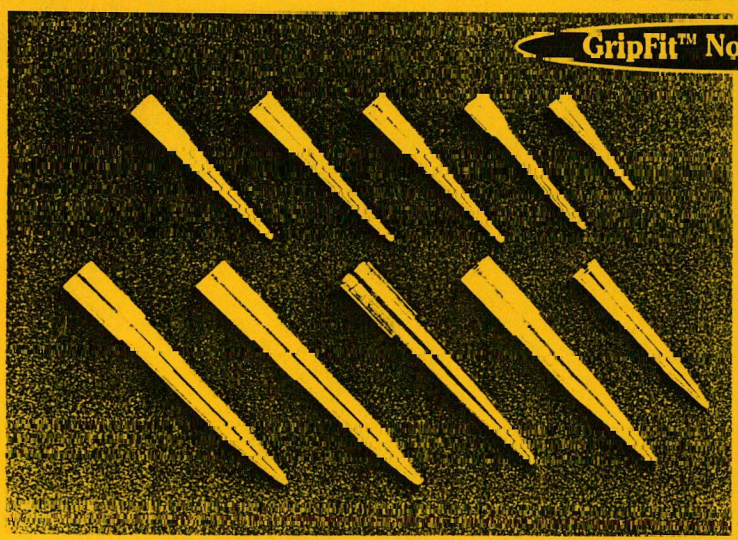

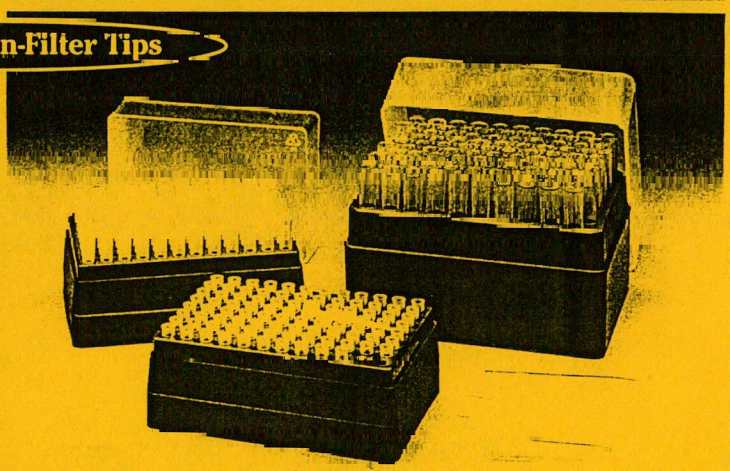

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PROGRAM

SUNDAY, 7 JULY 1996

7.00 - 10.00 pm **THE MIXER & REGISTRATION**
Junior Common Room, Queen's College
Sponsored by Bio-Rad Laboratories Pty. Ltd.

MONDAY, 8 JULY 1996

8.00 - 8.50 am **REGISTRATION**
Foyer of the Public Lecture Theatre, Old Arts Building, University of Melbourne

8.50 -9.00 am **Welcome** : Philip Batterham
Public Lecture Theatre, Old Arts Building

9.00 - 10.30 am **GENETIC APPLICATIONS**
Chair : Philip Batterham

9.00 - 9.45 am — **Nancy F. Millis**
Melbourne University and La Trobe University
(T1)
Designer genes - do they fit consumers?

9.45 - 10.30 am — **Peter Atkinson** and David O'Brochta
CSIRO (Entomology)
(T2)
Genetic engineering of insects

10.30 - 11.00 am ***Morning Tea - Old Arts Hall***

11.00 - 12.30 pm **GENOME ANALYSIS**
Chair : Viji Krishnapillai

11.00 - 11.45 am — **Simon Foote**
Australian Genome Research Facility - Walter And Eliza Hall Institute
(T3)
Phenotype-genotype interactions and the Australian Genome Research Facility

11.45 - 12.30 pm — **Tim Littlejohn**
ANGIS
(T4)
In silico genetics

12.30 - 2.00 pm ***Lunch (own arrangements)***

CONCURRENT SESSIONS

2.00 - 4.00 pm **Session 1A** — **Lecture Theatre E, Old Arts**
GENE REGULATION
Chair : Alex Andrianopoulos

2.00 - 2.40 pm — **Jim Pittard**
University of Melbourne (T5)
The TyrR protein of *Escherichia coli*. A genetic analysis of its role as repressor and activator

2.40 - 3.00 pm — **Peter Papagiannopoulos**, Alex Andrianopoulos, Julie A. Sharp, Meryl A. Davis and Michael J. Hynes (T6)
The *hap* genes of *Aspergillus nidulans* play a role in the regulation of *amdS* expression

3.00 - 3.20 pm — **Farrell, R.A.** and Winge, D.R. (T7)
Identification and characterisation of a conserved zinc-binding module in a family of copper regulated yeast transcription factors

3.20 - 3.40 pm — **Anna J. Small**, Michael J. Hynes and Meryl A. Davis (T8)
Nitrogen regulation and the *tamA* gene of *Aspergillus nidulans*

2.00 - 4.00 pm	**Session 1B** **GENOME MAPPING** *Chair : Marianne Frommer*	**Public Lecture Theatre, Old Arts**
2.00 - 2.20 pm	Weiyi Zhang and **Chris Moran** (T9)	Studies of CATS (Comparative Anchor Tagged Sequence) markers in the pig
2.20 - 2.40 pm	**Rachel Hawken**, Murray Broom, Tracey van Stijn, Pino Maccarone, Joanne Lumsden, Jennifer A Marshall Graves, Tom Broad and Jill Maddox (T10)	Evolution of a cytokine gene family
2.40 - 3.00 pm	**Jennifer Donald**, John Kwok, Judy Salmon, Philip Mitchell and Peter Schofield (T11)	Genetic mapping of a complex disease: Manic depressive illness
3.00 - 3.20 pm	**ZYH Wong**, M Stebbing and SB Harrap (T12)	Genetic analysis of fibrinogen and cardiovascular risk
3.20 - 3.40 pm	**C. L. Brubaker**, A. Paterson, and J. Wendel (T13)	Evolution of homoeologous chromosomes in polyploids: Evidence from comparative mapping of allopolyploid and diploid *Gossypium*
2.00 - 4.00 pm	**Session 1C** **POPULATION GENETICS** *Chair : Ben Oldroyd*	**Lecture Theatre B, Old Arts**
2.00 - 2.20 pm	**Craig Moritz**, Rob Slade, Noel Cortinas and Anita Heideman (T14)	Molecular population genetics of the cane toad (*Bufo marinus*): phylogeography and founder effects
2.20 - 2.40 pm	**Michael Cunningham** (T15)	Phylogeography, vicariance and introgression in Australian rainforest frogs
2.40 - 3.00 pm	**Susan Fuller**, Peter Mather and John Wilson (T16)	Gene flow among wild rabbit populations in a non-equilibrium system
3.00 - 3.20 pm	**Jane Hughes**, Stuart Bunn, David Hurwood, Cath Cleary and Mark Kingston (T17)	Gene flow and dispersal among populations of stream invertebrates
3.20 - 3.40 pm	**Margaret Heslewood** and Peter Baverstock (T18)	Rates of molecular evolution in Australian Passerines
3.40 - 4.10 pm	***Afternoon Tea - Old Arts Hall***	

CONCURRENT SYMPOSIA

4.10 - 5.10 pm	**Symposium A** **DEVELOPMENTAL GENETICS** *Chair : David Smyth*	**Public Lecture Theatre, Old Arts**
	Matthew Andrews *North Carolina State University* (T19)	Developing *Xenopus* embryos as a living test tube for transcription factor function
4.10 - 5.10 pm	**Symposium B** **POPULATION AND EVOLUTIONARY GENETICS** *Chair : Neil Murray*	**Large Botany Theatre, Botany School**
4.10 - 4.40 pm	**Richard ffrench-Constant** *University of Wisconsin, Madison USA* (T20)	Single versus multiple origins of insecticide resistance: Inferences from the cyclodiene resistance gene *Rdl*
4.40 - 5.10 pm	**Andrew Young** and Tony Brown *CSIRO (Plant Industry)* (T21)	Genetic diversity and mating system of remnant populations of the endangered grassland daisy *Rutidosis leptorrhynchoides*
5.10 - 7.00 pm	***Dinner (own arrangements)***	

CONCURRENT SYMPOSIA

7.00 - 8.00 pm — **Symposium A** — **Public Lecture Theatre, Old Arts**

DEVELOPMENTAL GENETICS
Chair : Len Kelly

Danny L. Brower, M.C. Brabant, T.A. Bunch and D. Fristrom
University of Arizona
(T22)

Diverse functions for integrins in *Drosophila* morphogenesis

Danny Brower's visit has been sponsored by **Commonwealth Serum Laboratories**

7.00 - 8.00 pm — **Symposium B** — **Large Botany Theatre, Botany School**

POPULATION AND EVOLUTIONARY GENETICS
Chair : Craig Moritz

7.00 - 7.30 pm — **Chris Schneider** and Craig Moritz
Cooperative Research Centre for Tropical Rainforest Ecology and Management and University of Queensland
(T23)

A genetic perspective on the historical biogeography and differentiation of rainforest-restricted reptiles

7.30 - 8.00 pm — **Eldredge Bermingham**
Smithsonian Tropical Research Institute USA
(T24)

The isthmus of Panama, molecular clocks and fish biogeography

8.00 - 8.20 pm — ***Supper - Old Arts Hall***

CONCURRENT SYMPOSIA

8.20 - 9.20 pm — **Symposium A** — **Public Lecture Theatre, Old Arts**

DEVELOPMENTAL GENETICS
Chair : Len Kelly

John Postlethwait
University of Oregon
(T25)

Zebrafish: A model for early human development

8.20 - 9.20 pm — **Symposium B** — **Large Botany Theatre, Botany School**

POPULATION AND EVOLUTIONARY GENETICS
Chair : Craig Moritz

Richard Frankham
Macquarie University
(T26)

Genetics and conservation

TUESDAY, 9 JULY 1996

9.00 - 10.00 am	*Chair : Michael Hynes*	**Copland Theatre, Economics & Commerce**
	Ron Morris *University of Medicine and Dentistry of New Jersey* (T27)	Cytoplasmic dynein and nuclear migration in fungi
10.00 - 10.30 am	***Morning Tea - Copland Theatre Foyer***	

CONCURRENT SESSIONS

10.30 - 12.10 pm	**Session 2A** **CONSERVATION GENETICS** *Chair : John Sved*	**Lecture Theatre E, Old Arts**
10.30 - 10.50 am	**Neil D. Murray (T28)**	Gene pools and conservation law: Some problems of definition
10.50 - 11.10 am	**England, Phillip R.,** Graham Osler, David A. Briscoe and Richard Frankham (T29)	The effect of population bottlenecks on allelic diversity and heterozygosity in *Drosophila melanogaster*
11.10 - 11.30 am	**Mark D.B. Eldridge,** Anne Loupis, Graham Hall and Juliet King (T30)	Australia's off-shore island mammal populations: Jewels in the conservation crown or genetic death-traps?
11.30 - 11.50 am	**Andrea Taylor** and Des Cooper (T31)	Microsatellite markers for marsupial population genetics: Case studies of macropods and possums in New Zealand
11.50 - 12.10 pm	**Nick J. H. Campbell** and P. R. Baverstock (T32)	Contrasting patterns of maternal gene flow when assessed over evolutionary and ecological time scales in fragmented and unfragmented populations of rainforest rodents
10.30 - 12.10 pm	**Session 2B** **THE DIVERSITY OF GENETIC SYSTEMS** *Chair : David Catcheside*	**Lecture Theatre B, Old Arts**
10.30 - 10.50 am	**Deborah C. A. Shearman,** John A. Sved and Marianne Frommer (T33)	The *Bactrocera tryoni* homologue of the *Drosophila melanogaster* sex determination gene *doublesex*
10.50 - 11.10 am	**Michele D. Binder,** Michael J. O'Neill and Andrew H. Sinclair (T34)	An avian W-specific gene isolated by differential display RT-PCR
11.10 - 11.30am	**Chris Gillies,** Trude Schwarzacher and Pat Heslop-Harrison (T35)	FISHing for meiotic pairing regions in rye
11.30 - 11.50 am	Hoa T. Le, Tieqiao Wu, A. Robertson, D. Bulach and **D. E. Tribe (T36)**	Several related *Helicoverpa* specific baculoviruses encode an asparagine rich zinc-finger protein, the coding region of which contains triplet-repeats that are subject to high rates of mutation
11.50 - 12.10 pm	**Y.M.Parsons,** D.W.Cooper and M.R.Fleet (T37)	Coat colour genetics of Merino sheep
10.30 - 12.10 pm	**Session 2C** **HUMAN MOLECULAR GENETICS** *Chair : Jim Camakaris*	**Copland Theatre, Economics & Commerce**
10.30 - 10.50 am	**Andrew H. Sinclair,** Fergus J. Cameron, Robyn M. Hageman, Claire Cooke-Yarborough, Linda L. Goodwin and David O. Sillence (T38)	A novel germ line mutation in *SOX9* causes familial campomelic dysplasia and sex reversal
10.50 - 11.10 am	**Rachel J. Waugh O'Neill,** Francine E. Brennan, Margaret L. Delbridge, and Jennifer A. Marshall Graves (T39)	*De novo* insertion of an intron into the mammalian sex determining gene, *SRY*

11.10 - 11.30 am	✳ **Nigel J. Parker**, C. Glenn Begley, Peter J. Smith and Richard M. Fox **(T40)**	Molecular cloning of a novel human gene at 11p15.5
11.30 - 11.50 am	✳ **Robert Kapsa**, A.S. Noer, D. Thyagarajan, P. Lertrit, S. Marzuki and Edward Byrne **(T41)**	Mitochondrial DNA polymorphism in human disease
11.50 - 12.10 pm	✳ **Michael Petris**, Julian Mercer, Janetta Culvenor, Paul Lockhart, Paul Gleeson and James Camakaris **(T42)**	Regulation of the Menkes copper transporter via copper-induced trafficking from the Golgi to the plasma membrane
12.20 - 4.30 pm	**Lunch, Posters and Trade Displays - Wilson Hall**	
4.30 pm	**Annual General Meeting**	**Copland Theatre, Economics & Commerce**
6.30 - 7.30 pm	Deans Lecture *Chair : Bruce McKellar* *(Dean, Faculty of Science)*	**Copland Theatre, Economics & Commerce**
	Anthony Griffiths *University of British Columbia* **(T43)**	Learning Genetics

Tony Griffiths' visit has been sponsored by **WH Freeman and Company, Publishers, New York and Macmillan Education, Australia**

WEDNESDAY, 10 JULY 1996

9.00 - 10.30 am	*Chair : Jenny Graves*	**Copland Theatre, Economics & Commerce**
9.00 - 10.00 am	**John Postlethwait** *University of Oregon* **(T44)**	Genetic analysis in the Zebrafish
10.00 - 10.30 am	**David G. Heckel** *Clemson University USA* **(T45)**	Mendel meets Mothra: The challenges of linkage mapping in the Lepidoptera
10.30 - 11.00 am	***Morning tea - Copland Theatre Foyer***	

CONCURRENT SESSIONS

11.00 - 12.40 pm	**SESSION 3A** **FUNGAL GENETICS** *Chair : Margaret Katz*	**Copland Theatre, Ecomonics & Commerce**
11.00 - 11.40 am	**Anthony Griffiths (T46)**	Natural plasmids of the fungus *Neurospora*
11.40 - 12.00 noon	**P. Jane Yeadon** and David E.A. Catcheside **(T47)**	Molecular analysis of meiotic recombination in the *histidine-3* region of *Neurospora* using natural sequence polymorphisms
12.00 - 12.20 pm	**A. M. Wyndham**, C. Vazques, E. Johnson and R. T. Baker **(T48)**	Characterisation of a yeast ubiquitin specific protease UBP6
12.20 - 12.40 pm	**Howlett B.J.**, Cozijnsen A.J., Rolls B.D. and Chen C.Y. **(T49)**	Characterisation of a gene of the blackleg fungus *Leptosphaeria maculans* conferring host specificity on indian mustard

11.00 - 12.40 pm	**SESSION 3B** **PHYLOGENETICS** *Chair : Mike Westerman*	**Public Lecture Theatre, Old Arts**
11.00 - 11.20 am	**John R. Kavanagh** and Mark S. Springer **(T50)**	Phylogenetic affinities of dromiciops based on mitochondrial rRNA genes and part of the nuclear interphotoreceptor retinoid binding protein
11.20 - 11.40 pm	**Angela Burk**, Michael Westerman, and Mark S. Springer **(T51)**	Phylogenetic relationships of the Macropodidae: Evidence from mtDNA sequences
11.40 - 12.00 noon	**Cushla J. Metcalfe**, Mark D.B. Eldridge and Peter G. Johnston. **(T52)**	The distribution of the telomeric sequence in twelve *Petrogale* taxa (Rockwallabies)
12.00 - 12.20 pm	**Paul Sunnucks**, Phillip R. England, Andrea C. Taylor and Dinah F. Hales **(T53)**	Microsatellite and chromosome evolution of parthenogenetic *Sitobion* aphids in Australia
12.20 - 12.40 pm	**Michelle Waycott** and Donald H. Les **(T54)**	Seagrasses evolution: A molecular phylogenetic perspective
12.40 - 2.00 pm	***Lunch***	

CONCURRENT SESSIONS

2.00 - 3.20 pm	**SESSION 4A** **GENETIC SYSTEMS IN MAMMALS** *Chair : Jon Martin*	**Copland Theatre, Economics & Commerce**
2.00 - 2.20 pm	**Roland Toder**, Marg Delbridge, Pino Maccarone and Jennifer A. Marshall Graves **(T55)**	The marsupial Y chromosome as a model mammalian Y
2.20 - 2.40 pm	**J.E. Deakin** and D.W. Cooper **(T56)**	Cloning the androgen receptor gene in the Common Brushtail Possum, *Trichosurus vulpecula*
2.40 - 3.00 pm	Catherine A. Forbes, Robert Cho, Michael G. Brown, Geoffrey R. Shellan, Wayne M. Yokoyama and **Anthony A. Scalzo (T57)**	A high resolution genetic map of the genomic region encompassing the natural killer cell gene complex (NKC) on mouse chromosome 6
3.00 - 3.20 pm	**Bronwyn Williams** and Peter Baverstock **(T58)**	Evolution of the aldolase A genes and pseudogenes in the Australian *Rattus*
2.00 - 3.20 pm	**SESSION 4B** **MOLECULAR GENETICS IN PLANTS** *Chair : Chris Cobbett*	**Lecture Theatre E, Old Arts**
2.00 - 2.20 pm	J Alvarez, **M. G. B. Heisler**, A Atkinson and D R Smyth **(T59)**	*Spatula* : A gene involved in carpel development in *Arabidopsis thaliana*
2.20 - 2.40 pm	**S. Sherson**, I. Gy, M. Kreis, A. Lecharny and C. Cobbett **(T60)**	Two sugar kinase genes of *Arabidopsis* : *ARA1* and *GAL1*.
2.40 - 3.00 pm	**Trevaskis, J.B.**, Andersson, C.R., Watt, R.A., Llewellyn, D.J., Dennis, E.S. and Peacock, W.J. **(T61)**	Cloning and characterisation of haemoglobins from *Arabidopsis thaliana*
3.00 - 3.20 pm	**Joanne Luck**, Jeff Ellis, Jean Finnegan and Greg Lawrence **(T62)**	LUTE-1, a transposable element isolated from spontaneous mutants of the L^6 flax rust resistance gene
2.00 - 3.20 pm	**Session 4C** ***DROSOPHILA*: LABORATORY AND NATURAL SELECTION** *Chair : Steve McKechnie*	**Lecture Theatre B, Old Arts**
2.00 - 2.20 pm	**Gawain McColl**, Ary A. Hoffmann and Stephen W. McKechnie **(T63)**	Response of two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*

2.20 - 2.40 pm	**Fiona M. Pyke**, Philip Batterham and John A. McKenzie **(T64)**	Diazinon resistance in *Drosophila melanogaster*
2.40 - 3.00 pm	**ME. Montgomery**, J. Ballou, RK. Nurthen, DA. Briscoe and R. Frankham **(T65)**	Conservation genetics : Comparing captive management options
3.00 - 3.20 pm	**Nicole L. Jenkins** and Ary A. Hoffmann **(T66)**	Testing species border hypotheses using *Drosophila serrata*
3.20 - 3.40 pm	***Afternoon Tea - Copland Theatre Foyer***	

CONCURRENT SESSIONS

3.40 - 5.00 pm	**SESSION 5A** **MOLECULAR EVOLUTION** *Chair : Peter Baverstock*	**Copland Theatre, Economics & Commerce**
3.40 - 4.20 pm	**Geoff McFadden**, Mike Reith and Naomi Lang-Unnasch University of Melbourne **(T67)**	Identification of a plastid in Apicomplexan parasites of humans
4.20 - 4.40 pm	**Mark Springer** and Michael Stanhope **(T68)**	The phylogeny of Eutherian orders based on 12S and IRBP sequences
4.40 - 5.00 pm	**John Mitchell** and Leah Earl **(T69)**	The Y of human evolution: male specific DNA polymorphisms and human origins and diversity?
5.00 - 5.20pm	**Jon Martin**, Rosemary Hoffman, Steven T. Case and Rob Hamilton **(T70)**	Evolutionary divergence and conserved motifs in the gene for the intermediate-sized silk protein sp185/220
5.20 - 5.40pm	**Carey Krajewski** and Michael Westerman **(T71)**	Protamine P1 gene sequences and the phylogeny of the Dasyurid marsupials
3.30 - 5.00 pm	**SESSION 5B** **CELL SIGNALLING** *Chair : Marie Phillips*	**Public Lecture Theatre, Old Arts**
3.40 - 4.20 pm	**David Bowtell** Peter McCallum Institute **(T72)**	A genetic analysis of the *ras* activating protein, *Sos1*, in mice
4.20 - 4.40 pm	**Anthony G. Uren**, Miha Pakush, Christine J. Hawkins, Kirsten L. Puls and David L. Vaux. **(T73)**	Cloning and characterisation of four novel cellular inhibitor of apoptosis protein (IAP) homologues
4.40 - 5.00 pm	**Coral G. Warr** and Leonard E. Kelly **(T74)**	Expression pattern of the *TRPL* cation channel of *Drosophila melanogaster*
5.00 - 5.20pm	**Warwick Grant** and Peter Hunt **(T75)**	Chemosensory mutants and feeding behaviour in *Caenorhabditis elegans*
7.30 pm	**Annual Dinner : Eakin's Hall, Queen's College**	

THURSDAY, 11 JULY 1996

9.30-10.30 am	*Chair : Alan Wilton*	**Copland Theatre, Economics & Commerce**
	Charles Langley *University of California, Davis* **(T76)**	The contribution of neurogenic loci to bristle number variation in natural populations of *Drosophila*
	Charles Langley's visit has been sponsored by the publishers of **GENETICA** (Kluwer Academic Publishers) and the **Dipteran Molecular Biology Group**	
10.30 - 11.00 am	***Morning Tea - Copland Theatre Foyer***	
11.00 - 12.00 noon	**The MJD White Address** *Chair : John McKenzie*	**Copland Theatre, Ecomonics & Commerce**
	Jennifer A. Marshall Graves *La Trobe University* **(T77)**	Mammals that break the rules: Genetics of marsupials and monotremes
12.00 - 12.30 pm	**Student Awards Presentation**	**Copland Theatre, Ecomonics & Commerce**
	Student awards have been sponsored by the **Promega Corporation**	
12.30	ZOO PICNIC	

POSTERS : TUESDAY JULY 9 12.30 - 4.30 PM

P1	**David J. Edwards** and Barbara A. Wilson	Genetic variability in a translocated population of Swamp Antechinus (*Antechinus minimus maritimus*) as assessed by microsatellite variation
P2	**J.A.Baril**, M. T. Ivanyi and N.D. Murray	Multilocus DNA fingerprinting of Orange-Bellied Parrot (*Neophema chrysogaster*)
P3	**M. Kruetzen**, W. Sherwin, R. Connor, R. Smolker, N. Gales and R. Shepherd	Kinship and alliance formation in male dolphins at Shark Bay, WA
P4	**Shiro Akiyama**, Tom R. Grant, Neil J. Gemmell, Jaclyn M. Watson, Jennifer A. Marshall Graves and Neil D. Murray	Molecular and ecological studies of family and population structure in the Platypus
P5	**Mark Ponniah**	Genetic differentiation within and among species of spiny crayfish belonging to the genus *Euastacus*
P6	**Elizabeth A. Sinclair**	Genetic variation and social structure in the quokka, *Setonix brachyurus* (Marsupialia: Macropodidae): two island populations
P7	**B. Costello** and W. Sherwin	Loss of genetic diversity in small populations of the greater stick-nest rat (*Leporillus conditor*)
P8	**J.A.Lade**, N.D. Murray, C.A. Marks and N.A. Robinson	Microsatellite differentiation between Phillip Island and mainland Australian populations of the red fox *Vulpes vulpes*
P9	**Belinda Mitterdorfer**, David Kendrick and Ross Cunningham	Stasis and symmetry in a Magpie hybrid zone
P10	**Catherine Oke**	Towards conservation priorities for the endangered stream-dwelling frog *Taudactylus eungellensis* using mtDNA data from it and a secure species, *Litoria lesueri*
P11	**Deirdre E. Sharkey**, Bronwyn A. Houlden and William B. Sherwin	Dispersal and parentage in a NSW koala population
P12	**Painter, J.**, Clarke, M. F. and Crozier, R. H.	Helping behaviour in the bell miner
P13	**Mark Dowton**	Factors influencing the rate of molecular evolution, and compositional bias in the Hymenoptera
P14	**Mark Dowton** and Andrew D. Austin	The evolution of parasitism in the Hymenoptera
P15	C.A. Tilley and **B.P. Oldroyd**,	Reproductive dominance in honeybees, *Apis mellifera*, detected by microsatellite analysis
P16	Michael M. Halford, Ary A. Hoffmann and **Stephen W. McKechnie**	Identification of a major gene affecting heat hardening in *Drosophila melanogaster*
P17	**P. O'Hanlon**, A.R. Smith-White and P. Adam	Population genetic structure of *Sporobolus virginicus* (L.) Kunth. within a single saltmarsh
P18	**D. M. Gilligan**, L. M. Woodworth, M. E. Montgomery, D. A. Briscoe and R. Frankham	Does mutational meltdown threaten the survival of finite sexually reproducing populations?
P19	**Steve Chenoweth**	Variation in the control region of the mitochondrial genome of the barramundi *Lates calcarifer* from Northern Australia
P20	**Linda Broadhurst** and David J. Coates	Genetic diversity and population genetic structure of *Geleznowia verrucosa* (Rutaceae) - an endemic monotypic genus
P21	**P.Fisher**, R.Gardner and T.Richardson	PCR-based microsatellite isolation using 5'-anchored primers
P22	**Leon J. Scott**, Corinna L. Lange and David K. Yeates	Genetic variation and origin of Siam Weed (*Chromolaena odorata*)
P23	**M.K. Tan** and P.T.W. Wong	Group I introns in 26S rRNA genes of *Gaeumannomyces graminis* as indicators of host range of *G. graminis* varieties

P24	**Christopher Cobbett** and Peter Goldsbrough	The roles of phytochelatins and metallothioneins in heavy metal detoxification in the plant *Arabidopsis*
P25	**R. Elliott**, A. Betzner, E. Huttner, M. Oakes, W. Tucker, D. Gerentes, P. Perez, and D. Smyth	*Aintegumenta*, an *apetala2*-like gene of *Arabidopsis* with pleiotropic roles in ovule development and floral organ growth
P26	**Tamzin M. Donald**, Carolyn R. Leach, Andreas Houben and Jeremy N. Timmis	Molecular organisation of a plant B chromosome
P27	**Donna V. Frost**, A.R. Hardham, J.G. Ellis, M.A. Ayliffe, G.J. Lawrence and E.J. Finnegan	Localisation of the protein products of the L6 gene
P28	**Rolls B.D.**, Plummer K.M. and Howlett B.J.	Organisation of ribosomal DNA (rDNA) of the blackleg fungus (*Leptosphaeria maculans*)
P29	**Rachael L. Murphy**, Meryl A. Davis and Michael J. Hynes	The *facB88* translocation: A fusion of two regulatory genes creating a transcriptional superactivator
P30	**Patricia A. vanKuyk** and Margaret E. Katz	The extracellular proteases of *Aspergillus nidulans*
P31	**Julie A. Sharp**, Meryl A. Davis and Michael J. Hynes	Interspecies sequence and functional comparisons of the acetamidase gene in *Aspergillus* species and the discovery of an orthologous amidase
P32	**Sarah J. Bugg**, Meryl A. Davis and Michael J. Hynes	Isolation and promoter analysis of acetamidase genes in *Aspergillus ustus*
P33	**Robert Shroff** and Joan Kelly	Characterisation of *creA* alleles in *Aspergillus nidulans*
P34	**Christopher J. Stemple**, Meryl A. Davis and Michael J. Hynes	The role of the *facC* gene of *Aspergillus nidulans* in acetate metabolism
P35	**Frederick J. Bowring** and David E.A. Catcheside	Conversion events at the *am* locus in *Neurospora crassa* rarely have an associated crossover
P36	**Alex Andrianopoulos**, Fiona Cooke, Janynke Brons and Michael J. Hynes	Functional dissection of transcriptional activation domains in *Aspergillus nidulans*
P37	N. Ozsarac, **M.J. Straffon** and I.W. Dawes	Transcriptional control elements of the yeast mid-sporulation gene *SPR3*
P38	**Margaret E. Katz**, Martin McLoon and Brian F. Cheetham	Genetic variation in *Aspergillus fumigatus*: Is Australia home to a unique new species of *Aspergillus*?
P39	**Julianne Camerotto**, Emily Wilson, Nipam Patel and Robert Saint	Characterisation of a novel cell cycle gene in *Drosophila melanogaster*: A role in chromosome condensation?
P40	**Michiko Smith** and Leonard Kelly	Subcellular localisation of the *Drosophila melanogaster stoned* gene products
P41	**A.M.Phillips** and L.E. Kelly	The *Suppressor* of *stoned*
P42	**Dennis J. Hulme**, Michelle J. Callaghan, Andrew J. Smith and Ken J. Beh	A search for genetic markers for resistance to *Trichostrongylus colubriformis* in sheep
P43	**Alexandra Gianakoulakos** and Nicholas Robinson	A comparison of the non-coding regions of the FGF 1 gene in super fine wool merino and border Leicester sheep
P44	**B.L. Fricke**, Y.M. Parsons, M. Fleet, I. Franklin and D.W. Cooper	Parentage studies in sheep
P45	Z. Chen, R. Newcomb, J. A. McKenzie and **P. Batterham**	Replaying the evolutionary tape - the insecticide resistance story
P46	**Z. Chen**, E. Forbes, R. Newcomb and P. Batterham	The sequence of the Acetylcholinesterase gene in the Australian sheep blowfly - consequences for insecticide resistance
P47	Z. Chen, T. Newsome, **K.Freebairn** and P. Batterham	Gene structure of the *Notch* homologue from the Australian sheep blowfly
P48	**Janet L. Yen**, Philip Batterham, Bridget Gelder and John A. McKenzie	Predicting resistance and managing susceptibility to cyromazine in the Australian sheep blowfly, *Lucilia cuprina*
P49	**Phillip J. Daborn**, Philip Batterham and John A. McKenzie	The isolation and genetic characterisation of cyromazine resistant mutants of *Drosophila melanogaster*
P50	**Besse, P**, McIntyre, C.L. and Berding, N.	Isolation and characterisation of repetitive sequences specific for the Erianthus genus (Saccharinae - Andropogoneae)

P51	**Alex C. Pinkerton**, Steven Whyard, Craig J. Coates, Hilary A. Mende, David A. O'Brochta and Peter W. Atkinson	The Homer transposable element of *Bactrocera tryoni* is a member of the hAT family of transposable elements
P52	**Rachel Hawken**, Kizanne Davies and Jill Maddox	Polymorphic markers for ovine cytokines genes
P53	**Rebecca N. Johnson** , John Sved and Marianne Frommer	DNA sequence polymorphisms in Queensland fruit fly populations
P54	**Craig Bennett** and Marianne Frommer	The *white* gene of *Bactrocera tryoni* a marker for transformation in the *Bactrocera* genus
P55	**R. A. Leopold** , K. J. Hughes, J. D. DeVault and P. W. Atkinson	Gene delivery to insect embryos using electroporation and a slot cuvette
P56	D. Bulach, A. Kumar, Bufeng Liang, C. Mansour, and **D. E. Tribe**	PCR amplification and direct DNA sequencing of DNA polymerase genes for inferring baculovirus phylogeny
P57	**David G. Heckel**, Linda C. Gahan, Fred Gould, and Arne Anderson	Identification of a linkage group with a major effect on resistance to *Bacillus thuringiensis* CryIAc endotoxin in the tobacco budworm *Heliothis virescens* (Lepidoptera: Noctuidae)
P58	**Murali Nayudu**, Terry Murphy and Julian Ash	Behaviour of genetically engineered *Pseudomonas* biological control bacteria in small scale field release
P59	**Jill R. Williams**, Nigel L Brown and Barry T.O. Lee	Characterisation of cryptic plasmids carrying *pco*-like copper resistance determinants in enteric isolates from pigs
P60	**Paul Gilson** and Geoff McFadden	The miniaturized nuclear genome of a eukaryotic endosymbiont contains genes that overlap, genes that are cotranscribed, and the smallest known spliceosomal introns
P61	**Tatyana Podolsky**, Sheik-Tao Fong and Barry T.O. Lee	*cutE*, the gene involved in divalent cations tolerance in *Escherichia coli*
P62	Marjory Martin and **Dawn Gleeson**	Genetics in the Victorian Year 12 Biology Course
P63	**Nauman J. Maqbool**, Chris Moran, Gavin E. Greenoak and Frank W. Nicholas	Creation of a highly fecund strain of hairless mice
P64	**Joanne M. Lumsden**, Eric A. Lord, Michael J. Dixon and Grant W. Montgomery	Mapping of DMP1 in sheep defines an inversion breakpoint with respect to human chromosome 4q
P65	**Alan N. Wilton**	Use of homologues of human Batten's disease gene to predict protein structure and amino acid sites important in function
P66	**Gasiamis, H.** and Bisucci, T.	Polymorphisms of the cholesteryl ester transfer protein (CETP) gene and their association with lipid phenotypes in Greek and Italian migrants to Australia
P67	**James Camakaris**, Peiyan Shen, Michael Petris, Leanne Bailey, Paul Lockhart and Julian F.B. Mercer	Studies on the Menkes (MNK; ATP7A) P-type ATPase in copper-resistant CHO cells
P68	**ZS Krozowski**, P Ferrari, VR Obeyesekere, K Li, R Smith, RC Wilson, MI New and JW Funder	Mutations in the 11s-Hydroxysteroid dehydrogenase Type II gene give rise to the syndrome of Apparent Mineralocorticoid excess
P69	**A. Kamitani**, ZYH Wong, P Dickson, L van Herwerden, MJ Abramson, EH Walters, J Raven, A Forbes and SB Harrap	Absence of genetic linkage of chromosome 5q31 with bronchial hyperreactivity and atopy in the general population
P70	V. Shridhar, S. Rivard, R. Shridhar, C. Mullins, L. Bostick, W. Sakr, D.Grignon, **O.J. Miller** and D.I. Smith	A specific point mutation in an imperfect trinucleotide repeat region of the ARP gene in sporadic human renal cancer
P71	**Rob Slade** and Hamish McCallum	MHC diversity and disease risk
P72	**K.E.Sloper** and R. Baker	A putative human ubiquitin specific-protease: HUBP
P73	**P. Dickson**, ZYH Wong, L van Herwerden, MJ Abramson, EH Walters, J Raven and SB Harrap	Absence of mutations in the coding regions of the high affinity IgE receptor gene in asthma
P74	**White JD**, Bower JJ and Austin L.	Expression of mutated and wild type dystrophin after myoblast transfer therapy in the *mdx* mouse

P75	**Katrina Goodge**, Angela Bruzzaniti, Philippe Jay§, Sylvie A. Taviaux, Mark H.C. Lam, Philippe Berta, T. John Martin, Jane M. Moseley and Matthew T. Gillespie	Identification of PC8 the seventh member of the convertase family
P76	**Rochelle L. Waite**, Judy M. Callaghan and Ban-Hock Toh	Detection of antibodies reactive to the early endosomal marker (EEA1) in disparate autoimmune sera
P77	**S.El-Osta**, P.Kantharidis and J.Zalcberg	Quantitating the number of mdr-1 molecules per cell using competitive PCR
P78	**Thomas Yong**, John Sentry, Yik-Yuen Gan and Ban-Hock Toh	Is the reported sequence of RMSA-1 a pseudo gene?
P79	**Veronika Gitlits**, John W. Sentry and Ban-Hock Toh	Characterisation of rat testis antigens reactive to serum from a patient with discoid lupus erythematosus (DLE)
P80	**Yugang Tu**, Veronika Gilitis, John Sentry, Yikyuen Gan and Ban-Hok Toh	Preliminary identification and characterisation of nervous system autoantigens reactive to a serum collected from a patient with discoid lupus erythematosus
P81	**Anita Quigley**, George Hale and Edward Byrne	Mitochondrial DNA deletion detection and quantification in cardiomyopathic hearts
P82	**Kaska, D.E.**, van Oorschot, R.A.H. and Mitchell, J.R.	Population studies and forensic application of the CTT multiplex STR system
P83	**Wilson-Wilde, L.M.**, van Oorschot, R.A.H. and Mitchell, J.R.	Population studies of a seven loci multiplex PCR system
P84	**Leeanne Mead**, Matthew Gillespie, Kathleen Rayeroux, Usha Rane, Jaclyn Hung, Lou Irving and Lynda Campbell	LOH at 9p21 Centromeric to CDKN2a: Another TSG?
P85	**Graham C. Webb** and Cynthia D.K. Bottema	PPD11 banding: Simply brilliant chromosomal identification
P86	**Jane E. Andrews**, Jill Kent, Susan C. Wheatley, Peter Koopman and Andrew H. Sinclair	Male-specific expression of *cSOX9* in chicken sex determination
P87	**Sharon Layfield**, Pino Maccarone, Roland Toder, Rachel O'Neill and Jennifer A. Marshall Graves	Localisation of the sex reversing *SOX9* gene to the 2q region of the Tammar Wallaby (*Macropus eugenii*)
P88	**Matthew J. Wakefield**	Mapping of human Xq13 genes in marsupials: Implications for X chromosome inactivation
P89	**Amanda Spurdle** and Jennifer A. Marshall Graves	Human DYZ1 Y-specific sequences are detected by PCR on the marsupial Y chromosome
P90	**Amanda Spurdle** and Jennifer A. Marshall Graves	Enrichment of a marsupial testis cDNA library for male-specific sequences by subtractive hybridization
P91	**Delbridge, M. L.**, Harry, J., Toder, R. and Graves, J.A.M.	Are candidate human spermatogenesis genes conserved on the Y in marsupials?
P92	**Cathy M. Watson**, Peter G. Johnston, Katherine A. Rodger, Louise McKenzie and Desmond W. Cooper	Sex determination and differentiation in intersexual marsupials
P93	**Andrew Pask**, Roland Toder, Stephen A. Wilcox and Jennifer A. Marshall Graves	Isolation and characterisation of the sex reversing *DAX*-1 gene in marsupials: Implications for the evolution of sex determination
P94	**Rachel J. Waugh O'Neill** and Jennifer A. Marshall Graves	Multiple copies of *SRY* in a Dasyurid marsupial
P95	**Rachel J. Waugh O'Neill**, Joanne Martin, James Cook, Mike Tristem, Ross H. Crozier and Jennifer A. Marshall Graves	Transposable element evolution in Rock Wallabies (Petrogale)
P96	**Tang, P.C.**, Sherwin, W.B. and Croft, D.B.	Analysis of matrilines in euros using mitochondrial DNA
P97	**Davies, K.P.**, Drinkwater, R., Harrison, B., Hulme, D. and Maddox, J.F.	Updating the sheep genetic linkage map
P98	**Shuliang Cui** and Rory Hope	Molecular biology and expression of the Leukaemia Inhibitory Factor (LIF) gene in the marsupial *Sminthopsis crassicaudata*

P99	**M-H. Lee** and R. Hope	The molecular biology and evolution of ß-globin genes in monotremes
P100	L.Young, J.Old, D.W.Cooper and **E.M.Deane**	T-cell cytokines in the marsupial, *Macropus eugenii*
P101	**Karen B. Firestone**	Taxonomy of quolls (Dasyuridae:Marsupialia) at the species, subspecies, and population level
P012	**Elizabeth A. Sinclair** and Michael Westerman	Phylogenetic relationships within the genus *Potorous* (Marsupialia: Potoroidae) using allozyme electrophoresis and sequence analysis of the cyrochrome B gene
P103	**David Hurwood**, Jane Hughes and Stuart Bunn	Comparative phylogeography of aquatic fauna in the Tully and Herbert Rivers - Evidence for an altered drainage pattern
P104	**Katrina McGuigan**	Evolutionary relationships within the *Litoria pearsoniana* complex
P105	**Merrin E. Spackman**, Marcus Matthews, Stephen W. McKechnie and John Trueman	Mitochondrial DNA for use in species identification and phylogenetic analysis of the Heliothinae (Lepidoptera: Noctuidae)
P106	**Glenn C. Graham**, Robert J. Henry, Ian D. Godwin and D. Garth Nikles	Phylogenetic position of Hoop Pine (*Araucaria cunninghamii*)
P107	**Roger Lowe** and Ross H. Crozier	The phylogeny of socially parasitic bees
P108	Alexander G. Blinov and **Jon Martin**	Phylogenetic relationships of non-LTR retrotransposons in the genus *Chironomus* (Diptera)
P109	**Andrea Crampton** and Steve Barker	FISHing the cattle tick genome
P110	Mohammad Reza Shariflou and **Chris Moran**	Evolution of the $(AC)_n$ microsatellite at the IGF-I locus in Artiodactyls
P111	**Pino Maccarone** and Jennifer A. Marshall Graves	Current status of the marsupial and monotreme genetic maps
P112	**Y. Chen**, W. Zhang, Muladno, B. Hoyheim, M. Yerle and C. Moran	Physical and linkage mapping of microsatellites in the Porcine genome
P113	Jing Cheng, Takao Kasuga and **Keith Mitchelson**	Heteroduplex polymorphism (HPA) and SSCP analyses using entangled solution capillary electrophoresis (ESCE) for rapid identification of intersterility group in *Heterobasidion annosum*
P114	Salvatore Moricca, Takao Kasuga, **Keith Mitchelson**, Alessandro Ragazzi and Stephanos Diamandis	Polymorphism in the ribosomal ITS distinguishes sexual forms of the pine blister rusts *Cronartium flaccidum* and *Peridermium pini*

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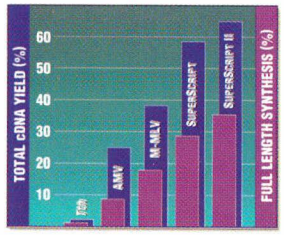

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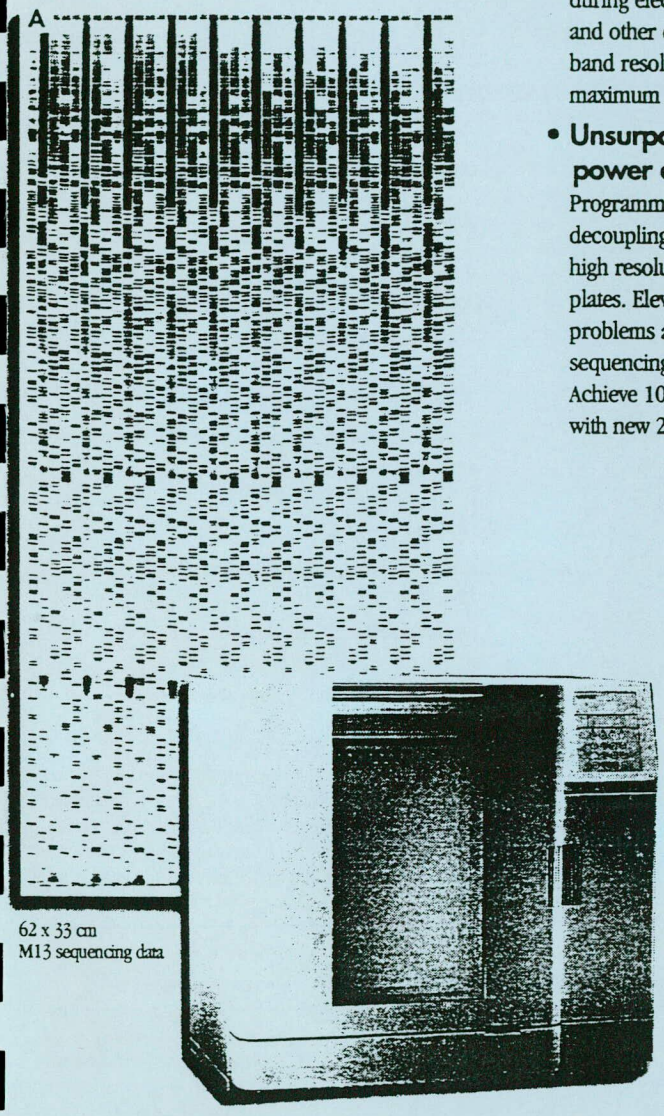

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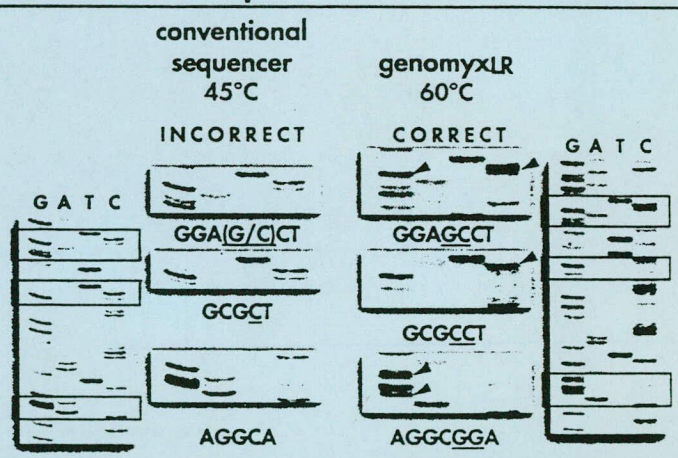
B

Eliminate compressions and data loss...



45°C sample run on conventional manual sequencing equipment under ambient conditions (6 hr, 60 W, 6% acrylamide, aluminum plate backing). 60°C sample run on **genomyxLR™ DNA Sequencer** (5 hr, 100 W, 6% HR-1000™ gel). Sequencing data derived from mouse cardiotrophin cDNA (Genentech, Inc.)

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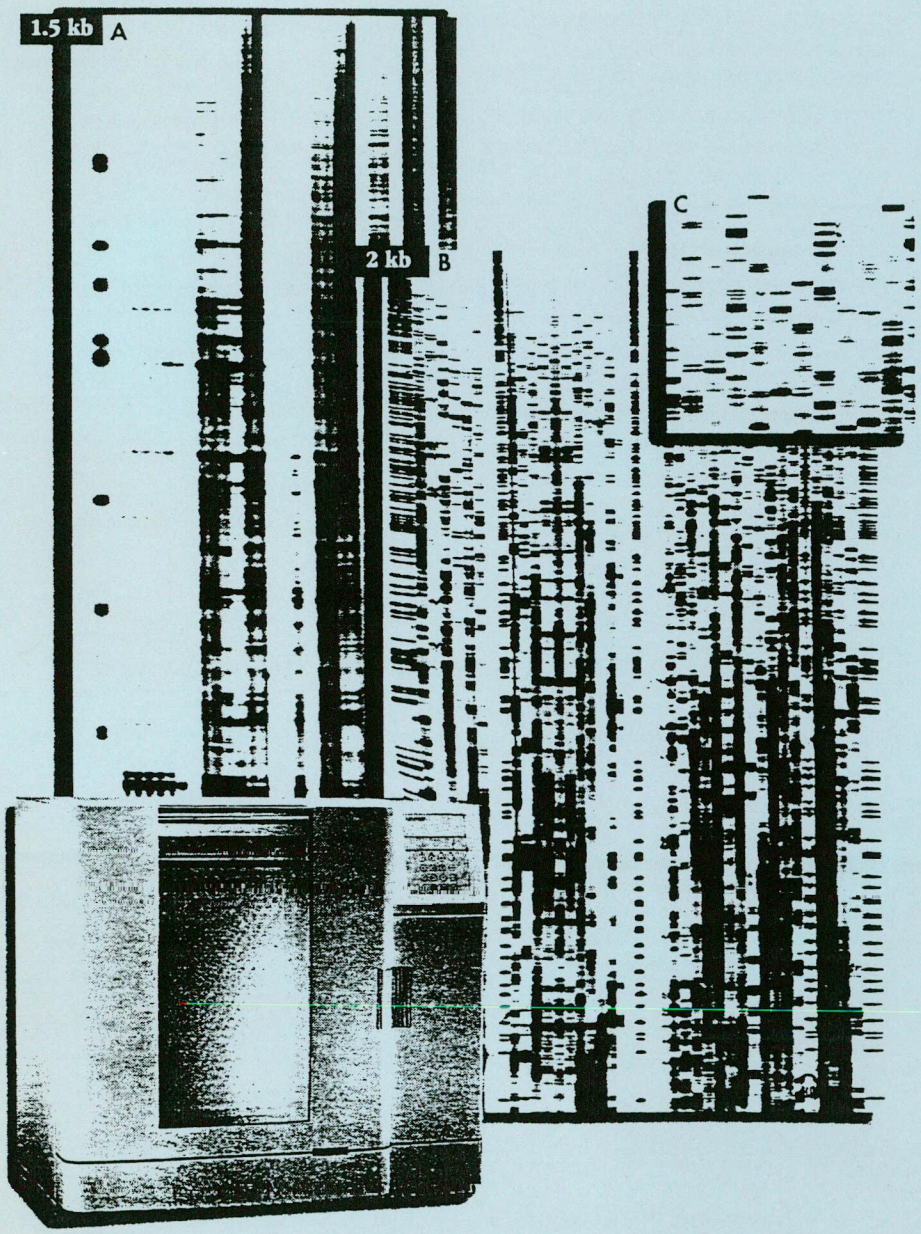

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Panel A: Long read, high resolution Differential Display data provided by Drs. Lidia Averboukh and Arthur Pardee, Division of Cell Growth & Regulation, Dana Farber Cancer Institute. Samples run on **genomyxLR** (2.5 hr, 100 W, 3 kV, 50°C, HR-1000™ 4.5% Denaturing gel). Data represent display patterns under different RT-PCR conditions using total cellular RNA from LNCaP-FGC prostate carcinoma cell line. All reactions performed with the GenHunter RNAimage™ Differential Display Kit.

Panel B: Long read, high resolution Differential Display patterns of total cellular RNA from HeLa and transformed rat fibroblast cells using GenHunter RNAimage Differential Display Kit. Samples run on **genomyxLR** (2.5 hr, 100 W, 2.7 kV, 50°C, HR-1000 6% Denaturing gel). Data provided by GENOMYX Corporation.

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T1

Designer Genes - do they fit consumers?

Nancy F. Millis

Historically, we have changed organisms by the tedious process of mating parents, and breeding selectively among the offspring, those displaying wanted properties. The others are destroyed. This callous process attracts no approbrium, far less restrictive legislation. So what is the fuss about changing the genetic make-up by a different, more precise method? It is important to recognise that the community is composed of many "publics", and that law makers tend to respond to the public's perception of risk, not to its scientific assessment. In Australia, surveillance of genetic engineering is provided by a Commonwealth Committee, GMAC. GMAC is a non-statutory body, but may be replaced by legislation in the future. Australian guidelines are designed to allow the risks in a proposal to be identified and managed. There is little public concern about research if it is in contained conditions, but the release of live modified organisms (GMO) is another matter, and their application is potentially important, particularly for Australian agriculture. For example,

Veterinary and Medical practice

- Pharmacologically active agents;
- vaccines (live, subunit)
- diagnostic kits and reagents
- enhanced growth rate and carcass composition

Agriculture

- resistance in crops to herbicides, insects, viruses, root pathogens
- change in starch composition in potato
- changes in fatty acid composition in oil seed
- changes in protein composition in pasture plants
- enhanced shelf-life in tomato
- tolerance of pH/temperature in rhizobia

Possible concerns associated with these applications will be discussed.

hobo ≡ Ac/Ds

Genetic Engineering of Insects

Peter W. Atkinson and David A. O'Brochta*

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Canberra ACT 2601

**Center for Agricultural Biotechnology*
University of Maryland Biotechnology Institute
College Park
Md 20742 USA

para transgenic insects

endosymbionts grown in culture, transformed + returned to host

Until recently the ability to genetically transform insects other the vinegar fly, *Drosophila melanogaster*, has eluded insect scientists. This inability has hampered the extension of the full repertoire of contemporary molecular biological and genetic techniques into entomology. Insects are the most abundant and diverse organisms on earth and so represent an untapped wealth of genetic and biochemical information. More importantly, a handful of insect species have and continue to affect our livelihood, either by competing for our food resources or by spreading disease. The need to control these pest insects has led to the widespread use of chemical insecticides and, as a consequence, the toxic residues of these are now affecting our environment. New approaches to insect pest control are urgently needed.

Recent developments in non-drosophilid insect transformation will be presented together with a discussion of what some of the possible consequences for both pest and beneficial species of insect transformation might be.

T3

Phenotype-genotype interactions and the Australian Genome Research Facility

Simon Foote

Australian Genome Research Facility - Walter and Eliza Hall Institute

The Human Genome Project has predicated a change in the direction that biological research is taking. The large amount of money and effort that the US and European Govemments have been investing in generating tools for genome research rneans that biologists can now use the genome and the information it encodes as a research tool. In human genetics it means that one can actually move straight from phenotype to genotype without the necessity of building up immense biological knowledge. This does not mean that a lack of understanding of biology will flow from genome science, on the contrary we can now add the organismal phenotype data to the enormous library of biological information that has accrued over the years about numerous biological traits.

However this increase in information comes at a cost and for we Australians that cost is that this research is done at a very large scale. While the fruits of genome research can be applied at an individual research level, the addition to genome information, the translation of genome techniques to other organisms and other systems requires a large technological and computational infrastructure. The Australian Government has recently invested $10 million into Australia's genome infrastructure in the guise of the Australian Genome Research Facility. This Facility will be housed at both Brisbane and Melbourne and will allow the translation of genome technological advances made overseas to Australia and also, hopefully, to capitalise on technological and experimental advances made here in Australia. This Genome Facility will have the ability to perform large scale DNA sequencing. The contribution of the facility to a researcher's needs will depend on the individual project but it is anticipated that the AGRF will be able to sequence genomic DNA from BACs or cosmids at one extreme, or at the other, be able to run sequencing reactions on pre-purified templates. The AGRF will carry out large scale genotyping, having the ability to genotype large pedigrees, herds, or crosses efficiently and rapidly. The genotyping technology will be heavily based on the analysis of simple sequence repeats. Mutation detection research will be performed on a large scale and possibly allow mutational analysis to be performed on large population samples. The AGRF will also be a storage facility in Australia for DNA libraries, be they genomic or cDNA libraries; it will have the ability to pick new libraries, grid and screen them. Other speciality services will be provided and there will be space within the facility for people to come and do their own projects using the technology housed within the AGRF.

The Facility will be accessible by anyone wishing to do genome research. In order to cover the running costs of the Facility (the initial grant only covers capital costs) an institutional subscription will be levied. Consumable costs for each project will be charged back to the user on a project by project basis. The cost of sequencing, genotyping and mutation detection reactions will be significantly lower than what is currently feasible in Australian laboratories due to increased economies of scale and also due to the use of automation, thereby decreasing labour costs.

T4

In silico genetics

Tim Littlejohn

Australian Genomic Information Centre, J03, University of Sydney, NSW, 2006
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Computers have become an essential component of modern genetics. They play central roles in the design of experiments, generation and management of data, analysis and extraction of genetic information, publication, communication and education. This "*in silico*" environment represents a great challenge and opportunity for the years ahead.

The first step in genetics research often involves querying databases for genomic information used in planning PCR, cloning, sequencing and genetic linkage experiments. Cloning strategies, primer design, pedigree simulations are all carried out on the computer. DNA sequence and genotyping data are increasingly generated on automatic sequencing machines, and the data generated from these devices is stored in electronic readable form in ever increasing volumes as sequencing facilities become more widespread and cost efficient. Raw data can be transmitted directly to geneticists' computers across the network where it can be manipulated, managed and analysed.

Genetic information is extracted from the data by sophisticated analysis systems querying a plethora of databases wrapped behind user-friendly software interfaces. Results are submitted electronically through networks to sequence, genome or organism specific databases, and publications submitted, reviewed and read through the Internet. Computers connected to international networks provide the most efficient means to communicate, collaborate and exchange data and ideas with our collaborators.

This new era of genetics is creating fertile ground for novel discoveries in genetics and bioinformatics (the application of computers to solving problems in biology). Our ability to generate genetic data is expanding rapidly, and this is reflected in the growth of databases of genetic information, which are increasing in size exponentially, as are the number of such databases. The number and quality of software tools to query these resources and interpret their biological information content is growing steadily, and more powerful and friendly means of accessing genetic resources are being developed, promoting the accessibility of these resources to geneticists.

This presentation will highlight some or the more recent developments in doing genetics "*in silico*", some of the research opportunities in genetics and bioinformatics, and some new projects underway at the Australian Genomic Information Centre (AGIC).

T5

The TyrR protein of *Escherichia coli*. A genetic analysis of its role as repressor and activator.

Jim Pittard

Department of Microbiology, University of Melbourne

The TyrR gene of *Escherichia coli* was identified initially by mutations which mapped in a single cistron, caused derepression of a number of genes concerned with aromatic amino acid biosynthesis and transport and were recessive to the wild-type allele in *trans*. The identification of some of these mutants as amber mutants supported the notion that the *tyrR* gene product was a protein. Since that time the structure and function of the protein has been probed by reference to its amino acid sequence as derived from the DNA, its homology with other known regulator proteins and more importantly, the specific alterations to DNA binding, repression and activation caused by different mutations. Genetic manipulation of target sites and alanine scanning of TyrR protein and the alpha sub-unit of RNA polymerase have added to our knowledge of the strategies used by this protein to modulate gene expression.

T6

The *hap* genes of *Aspergillus nidulans* play a role in the regulation of *amdS* expression.

Peter Papagiannopoulos, Alex Andrianopoulos, Julie A. Sharp, Meryl A. Davis & Michael J. Hynes.

Department of Genetics, The University of Melbourne, Parkville, 3052.

The *amdS* gene of *Aspergillus nidulans* encodes an acetamidase required for growth on acetamide as a sole carbon and nitrogen source. The 5' regulatory region of *amdS* contains a CCAAT sequence which is required for setting the basal level of transcription. Mobility shift studies have identified a factor in *A. nidulans* nuclear extracts which binds to this CCAAT sequence. In *Saccharomyces cerevisiae* the *HAP3* and *HAP5* genes encode components of a multisubunit complex which binds CCAAT sequences. An *A. nidulans* sequence with significant homology to the *HAP3* gene has been identified adjacent to the previously cloned regulatory gene *amdR*. This gene has been named *hapC*. A haploid carrying a *hapC* gene deletion has been created and is viable. The deletion strain grows weakly on acetamide as a sole carbon and nitrogen source, indicating that *hapC* may play a role in *amdS* expression. Consistent with this it has been shown that the *hapC* deletion results in reduced levels of expression of an *amdS::lacZ* reporter gene, particularly under carbon limiting conditions. Nuclear extracts prepared from the haploid gene deletion strain show no CCAAT specific binding activity to the *amdS* promoter, indicating that *hapC* may encode a component of the complex binding at this sequence. Using a heterologous probe we have cloned a homologue of the yeast *HAP5* gene, which we have named *hapE*. We intend to disrupt this gene in order to determine whether it has similar effects to those seen with *hapC*.

Identification and Characterisation of a Conserved Zinc-Binding Module in a Family of Copper Regulated Yeast Transcription Factors.

Farrell, R.A.*, and Winge, D.R.

Departments of Medicine and Biochemistry, University of Utah Health Sciences Center, Salt Lake City, Utah, 84132, U.S.A..
**Current Address: Department of Genetics, The University of Melbourne, Parkville, VIC, 3052, Australia.*

The AMT1 metalloregulatory *trans*-acting factor of *Candida glabrata* is functionally and structurally homologous to the ACE1 transactivator of *Saccharomyces cerevisiae*. AMT1 regulates the expression of three metallothionein genes in *C. glabrata* in a Cu(I)-dependent manner. Cu(I) binding to AMT1 stabilises a conformation capable of binding to specific upstream activation sequences (UAS) in the MT gene promoters.

We have shown that both the AMT1 and ACE1 proteins bind four Cu atoms, as a tetracopper center, and one Zn atom within the DNA-binding, N-terminal half of each molecule (1). Site directed mutagenesis of a synthetic gene, encoding the N-terminal metal-binding domain of AMT1, has been utilised to identify residues that may act as Zn- and/or Cu-binding ligands. These studies indicate the N-terminal third of the metal-binding domain is critical for Zn-binding.

Studies of synthetic peptides, corresponding to residues 1-42 of both AMT1 and ACE1, have confirmed that this region is responsible for Zn-binding. ^{113}Cd NMR spectroscopy of the Cd:AMT1 peptide complex has shown that three cysteinyl and one histidyl imidazole act as metal ligands. The sequence forming the zinc module is conserved in another putative Cu-binding transcription factor, MAC1, as well as three other putative yeast transcription factors that have not yet been characterised. Studies are under way to further elucidate the role of Zn(II) in the proteins.

References:

1. Farrell, R.A., Thorvaldsen, J.L., and Winge, D.R. (1996) *Biochemistry* **35**: 1571-810.

T8

Nitrogen regulation and the *tamA* gene of *Aspergillus nidulans*

Anna J. Small, Michael J. Hynes and Meryl A. Davis

Department of Genetics, University of Melbourne, Parkville 3052

In *Aspergillus nidulans*, the expression of many genes involved in nitrogen utilization is increased in the absence of easily metabolized nitrogen sources such as glutamine. This activation is mediated by the positively acting AreA protein, which binds to 5'GATA3' sequences in the promoters of regulated genes and activates transcription under nitrogen limiting conditions. The *tamA* gene in *A. nidulans* may also play a role in nitrogen regulation in addition to *areA*. *tamA* mutants have been identified which have low levels of a number of nitrogen metabolic enzymes, including NADP-glutamate dehydrogenase which is required for ammonium metabolism. Mutants are also resistant to the toxic ammonium analogue methylammonium.

tamA has been cloned, and sequence analysis revealed a number of features suggestive of regulatory function. The predicted protein contains a Zn(II)2Cys6 zinc cluster motif similar to others known to bind DNA, and regions of similarity to fungal regulatory proteins. TamA shows strongest homology to UGA35, a nitrogen regulatory protein in *Saccharomyces cerevisiae*. The importance of these regions to TamA function is being investigated. Inverse PCR mutagenesis has been used to demonstrate that the zinc cluster motif is not required for TamA function. Substitution of a crucial cysteine residue did not prevent an altered *tamA* construct from complementing a *tamA* mutant strain in cotransformation experiments. An internal deletion has demonstrated that sequences towards the C-terminal of the protein are required for function. The precise nature of changes in a range of *tamA* mutants are being determined to further identify areas important in function.

Pig radiation hybrid panel
- McDowell

T9

Studies of CATS (Comparative Anchor Tagged Sequence) markers in the pig

Weiyi Zhang and Chris Moran

Department of Animal Science, University of Sydney NSW 2006

Lyons *et al* (1996) have developed "universal" PCR primers from exon sequences conserved in at least two eutherian species. The primers have been chosen to include about 50 bp of exon for verification of the identity of PCR products by sequencing and to flank introns, which will hopefully be the source of intraspecific polymorphisms. These comparative anchor tagged sequence ("CATS") primers have been developed for 384 loci including many of the human comparative anchor loci selected by O'Brien *et al.* (1993).They are being distributed to gene mapping laboratories throughout the world to facilitate mapping of a standard set of loci in as many mammalian species as possible. This will permit the detection of conserved synteny, the recognition of evolutionary breakpoints and the reconstruction of the evolutionary history of chromosomes within the mammals.

As part of a co-ordinated programme of mapping of these markers in the pig, we are working on 36 primer pairs from loci which map to human chromosomes 9 and 10. The strategy for using the primers and progress in PCR amplification of products, verification of identity of products and the search for polymorphisms will be described.

References

Lyons, L.A., Laughlin, T.F., Copeland, N.G., Jenkins, N.A., Womack, J.E. and O'Brien, S.J. Comparative Anchor Tagged Sequences for integrative mapping of mammalian genomes. *Science* (In press)

O'Brien, S.J., Womack, J.E., Lyons, L.A., Moore, K.J., Jenkins, N.A. and Copeland, N.G. (1993) Anchored reference markers for comparative genome mapping in mammals. *Nature Genetics* **3:** 103-112

Evolution of a Cytokine Gene Family

[1]Rachel Hawken, [2]Murray Broom, [2]Tracey van Stijn, [3]Pino Maccarone, [2]Joanne Lumsden, [3]Jennifer A Marshall Graves, [2]Tom Broad and [1]Jill Maddox.

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Cytokines are a diverse group of proteins and polypeptides that, amongst other functions, are important in immune and inflammatory responses. From the limited number of species studied so far, it has been postulated that many cytokines evolved from one primordial gene by gene duplication. Comparative mapping of a group of cytokine genes located on human chromosome 5q supports this hypothesis. The relative position of the genes encoding IL3, IL4, IL5 and CSF2, which are located on human chromosome (chr) 5q31.1, have remained conserved in the rat (chr 10), mouse (chr 11) and cattle (chr 7) genomes. In order to further investigate the evolution of the IL3, IL4, IL5 and CSF2 genes, two approaches have been taken.

The first has been to examine the extent of conservation of gene clustering by extending the comparative gene map for this region in both eutherian (sheep) and metatherian (Tammar Wallaby) mammals.

The second approach has been to compare the rates of evolution of these genes. Sequence comparisons among a number of species (including primates, rodents, and artiodactyls) have been made using coding sequences available in GenBank, and sequence data obtained for these genes in the Tammar Wallaby.

GENETIC MAPPING OF A COMPLEX DISEASE: MANIC DEPRESSIVE ILLNESS

Jennifer Donald[1], John Kwok[2], Judy Salmon[2], Philip Mitchell[3] and Peter Schofield[2]

1 School of Biological Sciences, University of Sydney, NSW
2 Garvan Institute of Medical Research, Darlinghurst NSW
3 School of Psychiatry, University of New South Wales, NSW

Manic-depressive illness (MDI) or bipolar disorder is a mental disturbance characterised by severe mood swings. There is evidence for a significant genetic component to susceptibility to this disease, although the genes involved are not known. It is very likely that there is significant genetic heterogeneity in MDI. Recently candidate susceptibility sites have been identified on a number of different human chromosomes.

One of the areas of the genome identified as a possible site for a susceptibility gene is chromosome 21. Straub *et al* (1994) found significant evidence for linkage of disease susceptibility to a chromosome 21 marker in a small proportion of families. The combined dataset did not support linkage to chromosome 21 with lod score analysis, but support was found with nonparametric tests, such as the Affected Pedigree Member method (APM).

As part of a wider genome screen for susceptibility genes, we have studied 6 markers located in a 20cM region of chromosome 21q in 12 multigenerational Australian pedigrees with MDI. Linkage results for the whole dataset do not support linkage, although 4 families give consistently positive lod scores. The APM method, however, gives statistically significant support for linkage of a susceptibility gene to part of this region.

We are currently investigating this apparent discrepancy between results obtained from conventional lod score analysis and those from the non-parametric APM method, an effect found in other studies of MDI. The significance of the differing results obtained from the different approaches will be discussed, as well as implications for further studies.

Straub R. *et al* (1994) *Nature Genet.* **8**:291-296

T12

Genetic Analysis of Fibrinogen and Cardiovascular Risk.

ZYH Wong, M Stebbing, SB Harrap

Victorian Family Heart Study, Department of Physiology, University of Melbourne, Parkville, Victoria, Australia.

Plasma fibrinogen has been identified as an important determinant of risk for coronary disease. To understand the familial and genetic factors that might affect plasma fibrinogen we studied 603 families comprising 1137 offspring recruited from general population as part of the Victorian Family Heart Study. Plasma fibrinogen was measured by the clot opacity method and molecular variation in the fibrinogen gene was assessed by the presence or absence of two alleles *(Hl & H2)* that were detected after PCR amplification of a *Hae*III restriction site. Plasma fibrinogen was corrected for age and sex before inclusion in analyses. There was a significant correlation between plasma fibrinogen ($r = 0.13$, $P < 0.001$) between spouse pairs. The magnitude of correlation of plasma fibrinogen between mothers and offspring ($r = 0.15$, $P < 0.001$) and fathers and offspring ($r = 0.13$, $P < 0.001$) was of similar magnitude. The correlation between sibling pairs was slightly higher ($r = 0.19$, $P < 0.001$) than other intrafamilial correlations. The frequencies of the fibrinogen genotypes in 274 individuals chosen at random were *H1H1:* 64.6%, *H1H2:* 34.4% and *H2H2:* 1.0% with average levels of plasma fibrinogen of 2.85 g/l, 3.34 g/l and 2.83 g/l respectively. After correction for age and sex, there remained a marginally significant contribution ($F_{2,273} = 3.43$, $P = 0.03$) of fibrinogen genotype to plasma fibrinogen. These findings indicate that in the general population, shared environment within families contributes significantly to variation in plasma fibrinogen. Our observations also suggest a lesser influence of shared genes which in part is associated with molecular polymorphism in the fibrinogen gene.

T13

Evolution of homoeologous chromosomes in polyploids: evidence from comparative mapping of allopolyploid and diploid *Gossypium*.

C. L. BRUBAKER, A. PATERSON, AND J. WENDEL.

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Allopolyploidy is prevalent in plants, yet little is known about the consequences of uniting two divergent genomes in a common nucleus and cytoplasm. To the extent chromosome homologies influence meiotic pairing, theory predicts that in allotetraploids residual homologies that allow multivalent formation between progenitor chromosomes would be selected against, thus accelerating genomic differentiation. The AD-genome allotetraploid (N=26) cotton species, *Gossypium barbadense* and *G. hirsutum*, belong to a 1-2 million-year-old lineage that recombines two diploid (N=13) genomes (A and D) that diverged from a common ancestor 4-9 million years previously. Comparative mapping of the tetraploid A and D subgenomes and their A and D genome diploid progenitor genomes provides an ideal system for characterizing the extent and type of genomic rearrangements that accompany the divergence of diploid genomes as well those that arise from recombination of subgenomes in an allopolyploid. Using a common set of RFLP probes, linkage maps for the A and D genome diploids and the A and D subgenomes of the allotetraploids were organized into the 13 suites of homoeologous linkage groups. Comparisons confirm the presence of two reciprocal translocations among four tetraploid A subgenome chromosomes and provide indirect evidence of a third translocation between the two A genome diploid species, *G. herbaceum* and *G. arboreum*. Nineteen inversions were provisionally identified among the four genomes. Synteny is highest between the A and D genomes of the diploids and is lowest between the A and D subgenomes of the allotetraploids, indicating that polyploidy is associated with an increased rate of structural differentiation. This result is also evidenced by the fact that the A and D genome diploids are more similar to each other than they are to their respective subgenomic homoeologs. The length of conserved regions of A and D genomes and the A and D subgenomes differ by 6% and 5%, respectively. Lengths of conserved regions of the A-genome and the A-subgenome and the D-genome and the D-subgenome differ by 51% and 59%, respectively, suggesting that both tetraploid subgenomes are recombinationally more active that their diploid progenitors. Differentiation of the tetraploid subgenomes appears not to differ qualitatively from the divergence of their progenitor diploid genomes, but the rates at which syntenic and colinear associations are lost has been accelerated.

T14

Molecular population genetics of the cane toad (*Bufo marinus*): phylogeography and founder effects.

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[1] Molecular Zoology Lab, Dept. of Zoology, University of Queensland; [2] current address: Queensland Institute for Medical Research; [3] Lab de Evolucion, Universidad de la Republic, Uruguay.

The cane toad has been introduced to numerous Carribean and Pacific Ocean islands from its natural range in South and Central America. In most cases, the year of introduction and founder sizes are known (Easteal and Floyd 1981), making this an ideal system with which to examine the phylogenetic and population genetic consequences of range expansions coupled with serial bottlenecks. Previous studies by Easteal (1985, 1988) exploited this system to examine patterns of allozyme variation among introduced populations from Hawaii and Australia, but did not extend to populations in the natural range. We have examined variation in mtDNA ND3 sequences across the natural range from Texas to south-east Peru and introduced populations from Hawaii and Australia. Tests for selection indicated that the ND3 diversity reflects neutral evolution. The mtDNA phylogeny demonstrated long-term historical separation of populations on either side of the Venezuelan Andes. Other aspects of the phylogeography reflect exactly the known colonisation history; the Hawaiian and Australian populations are related to variants from French Guyana and east Venezuela, the known source, and diversity in the introduced populations is zero, compared to substantial sequence diversity in populations from the source area. These observations attest to the power of mtDNA phylogeography for reconstructing population history, despite the fact that it represents a single gene. We are currently extending this study to examine the effects of introductions and population expansions on hypervariable nuclear microsatellite loci.

Easteal, S. and R. Floyd (1981) Biol. J. Linn. Soc. 16:93-113.

Easteal, S. (1988) Evol. Biol. 23:49-84.

Phylogeography, vicariance and introgression in Australian Rainforest frogs

Michael Cunningham

Department of Zoology, The University of Queensland

Repeated pleistocene climatic fluctuations have had a major effect on the distribution of animals and species diversity in Australian rainforests. The controversial 'vicariant refuge' model proposes that the diversity of rainforest organisms results from allopatric speciation in refugial areas during dry, glacial times. Analysis of mitochondrial sequence variation in three rainforest frogs has shown congruent phylogenetic patterns consistent with independent evolution of populations isolated in different pleistocene refuges. The depth of divergence among rainforest areas differs widely among species; *Litoria nannotis* and *L. genimaculata* show ancient divergences, whilst coalescence of mtDNA haplotypes in *L. rheocola* is more recent. Zones of introgression between divergent mtDNA clades have been found in both *L. nannotis* and *L. genimaculata.*

In this paper I compare mtDNA genealogies from *L. nannotis, L. genimaculata* and *L. rheocola* and present haplotype frequency data across a 'hybrid' zone in *L. nannotis* . From these data I infer relative rates of gene flow ($\mathbf{N_em}$) for these species, and contrast these with expectations based on ecological studies. I interpret the results as showing strong support for vicariance within species but not for refugial speciation.

GENE FLOW AMONG WILD RABBIT POPULATIONS IN A NON-EQUILIBRIUM SYSTEM.

Susan Fuller*, Peter Mather and John Wilson

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Rabbits are a significant pest species which cause considerable environmental and economic loss, particularly in arid and semi-arid Australia. In these dynamic environments rabbit populations fluctuate, experiencing a high rate of local extinction and varying probabilities of recolonisation. Mitochondrial DNA haplotype frequencies (developed using heteroduplex analysis and temperature gradient gel electrophoresis) were used to estimate gene flow among populations. Frequencies were homogeneous throughout arid western Queensland, with populations connected by high gene flow (Nm=250) forming one large management unit. In contrast, frequencies were significantly heterogeneous in semi-arid eastern Queensland with less gene flow (Nm=3.8) among populations, suggesting that this region may be comprised of many small management units. However, according to the theoretical guidelines of Wright's equilibrium model, an Nm>1 indicates sufficient gene flow to counteract local differentiation due to drift. In dynamic systems where populations are not constant and frequencies have not reached equilibrium, gene flow estimated using this model may be inflated. These results suggest that in non-equilibrium systems, estimates of gene flow must be accompanied by an understanding of the spatial and temporal scales over which population processes operate and that less importance should be placed on the actual levels of gene flow and more on the relative differences between estimates.

T17

Gene Flow and Dispersal among Populations of Stream Invertebrates

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Patterns and levels of dispersal among populations of a species can be inferred from data on genetic differentiation. This study uses genetic variation at allozyme loci to investigate dispersal among populations of stream invertebrates in the Conondale Range, south East Queensland. Initially sampling was from three streams in each of two subcatchments in each of two major drainages, the Brisbane River and the Mary River. Later, pools sampled were 100-200 metres apart along some streams. This paper will discuss results for three species, the glass shrimp *Paratya australis*, a stonecase caddis fly, *Tasiagma* sp. and a mayfly *Baetis* sp.

For the shrimp, the levels of differentiation among populations in the headwater streams was huge, sometimes even within single subcatchments. The lowest levels of differentiation were within streams and the largest between subcatchments and catchments, as may be expected for an animal restricted to instream dispersal and with a limited capacity for movement.

For the other two species, the levels of differentiation overall were an order of magnitude lower than for the shrimp, suggesting extensive movement, both within and between streams. These results suggest that dispersay by adult flight, rather than within stream movement of larvae, is the major mechanism of dispersal. In contrast to the shrimp, both insect species exhibited the greatest genetic differentiation on the smallest spatial scale, i.e., among pools within streams. Possible reasons for these patterns will be discussed.

RATES OF MOLECULAR EVOLUTION IN AUSTRALIAN PASSERINES

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The aim of this research is to investigate the relative rates of molecular evolution of different forms of non-coding DNA in birds.

Here I will compare and contrast rates of molecular evolution in two classes of non-coding DNA :- mitochondrial control region and the second intron of the nuclear myoglobin gene in Australian passerines.

The results I will present show that the nucleotide substitution rate is much higher in the mitochondrial control region than in the myoglobin intron in these birds. These results suggest that either there is selection occurring on the intron sequences, OR that the mutation rate of this intron is much lower than that of control region in passerines.

5S expression.
1,2,3 mutants ← wildtype
4,5 ≫ wildtype.
6 ⋙ wildtype.
7,8,9 ≪ wildtype.

Mutations in middle of protein increase activation

Developing *Xenopus* embryos as a living test tube for transcription factor function.

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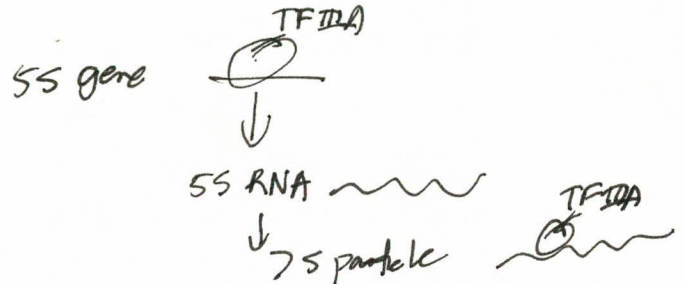
The *Xenopus* 5S RNA gene-specific transcription factor IIIA (TFIIIA) has nine consecutive cys_2his_2 zinc finger motifs. Developing *Xenopus* embryos were used as a homologous system in which to assess the structural and functional relationships between the nine different zinc fingers of TFIIIA in a physiological setting. Nine separate TFIIIA mutants were expressed in *Xenopus* embryos following microinjection of their respective *in vitro*-derived mRNAs. Each mutant contained a single histidine-to-asparagine substitution in the third zinc-ligand position of an individual zinc finger. These mutations result in structural disruption of the mutated finger with little or no effect on the other fingers. The activity of mutant proteins *in vivo* was assessed by measuring transcriptional activation of the endogenous 5S RNA genes. We found that differences in the level of 5S RNA synthesis were due to the ability of the various TFIIIA mutants to nucleate transcription complexes on the chromosomal 5S RNA genes. The most significant activation was seen with the finger-6 mutant (H183N) which activated the embryo's 5S RNA genes at a level 5-6 times greater than that seen by the overexpression of wild-type TFIIIA. High levels of transcription seen with H183N result from the formation of transcription complexes on the normally inactive oocyte-type 5S RNA genes. The remarkably high activity of the finger-6 mutant can be reproduced *in vitro* when transcription is carried out in the presence of 5S RNA. Disruption of zinc finger 6 results in a form of TFIIIA that exhibits reduced susceptibility to feedback inhibition by 5S RNA and therefore increases the availability of the transcription factor for transcription complex formation. We are now studying other RNA-protein feedback loops in *Xenopus* embryos to investigate coordination of ribosomal protein synthesis with the transcription of 5S RNA *in vivo*.



i.e. excess 5S RNA binds TFIIIA.
- mutation at finger 6 prevents binding to 5S RNA
∴ stops feedback inhibition

T20

Single versus Multiple Origins of Insecticide Resistance: Inferences from the Cyclodiene Resistance Gene *Rdl*

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The number of independent origins of insecticide resistance alleles is currently the subject of intense debate. Support for the importance of a single point of origin and spread of resistance through insect populations comes from studies of amplified esterases and insensitive acetylcholinesterase in *Culex* mosquitoes. Here we argue that it is difficult to determine precisely the number of origins of resistance alleles due to the complexity of the two mechanisms studied in *Culex*. The repeated replacement of the same amino acid in the Resistance to dieldrin (*Rdl*) gene, conferring resistance to cyclodiene insecticides, offers a model system within which to examine the diversity and origins of resistance alleles. By comparing *Rdl* alleles in two *Drosophila* species, two beetle species and the *Bemisia tabaci* whitefly complex we present repeated evidence for multiple independent origins of resistance. Evidence for independent origins comes not only from the finding of different replacements of this same amino acid but also flanking sequence data supporting multiple origins of the same amino acid replacement. Further, we emphasize that the life history of the insect under consideration can play a major role in determining the likely origin and spread of different resistance alleles.

Genetic diversity and mating system of remnant populations of the endangered grassland daisy *Rutidosis leptorrhynchoides*

Andrew Young and Tony Brown

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Allozyme markers were used to assess the genetic diversity and structure of 20 populations of the endangered daisy *Rutidosis leptorrhynchoides* occupying remnant grassland patches in Victoria, NSW and the ACT. Polymorphism and allelic richness were positively related to remnant population size. Six Victorian populations included polyploid plants with both putative autotriploids (3n=39) and autotetraploids (4n=52) being present. Populations from NSW and the ACT were exclusively diploid (2n=26). Multilocus outcrossing rates were estimated for eight populations ranging in size from 5 to 70 000 plants. Large populations (~10 000 - 70 000 plants), and small populations (~100-200 plants) close to large ones, were primarily outcrossed (t_m:0.8-0.90). One of two small isolated populations, and one very small population (5 plants) close to a large one, showed increases in inbreeding of 10-15% (t_m:0.68-0.69). The conservation value of small isolated populations may be limited by their low genetic diversity and the possibility of inbreeding depression. Any attempts to increase population sizes to increase genetic diversity and avoid inbreeding in these populations must take account of the chromosomal complement of source and recipient populations.

Diverse functions for integrins in *Drosophila morphogenesis*

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Integrins are a large, phylogenetically conserved family of cell surface receptors. Most integrins are receptors for extracellular matrix proteins, and form connections between the matrix and the cytoplasmic cytoskeleton. The PS1 and PS2 integrins of *Drosophila* are expressed in many cell types during development, and bind to specific (and so far distinct) matrix proteins. Genetic studies have demonstrated integrin requirements in the morphogenesis of numerous *Drosophila* tissues, including the wing. Here, clonal analysis shows that integrins are required to maintain the tight association of the apposed dorsal and ventral epithelia. Using a combination of genetic and molecular genetic approaches we find that the primary roles of the PS integrins appears to change during prepupal and pupal stages of wing morphogenesis. The late requirement appears to correspond to a relatively nonspecific adhesion role, although earlier, integrins seem to be involved in a regulatory event that is necessary to permit subsequent morphogenesis. This earlier role requires the complementary expression of dorsal PS1 and ventral PS2 seen at the beginning of prepupal development, and is discussed with respect to signalling functions of integrins generally.

A Genetic Perspective on the Historical Biogeography and Differentiation of Rainforest-Restricted Reptiles.

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The pattern and distribution of genetic variation within and among populations preserves the record of historical changes in population distribution, size, and connectivity. We are using mitochondrial nucleotide sequence data to examine historical biogeographic and population processes in a number of rainforest-restricted taxa. Analyses within taxa reveal patterns that are consistent with vicariance playing a strong role in structuring genetic variation, but areas of secondary contact and introgression highlight the importance of the latter processes in maintaining genetic cohesion among populations and in elevating local variation. Comparisons across taxa reveal concordant patterns that suggest similar processes acting across taxa, but over different time scales (unless neutral rates of nucleotide substitution are very different). Life history parameters are generally correlated with patterns of geographic genetic variation, though notable exceptions exist. Levels and patterns of divergence suggest that Pleistocene fluctuations in rainforest habitat acted to modify the distribution of pre-existing diversity rather than to generate new diversity. This is contrary to the Refuge Hypothesis but consistent with patterns of genetic variation in neo-tropical small mammals.

T24

THE ISTHMUS OF PANAMA, MOLECULAR CLOCKS AND FISH BIOGEOGRAPHY

Eldredge Bermingham

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The Pliocene rise of the isthmus of Panama separated neotropical marine habitats and joined terrestrial ones. For several years we have studied the influence of this recent earth history event on molecular evolution in marine fish populations split by the rising isthmus and in freshwater fish populations invading a new terrestrial landscape. We have measured protein divergence by electrophoresis and mitochondrial DNA differentiation by direct sequence analysis for 20 species pairs of fish separated by the Panamanian isthmus. By using data from two classes of macromolecules, we are better able to distinguish between genetic dissimilarities arising from different dates of separation and dissimilarities that may be due to different rates of evolution. Because many of the transisthmian species pairs are representatives of circumtropically distributed species groups, our transisthmian fish data also provide a logical jumping-off point for historical biogeographic analyses of tropical marine fishes. We will exemplify this approach with our molecular systematic studies of the *Abudefduf saxatilis* and *A. sordidus* species complexes. In addition, our marine fish data provide fruitful comparisons to our studies of the historical biogeography of Panama, a landscape thought to have been colonized by freshwater fishes over the past three million years. In turn, we will argue that molecule-based, historical biogeographic analyses of tropical faunas and floras provide the most efficacious means of determining whether species richness is principally controlled by regional or local processes and for identifying priority areas for biodiversity conservation.

ZEBRAFISH: A MODEL FOR EARLY HUMAN DEVELOPMENT

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The systematic collection of mutations affecting embryonic development provides material for understanding the molecular mechanisms of embryogenesis. Embryonic lethal mutations are readily collected in zebrafish, and molecular, genetic, and cellular mechanisms of early development and organogenesis are very similar in zebrafish and humans. The analysis of mutations affecting patterning in the embryonic midline will be discussed, including mutations leading to cyclopia and spinal cord defects. The studies lead to a model for the development of the midline axial mesoderm and ventral neural tube. The construction of a genetic map in zebrafish has demonstrated that nearly entire chromosomes may have been inherited by zebrafish and humans essentially intact from their common ancestor 420 million years ago. Thus, if a zebrafish mutation lies in a conserved chromosome segment, human genes located in this segment become candidates for the zebrafish mutation. Reciprocally, and importantly, the phenotype of the zebrafish mutation, which can be understood with great cellular precision, suggests functions for the orthologous gene in human embryos.

T26

Genetics and conservation

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The biological diversity of the planet is being depleted due to habitat loss, over exploitation, pollution and introduced species. The final *coup de grâce* for endangered species is usually due to stochasitic effects, whether environmental, demographic or genetic. Island species are particularly prone to extinction and so are critical to delineating causes of extinction. Neutral genetic theory suggests that finite populations lose genetic variation and become inbred, such that they would be expected to suffer increased extinction risk. However, the validity of this theory is unclear, and the importance of genetic issues in conservation is controversial. Evaluating these issues in threatened wildlife is difficult are they are typically slow breeders, expensive to maintain and present in low numbers. Consequently, we have turned to a combination of modelling problems in *Drosophila*, and doing meta-analyses on published wildlife data. *Drosophila* studies on the relationship between finite population size, inbreeding, loss of genetic variation, and evolutionary potential have validated the predicted effects of finite population size in reducing genetic variation, reproductive fitness and evolutionary potential. Deliberate inbreeding has been shown to greatly increase extinction risk in several species of laboratory animals. Several predictions relating to genetic factors in endangerment have been evaluated using meta-analyses of wildlife data. Population size within and among wildlife species has been shown to be related to genetic variation. A majority of endangered species have lower genetic variation than non-endangered species. If the extinction proneness of island populations is due to genetic causes then they must have lower levels of genetic variation than mainland populations; this prediction has been validated. Inbreeding levels for island population have been estimated; many are at levels where laboratory species suffer elevated extinction risks. In no case could genetic factors be excluded as a potential cause of endangerment and extinction.

T27

Cytoplasmic dynein and nuclear migration in fungi

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Intracellular nuclear migration is a prominent feature of eukaryotic development. We have undertaken an analysis of this process in the filamentous fungus *Aspergillus nidulans* by isolating and characterizing a set of temperature sensitive (*ts*) nud (nuclear distribution) mutants in which nuclear migration through the mycelium is defective. These mutants grow very slowly and fail to sporulate. The *nudA* and *nudG* genes encode the heavy and light chains respectively of the microtubule-dependent molecular motor cytoplasmic dynein, indicating that dynein is the main motor for nuclear migration in *A. nidulans*. Deletion of the *nudA* gene produces a slow growth phenotype indistinguishable from that of the *ts nudA* mutations. Thus, although dynein is important for growth and development in *A. nidulans,* it is not essential. This results suggests the possibility of a backup system that becomes operative when dynein function is defective. A synthetic lethal hunt for genes defining such a system identified mutations in at least seven genes that cause lethality only in the absence of the cytoplasmic dynein heavy chain. Antibodies against the *nudA* and *nudG* genes were used to characterize the cytoplasmic dynein gene products and to determine where dynein is located in the cell. Interaction between the dynein heavy and light chains was demonstrated by coimmunoprecipitation of these chains from cell free extracts. Antibodies against the heavy chain specifically stained the mycelial tips. The light chain is apparently necessary for targeting of the heavy chain to the tip, as the *ts nudG8* mutation, which lacks the light chain at restrictive temperature, does not exhibit heavy chain tip staining.

T28

Gene Pools and Conservation Law: Some Problems of Definition

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Increasingly, Acts of Parliament relating to the conservation of biodiversity include phrases such as "genetic diversity" and "potential for evolutionary development". Geneticists differ amongst themselves in what such terms mean, and how (if at all) they can be related to conservation objectives. Nonetheless wildlife authorities and courts are obliged to interpret these laws clearly and consistently.

I will illustrate some of the ambiguities and inconsistencies in the conservation status of distinct gene pools within species as enacted in Australian Commonwealth and State laws. They reveal a clear need for research that targets some general and unanswered questions.

Informed advice based on such studies is needed if biodiversity is to be conserved and managed effectively. Geneticists also need to realize that they will be asked for such advice more frequently than they have been in the past.

The effect of population bottlenecks on allelic diversity and heterozygosity in *Drosophila melanogaster.*

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To persist in the long term, a population must harbour sufficient genetic variation to permit continued evolution. Population bottlenecks, through which many imperilled species are forced to pass, threaten to erode genetic variation below critical levels. How much genetic variation is enough to maintain evolvability, and how should it be measured? *Drosophila melanogaster* populations were subjected to bottlenecks to achieve various theoretical changes in heterozygosity and allelic diversity. The bottlenecks were designed to cause the same loss of heterozygosity via either a small number of individuals for one generation (intense bottlenecks) or a larger number for many generations (diffuse bottlenecks). Greater loss of allelic diversity was expected in intense bottlenecks. Highly polymorphic microsatellites were developed to accurately assess whether genetic diversity changed as predicted. Computer simulation was also used to model the effects of the bottlenecks on microsatellite diversity. The loss of genetic diversity caused by intense bottlenecks in both *Drosophila* populations and simulations conformed closely to predictions, demonstrating the value of microsatellites as neutral genetic markers and validating the use of *Drosophila* for the evaluation of conservation genetic theory. Loss of allelic diversity was greater than expected in diffuse bottlenecks, contradicting the intuitive prediction that they would preserve much greater allelic diversity than intense bottlenecks. Our results warn that the theory used to maximise heterozygosity in small populations inadequately accounts for changes in allelic diversity.

AUSTRALIA'S OFF-SHORE ISLAND MAMMAL POPULATIONS: JEWELS IN THE CONSERVATION CROWN OR GENETIC DEATH-TRAPS?

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The black-footed rock-wallaby *Petrogale lateralis lateralis* was formerly widespread and common throughout much of south-western Western Australia. Since European settlement this species has declined significantly with remnant populations only remaining at five localities (including two offshore islands). Preliminary genetic analysis of these remnant populations using 6 highly variable microsatellite loci has revealed a complete lack of genetic variation in *P. l. lateralis* (n = 14) from Barrow Island (202 000 ha). Additionally the Barrow Island animals are characterised by unique alleles at most loci. By comparison, mainland populations have heterozygosity values of 50-60% and up to 6 alleles per locus. It is suggested that founder effects, population fluctuations and/or genetic drift have effectively eliminated genetic variation from the Barrow Island population since it was separated from the mainland approximately 10, 000 years ago. Should this level of genetic variation prove to be typical of other macropod populations on off-shore islands it calls into question the usefulness of island populations in the long-term management of endangered species.

Microsatellite markers for marsupial population genetics: case studies of macropods and possums in New Zealand.

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Cooperative Research Centre for Conservation and Management of Marsupials.

Populations of several Australian marsupial species were established in New Zealand (NZ) last century. Several translocated wallaby species have subsequently become endangered, or at least severely range-restricted in Australia, with possible important roles in reintroduction for the NZ populations. However, although exact numbers of translocated wallabies were often not documented, it is likely that numbers of founders were small, and population genetic theory tells us that genetic diversity in such populations should be low. Information about genetic variation should be available to assess the usefulness of these animals in possible reintroduction schemes. Also identification of the origin of founding NZ animals is of vital importance if they are to be considered in a conservation strategy. We are therefore in the process of characterising genetic variation in various NZ wallabies. A separate issue concerns the common brushtail possum *Trichosurus vulpecula*, which has reached pest proportions in NZ. In managing populations of this animal, and controlling the various harmful effects it has on the NZ native biota, information about population subdivision, migration and mating system would be invaluable. To address these issues we have developed 8 highly variable microsatellite markers from the tammar wallaby *Macropus eugenii*, and 7 from the common brushtail possum. PCR primers designed to amplify the tammar microsatellites detect variation in a wide variety of other macropods, including rock wallabies. This paper reports on the progress of genetic characterisation of NZ marsupial populations, and discusses some of the implications of our findings for *in situ* conservation of the species in Australia.

T32

Contrasting Patterns of Maternal Gene Flow when assessed over Evolutionary and Ecological time scales in Fragmented and Unfragmented Populations of Rainforest Rodents

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The current distribution of Australian rainforests has been severely affected by anthropogenic fragmentation. To help evaluate the genetic and ecological consequences of habitat fragmentation for rainforest taxa, we used Outgroup Heteroduplex Analysis / Temperature Gradient Gel Electrophoresis (OHA/TGGE) and DNA sequencing to examine Control Region variation in two species of Mosaic-tailed Rats - the Fawn-footed Melomys (*Melomys cervinipes*) and the Giant White-tailed Rat (*Uromys caudimaculatus*). These studies focussed on fragmented and unfragmented populations from the Atherton Tableland (*M. cervinipes* - 20 populations, 202 individuals; *U. caudimaculatus* - 17 populations, 244 individuals) and produced some notable, and somewhat surprising, results: (a) levels of gene flow per generation among populations of both species are an order of magnitude greater when assessed over an evolutionary compared to an ecological time scale, (b) fragmented and unfragmented populations of both species demonstrate highly significant genetic structure ($P < 0.0001$), in some cases over distances less than 1km, (c) gene flow among fragmented populations was reduced compared to levels among unfragmented populations in both species, (d) populations of *U. caudimaculatus* in very large rainforest fragments ($\approx$ 500ha) have significantly reduced variation compared to unfragmented populations, (e) two highly divergent Control Region clades, apparently too deep in origin to be attributed to late-Pleistocene refugia, are distributed throughout populations of *U. caudimaculatus* on the Atherton Tableland. The reduced gene flow and loss of genetic variation in fragmented Mosaic-tailed Rat populations suggest that even species that survive well in fragmented landscapes in the short term, may be subject to future genetic and demographic problems which could ultimately lead to extinction in this landscape.

The *Bactrocera tryoni* homologue of the *Drosophila melanogaster* sex determination gene *doublesex*.

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Fruit Fly Research Centre, School of Biological Sciences, A12
The University of Sydney, 2006.

The *doublesex* (*dsx*) gene of *Drosophila melanogaster* is the last gene in the heirachy of genes that control somatic sexual differentiation. The sex specificity of the protein products of this gene is brought about by sex-specific splicing of the pre-mRNA which results in products with a common amino terminus and a sex-specific carboxyl terminus.

Recently the sequence of the *doublesex* gene was reported for *D. virilis* although this gene has yet to be identified in insect species other than *Drosophila*. The *D. virilis* sequence was found to contain regions of strong amino acid identity with that of *D. melanogaster*, particularly across the putative DNA binding region within the common region of the proteins. Degenerate nested primers were made across this region and a PCR product of 287bp was isolated, cloned and sequenced. The identity of the sequence across this region to *D. melanogaster* (excluding the primer regions) is 93% for protein and 76% for DNA. Specific primers were made to the *B. tryoni* sequence and 3' RACE carried out on adult and larval cDNA. Seven PCR products were isolated and cloned, and sequencing is currently underway.

There are a number of genes in the sex determination pathway of *D. melanogaster* of which *Sex-lethal* has been identified as the key binary switch. Homologues of this gene have been reported in other insect species but the expression of these homologues has not been proven to be sex-specific. Therefore work is underway to establish whether sex-specific transcripts of the *dsx* gene are present in *B. tryoni*.

In *D. melanogaster* both the sex-specific protein products of these genes have been shown to interact directly with an enhancer region of one set of target genes, the yolk protein genes, that has previously been shown to direct sex- and tissue-specific transcription of these genes (Coschigano and Wensick, 1993). In males transcription of the genes is repressed while in females the genes are activated through these proteins.

T34

AN AVIAN W-SPECIFIC GENE ISOLATED BY DIFFERENTIAL DISPLAY RT-PCR

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Sex in birds is determined by a chromosomal system in which the male is the homogametic sex (ZZ) and the female is the heterogametic sex (ZW). The W chromosome in birds seems to act as a dominant determinant of female sexual differentiation. The aim of this project is to elucidate the mechanisms controlling gonadal development using chicken *(Gallus domesticus)* embryos as a model system.

Genital ridges were dissected from 4.5, 5.5 and 6.5 days of chicken embryonic development. These represent periods just before, during and after sex determination. Differential display RT-PCR was used to examine these genital ridges for sex specific transcripts. A female specific differential display transcript was identified and cloned. This transcript was not seen in the displays of male genital ridge. RACE PCR was used to clone the 5' end of the cDNA. The RACE clones contained a putative open reading frame of 130 amino acids. This ORF was compared to the SWIS-PROT database and found to contain homology (~36%) to the bovine and rat protein kinase C inhibitor. Northern blotting confirmed sex specific expression of the clone. The clone was expressed at all times examined (3.5 to 11.5 days) in the female but not the male. A genomic clone was obtained from a female chicken library. A 2.1kb subclone has been sequenced and compared to the cDNA. The genomic clone contained a single intron and the exons were identical to the cDNA sequence. Southern blotting analysis of chicken DNA revealed the clone to be W-linked. This analysis was extended to numerous other bird species and has shown almost universal W-linkage. The clone has been accordingly named *ASW* (Avian Sex specific gene on the W).

FISHing for meiotic pairing regions in rye

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Recent research in yeast has suggested that homology search leading to meiotic pairing procedes by initial double strand break formation. Because of the physical distances, amounts of DNA and packaging of chromatin in higher eukaryote genomes it appears that telomeres may have important roles in aligning homologues via nuclear envelope clustering and bouquet formation. Previous studies with EM serial sectioning and spreading of synaptonemal complexes have indicated that pairing begins near telomeres. We are attempting to use Fluorescent In Situ Hybridization (FISH) to pin down subtelomeric sites of homology search in cereal chromosomes during prophase I.

The initial work described here has used a number of repeated sequences located close to the chromosome telomeres of rye. The sequences used, in order from the telomere, were telomeric repeat oligomers, pSc 200 - a 379bp repeat, pSc 250 - a 466bp repeat and pSc 119.2 - a 120bp repeat. They were labelled using nick translation or PCR to incorporate biotin or digoxigenin or direct-labelled fluorochromes. One to four labelled probes were hybridized with squashes and spreads of pachytene to anaphase I stages of different rye lines and wheat-rye substitution lines. Examination was by fluorescent light microscopy after incubation with the appropriate fluorochrome tagged antibodies. Images were recorded on colour print film and subsequently computer enhanced in some cases.

At pachytene there appeared to be overlaps or foldbacks in the packaging of adjacent sequences. Computer enhancement of FISH on synaptonemal complex spreads revealed loops of repeated sequences. At metaphase I chiasmata were present at or close to the sites of subtelomeric probes.

Several related *Helicoverpa* specific baculoviruses encode an asparagine rich Zinc-finger protein, the coding region of which contains triplet-repeats that are subject to high rates of mutation.

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Larvae of various noctuids of the genus *Helicoverpa* are important agricultural pests in sub-tropical regions. Baculoviruses such as HzSNPV are potentially useful tools for controlling crop damage caused by these insects. We have characterised a region that is present in five different baculoviruses isolated from natural *Helicoverpa* populations over several decades. The polymerase chain reaction (PCR) was used to determine nucleotide sequence at both the polyhedrin and DNA polymerase loci of these isolates, and they were both shown to be highly similar to one another (≈ 99.9% identity at the nucleotide level). In contrast, a region termed ORF2136, encoding an unusual Zinc-finger motif protein, was found to be polymorphic. The different ORF2136 homologues displayed changes largely due to the loss or gain of nucleotide triplets but the changes still preserved the reading frame. The ORF contains many repeated triplets, and has no close homologues in viruses AcMNPV or BmNPV. It is downstream from a promoter like element that is linked to the IE-N gene in AcMNPV, and the flanking protein kinase and immediate early genes are also present in distantly related viruses. To assist characterisation of the ORF2136 protein, we have expressed it at a high level in *Escherichia coli* using the pRSET expression system, and have obtained a 93 kDa histidine-tagged fusion protein for purification.

We propose that the ORF2136 protein is an essential virus product whose polymorphism has an important role in pathology of polyhedrosis disease. PCR targeted at the locus provides a diagnostic tool for tagging and tracing various *Heliocoverpa* viruses circulating in the environment.

T37

Coat Colour Genetics of Merino Sheep

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Coat colour variation in mammals has long been a source of fascination particularly for scientists and animal breeders. Over 50 mouse mutations have been recognised through their effect on coat colour and most of our knowledge of the genetics of pigmentation has come from mouse research. Many genes that effect coat colour act early in embryogenesis and/or affect fundamental processes unrelated to pigmentation. The study of the pleiotropic effects of pigmentation genes can provide us with information related to human development and disease processes. Our interest in coat colour genetics stems from the financial losses associated with pigment contamination of the Australian wool clip. We are working on a joint project to identify rams heterozygous for the gene allowing recessive expression of black wool in Merino sheep. Several pigmentation genes, including agouti, kit and steel, have been identified as possible candidates, the most likely being agouti. Initial work has involved the development of a PCR-based clone for hybridisation studies and microsatellite analysis of ovine chromosomal regions homologous to the mapped position of human and mouse genes.

A NOVEL GERM LINE MUTATION IN *SOX9* CAUSES FAMILIAL CAMPOMELIC DYSPLASIA AND SEX REVERSAL

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Mutations in the gene *SOX9* result in the syndrome of campomelic dysplasia (CD) which includes sex-reversal in 75% of 46,XY affected individuals. The *SOX9* gene maps to the long arm of human chromosome 17 and translocations in this region also result in C.D. We report a family in which there were three affected patients, two of whom showed 46,XY sex-reversal. The propositis was found to have 46,XY true hermaphroditism with ambiguous genitalia. The two sibs were 46,XY and 46,XX patients both of whom had bilateral ovaries with a normal female genitalia. Interestingly, despite these widely varying phenotypes we showed that all three patients were heterozygous for a novel mutation in *SOX9,* which involved the insertion of a cytosine residue at nucleotide position 1096. By contrast, the somatic cells in both parents revealed wild-type *SOX9* nucleotide sequences. However, analysis of the father's germ cells revealed they were mosaic for mutant and wild-type *SOX9* sequences. This family is particularly informative as it demonstrates that the same *SOX9* mutation can produce very different 46,XY gonadal phenotypes. Presumably, the variable penetrance of CD between these sibs reflects differences in genetic background in which the *SOX9* gene operates. Finally, these mutations only affect a single allele of *SOX9* suggesting a dominant mode of inheritance for this syndrome. Consequently, CD and autosomal sex reversal may result from haploinsufficiency of *SOX9.* These results are the first to demonstrate that a mosaic, *de novo* germ cell mutation in *SOX9* results in CD.

De Novo Insertion of an Intron into the Mammalian Sex Determining Gene, *SRY*

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Two theories have been proposed to explain the evolution of introns within eukaryotic genes: the introns late theory, or "insertional theory of introns"[1,2,3], and the introns early theory, or "exon theory of genes"[4,5]. The mammalian sex determining gene, *SRY*, is intronless in all mammals studied to date[6]. However, comparisons of genomic and cDNA sequences now provide evidence of a *de novo* insertion of an intron into the *SRY* gene of dasyurid marsupials. This recent (<45 MYA) insertion bears no homology to known transposable elements and illustrates that introns may be inserted as spliced units within a crucial gene without disrupting its function.

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T40 gene on 11 GOK

Molecular cloning of a novel human gene at 11p15.5

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A novel gene (currently named *GOK)* has been cloned after identity was found between expressed sequence tags and the promoter region of the *RRM1* gene. *GOK* and *RRM1* genes map to a region of chromosome 11 (11p15.5) which is believed to contain a gene or genes associated with Wilms' tumour and other paediatric malignacies. A 4-kb *GOK* cDNA has been cloned and it encodes a predicted protein of ~84-kDa that could be translated *in vitro.* Database searches have failed to identify any known gene or protein with significant homology to *GOK* Computer analysis predicted that the protein had a leader peptide and may contain a transmembrane helix. The protein also appears to contain a coiled coil domain, suggesting that it forms a stable contact with itself or another protein. Restriction mapping by pulsed field electrophoresis indicates that *GOK* is located telomeric of *RRM1. GOK* displays high evolutionary conservation: cloning and partial sequencing of a mouse genomic clone revealed 90% identity with the human gene at both nucleotide and predicted amino acid levels. Expression of *GOK* mRNA was found, by RT-PCR, in placenta, kidney and several cell lines. ESTs showing *GOK* homology have been isolated from melanocytes, infant brain, placenta, pancreatic islets and HL60 cells.

MITOCHONDRIAL DNA POLYMORPHISM IN HUMAN DISEASE

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Human mitochondrial DNA sequence polymorphism was analysed in 33 subjects over the entire coding region (15446 bp). Analysis of a group of these sequences from patients with confirmed (n=16) mitochondrial cytopathies showed polymorphic frequency (sites/100 bp/sequence) of 0.34 ± 0.02 collectively in the subunits of Complex I, which differed significantly ($p<O.O5$, *t)* from a control group (n=4) polymorphic frequency of 0.17 ± 0.04. A group of 11 published mtDNA sequences from Cardiomyopathy patients also differed significantly in collective Complex I genes ($p<0.05$, *t*) from the controls. Collective Complex I polymorphic frequencies in cytopathic groups (MERRF n=5, MELAS n=4 and LHON n=5), were also greater than that displayed by the control group. Elevated polymorphic frequencies in disease groups were mainly focussed to ND6 and ND3 coding frames. Polymorphic site frequency differed between the various mtDNA subgroups in several respiratory subunit genes, suggesting differential subunit involvement in phenotypic expression. Pairwise divergence was elevated in many mitochondrial genes, particularly in the ND3 and ND6 subunits of Complex I (0.48% and 0.70% respectively) in the cytopathy mtDNA group (n=16) compared to controls. Nonconservative missense polymorphic sites were of higher mean evolutionary conservation in disease subgroups than in the control group. This supports the possibility that polymorphisms of low individual deleterious potential at conserved sites in mitochondrial respiratory subunit genes may contribute to cumulative impairment of normal respiratory function. As a corollary to this, the variable expression of known major deleterious polymorphisms may be mediated by polymorphism at multiple sites of moderate to high evolutionary constraint.

Regulation of the Menkes Copper Transporter via Copper-Induced Trafficking from the Golgi to the Plasma Membrane.

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Menkes disease is a genetic disorder affecting copper transport in humans. Patients with Menkes disease are unable to transport essential dietary copper across the serosal membrane of intestinal epithelial cells. Consequently, copper accumulates in these cells resulting in a systemic copper deficiency. Cultured fibroblasts from Menkes patients hyper-accumulate copper even when grown in low levels of copper and show evidence of reduced copper efflux (1).

The Menkes gene encodes a P-type ATPase, containing six putative copper-binding Cys-X-X-Cys motifs (2). Analogies in structure to other P-type ATPases has led to proposals that the Menkes gene product has a role in the transmembrane pumping of copper (2). Evidence for this has been found through studies in cultured Chinese hamster ovary (CHO) cells from which copper-resistant variants have been isolated. In these copper-resistant cells, over-expression of the MNK P-type ATPase has resulted in enhanced rates of copper efflux (3). Using indirect immunofluorescence, we have demonstrated that this elevated copper efflux is accompanied by an ATP-dependent change in the subcellular location of the MNK protein. When cells are grown in media with no additional copper the MNK protein localises to the *trans*-Golgi network, however, when copper is added to the medium there is rapid and reversible trafficking of the protein to the plasma membrane. These results reveal a novel system of regulated protein trafficking which ultimately leads to the efflux of an essential yet potentially toxic ligand, where the ligand itself appears to directly and specifically stimulate the trafficking of its own transporter.

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Learning Genetics.

Tony Griffiths

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Students find genetics the most difficult subject in the biological sciences and often become disenchanted with it as a result of their frustrations. However, because genetics is so central to the life sciences and to liberal education generally, instructors need to make special efforts for students to develop a deep understanding of genetics. I suggest that most of the students' difficulties lie in their passive approach to learning, instilled by traditional teacher-centered educational practices at university and at school. In most biology courses, students are bombarded with a blizzard of facts that are remembered, recalled for exams, and then forgotten at the end of the course. Because of the analytical nature of genetics, this fact-based approach is not effective, but many students have not acquired the necessary skills to apply to analysis. I propose that active processing of hereditary data and concepts are essential to understand genetics. Furthermore students have difficulty switching into this active mode of thought unaided. Therefore, several methods will be discussed that are designed to shift the educational emphasis away from teaching genetics and into the facilitation of learning genetics.

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Contents

continued over page

Recombinant DNA Technology
Applications of Recombinant DNA Technology
The Structure and Function of Eukaryotic Chromosomes
Genomics
Control of Gene Expression
Mechanisms of Genetic Change I: Gene Mutation
Mechanisms of Genetic Change II: Recombination
Mechanisms of Genetic Change III: Transposable Genetic Elements
The Extranuclear Genome
Gene Regulation During Development
Genetics and Cellular Differentiation
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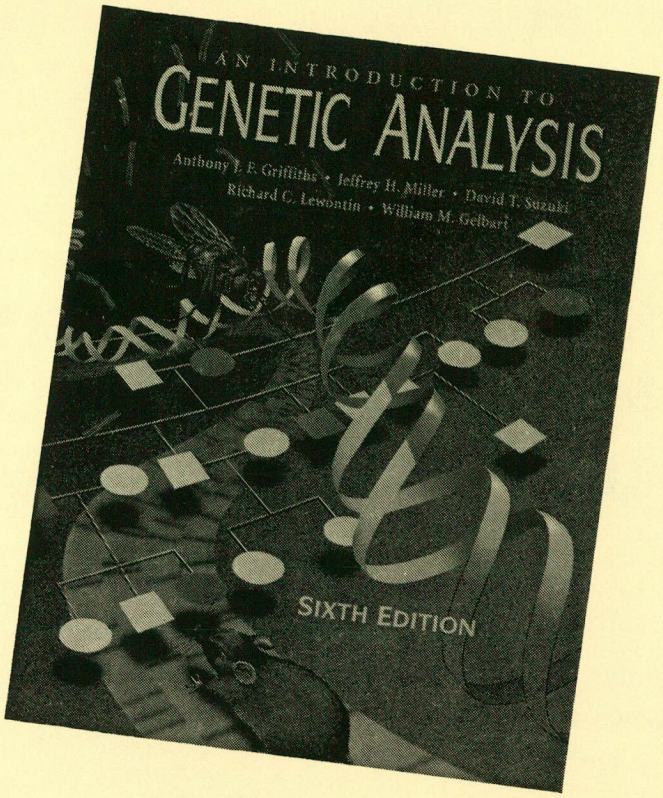

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T44

GENETIC ANALYSIS IN THE ZEBRAFISH

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A collection of mutations affecting any biological process provides materials for understanding the molecular mechanisms driving that process. Mutations affecting embryonic development in the zebrafish are readily collected and promise to further our understanding of development in vertebrate embryos. Mutant screens are facilitated by optically clear, externally developing embryos. Furthermore, mutant hunts in zebrafish can exploit first generation screens using haploid or gynogenetic diploid animals. Over 2000 mutations in more than 400 genes affecting zebrafish development have recently been collected in several laboratories.

Molecular isolation of mutated genes can occur using either the candidate gene approach or the positional cloning approach, both of which require a genetic map. We have constructed a genetic map for the zebrafish that has about 800 markers and has the same number of linkage groups as chromosomes (25). We have localized centromeres on the linkage map, and have used DNA polymorphisms closely linked to centromeres to map mutations rapidly and efficiently in gynogenetic half-tetrad zebrafish using tetrad analysis as practiced in the genetic analysis of fungi. We have developed ways to screen thousands of DNA polymorphisms for close linkage to mutations, usually identifying a marker within a centiMorgan (cM) of the mutation. Because a cM is about 600 kb, a marker about a half cM distant should be close enough to initiate a chromosome walk using YAC or PAC clones for positional cloning.

To facilitate the candidate gene approach for the molecular isolation of mutated genes, we have begun to add cloned genes to the linkage map of anonymous DNA polymorphisms. Genes mapping in a chromosome that includes a mutation become candidates for the mutated gene which can be tested by molecular genetic experiments. This approach for cloning mutations would be enhanced if we could draw upon the map-rich human and mouse genomes to identify candidate genes. This requires the identification of chromosome segments that have been conserved intact during the 420 million years since the last common ancestor of zebrafish and mammals. Results show a remarkable number of shared syntenies, situations in which the orthologues of a group of genes that reside on a single chromosome in one species also occupy a single chromosome in another species. For example, *engrailed1*, *hoxD4*, *dlx1*, *dlx2*, *activinBB*, and *twist* all reside on Linkage Group 9 in zebrafish, and their human orthologues EN1, HOXD4, DLX1, DLX2, INHBB and, by comparative mapping with mouse, the human orthologue of mouse Twi, all encompass most of human chromosome 2, about 5% of the human genome. Analysis suggests that syntenic conservation between human and zebrafish genomes may not be much less than that between the human and rapidly rearranging rodent genomes. Thus, if a zebrafish mutation lies in a conserved chromosome segment, human genes located in this chromosome segment become candidates for the zebrafish mutation. Reciprocally, and importantly, the phenotype of the zebrafish mutation, which can be understood with great cellular precision, suggests functions for the orthologous gene in human embryos.

Mendel meets Mothra: The challenges of linkage mapping in the Lepidoptera

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Construction of linkage maps in Lepidoptera is complicated by the large number of small chromosomes, and the lack of general computational techniques accounting for achiasmatic oogenesis in females. But progress has been greatly facilitated by development of molecular markers based on DNA polymorphisms, and our extension of the lod score method to deal with the unique genetic system of Lepidoptera. We describe the current status of the linkage map of the tobacco budworm *Heliothis virescens* (F), a polyphagous noctuid crop pest, and its application to localizing genes conferring resistance to chemical insecticides and endotoxins from *Bacillus thuringiensis* (Bt). Utilizing a set of RFLP probes spanning the genome of *Heliothis*, we propose to construct a homologous map for the closely related *Helicoverpa armigera* in the coming year. Recently-developed computational methods and molecular techniques have also aided linkage mapping in *Bombyx mori, Yponomeuta padellus*, and *Plutella xylostella*, and aided in the localization of Bt resistance genes in the latter species. The prospects for comparing the linkage maps of these Lepidoptera are discussed, and a system of universal anchor loci is proposed for this purpose.

Natural Plasmids of the fungus *Neurospora*.

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Plasmids are interesting as elements of parasitic DNA and because of the novel genetic and molecular mechanisms they reveal. *Neurospora* has become a model organism for the study of eukaryotic plasmids. They are much more widespread globally than previously believed. There are different types and families and their distribution patterns can be explained by vertical descent from common ancestors or by horizontal transmission. Some plasmids are circular, some linear. Circular plasmids are rare; they encode a single ORF encoding a presumptive DNA polymerase or reverse transcriptase. Linear plasmids carry two ORFs, a DNA polymerase and an RNA polymerase, and have perfect inverted terminal repeats. The 'kalilo' family of linear plasmids is distributed across species and genera. It is particularly interesting in that some members of the family have the rare property of killing the host fungus by integration into its mitochondrial DNA. MtDNA with integrated plasmid soon becomes the dominant type and death ensues.

T47

MOLECULAR ANALYSIS OF MEIOTIC RECOMBINATION IN THE *HISTIDINE-3* REGION OF NEUROSPORA USING NATURAL SEQUENCE POLYMORPHISMS.

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Meiotic recombination in *Neurospora crassa* is differentially regulated by at least three *trans* acting genes: *rec-1*, *rec-2* and *rec-3* each polymorphic in the wild-collections used to establish the commonly used laboratory strains (reviewed by Catcheside 1977, 1986). In each case dominant alleles (rec^+) are known to reduce recombination in specific target regions on at least two chromosomes. Collectively, the three *rec* loci influence recombination in approximately one third of the genome sampled and cause about ten fold variation in recombination at their target sites. Recombination in the *his-3* region is influenced both by *rec-2*, which affects crossing over between *his-3* and *ad-3* and recombination across the *his-3* gene, and by the *cis* acting element *cog*, which is also polymorphic amongst laboratory strains. The dominant allele cog^+ permits higher recombination but events initiated by either allele are blocked by $rec\text{-}2^+$. It is presumed that *cog* like recombinators with the relevant *rec* gene sensitivity exist within all DNA segments under *rec* control.

We have found that *cog* is within highly polymorphic DNA and have used the polymorphisms to map *cog* to a region 2.3 - 3.2 kb 3′ of *his-3* (Yeadon and Catcheside 1995). We have also found substantial polymorphism within the *his-3* coding region, providing markers for determining the position of recombination events. We report here that although events stimulated by *cog* which lead to recombination between *his-3* alleles only rarely have termini within *cog*, there are regions where termination is common; that conversion tracts are discontinuous in some recombinants; and that the chromosome carrying cog^+ is preferentially the recipient of information unless $rec\text{-}2^+$ is present.

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CHARACTERISATION OF A YEAST UBIQUITIN SPECIFIC PROTEASE UBP6.

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Ubiquitin-mediated proteolysis is the main mechanism of selective protein turnover in the eukaryotic cell[1]. Proteolysis is triggered by post-translational conjugation of ubiquitin (Ub) moieties to the substrate[2]. A family of yeast thiol proteases has been identified which cleave Ub from substrates. These enzymes are the Ubiquitin-Specific Proteases (Ubps). Up to 14 potential Ubps have been identified in yeast alone through experiments[3,4,5] and database searches. Ubps potentially have multiple roles in the cell including Ub precursor processing, replenishing of the cell's free Ub pool and regulation of proteolysis. We have identified a novel *UBP* gene in *Saccharomyces cerevisiae*, *UBP6*. The *UBP6* gene has a 1.5 kb ORF encoding a 499 amino acid product containing Ubp-like conserved domains. Homologues are present in humans, rabbits, rice, *Arabidopsis*, and *Caenorhabditis*. Ub fusion cleavage activity has been demonstrated both *in vivo* and *in vitro* in *E. coli* expressing recombinant *UBP6*. Pulse-chase and ONPG assays show that overexpression of *UBP6* in *S. cerevisiae* stimulates Ub-mediated degradation of Ub-Pro-βGal. *UBP6* was also isolated from a yeast mutant defective in the degradation of non-N-end rule substrates, suggesting a regulatory role for Ubp6. The haploid *ubp6* deletion mutant is viable, in keeping with the redundant and overlapping functions of Ubps. The mutant strain has no abnormal growth phenotype and is viable in 1.5M salt conditions. ONPG and pulse-chase assays demonstrated that the normally unstable substrate Ub-Pro-βGal was stabilised in the *ubp6* mutant. A series of multiple ubp mutants in yeast have been constructed with deletion of up to five Ub proteases viable in a haploid strain. No Ub cleavage activity was detected in *E. coli* transformed with clones of either the rabbit or human homologue of Ubp6. Further analysis of rabbit and human Ubp6 homologues will determine whether these are functional Ubps and may reveal possible links with human disease and Ub-mediated proteolysis.

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CHARACTERISATION OF A GENE OF THE BLACKLEG FUNGUS *LEPTOSPHAERIA MACULANS* CONFERRING HOST SPECIFICITY ON INDIAN MUSTARD

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The ascomycete, *Leptosphaeria maculans* causes blackleg disease of oilseed *Brassica* crops such as canola worldwide. This fungus is an excellent model system as it has a haploid vegetative state, is outcrossing, can be cultured on defined media, has a relatively small genome size and a high efficiency DNA transformation system. We are examining its interaction with Indian mustard (*B. juncea*). In a cross between a virulent blackleg isolate (attacks Indian mustard) and an avirulent isolate (cannot attack), the F_1 and backcross progeny segregate in a 1:1 ratio, suggesting the presence of a single gene (Chen et al. 1996). Analysis of resistance responses during infection suggests that this gene is an avirulence gene encoding an elicitor that interacts with the product of a host resistance gene (Chen and Howlett 1996). We are using three independent strategies to isolate this gene. Firstly, tagged mutants are being generated using restriction endonuclease - mediated integration (REMI), where a plasmid integrates into the fungal genome and inactivates genes at random. We are screening 2,000 tagged transformants for a change in phenotype from avirulence to virulence on Indian mustard. Secondly, Random Amplified Polymorphic DNA (RAPD) markers linked to the gene are being sought using Bulked Segregant Analysis. We are determining the distance from this gene of 12 RAPD markers that segregate with avirulence. Thirdly, we are trying to purify an elicitor from infected plants and to use its N-terminal sequence to clone the gene. The identity of the isolated gene as an avirulence gene will be confirmed by complementing virulent isolates with it.

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T50

Phylogenetic Affinities of Dromiciops Based on Mitochondrial rRNA Genes and Part of the Nuclear Interphotoreceptor Retinoid Binding Protein

John R. Kavanagh and Mark S. Springer

Biology Department, University of California Riverside, Riverside, CA, USA

Most recent classifications divide extant marsupials into two cohorts, the Ameridelphia and Australidelphia. In addition to the Australasian lineages (e.g., bandicoots, dasyurids, diprotodontians), South American microbiotheres (e.g. Dromiciops australis) is included in the Australidelphia. The initial impetus for this arrangement was Szalay's work on tarsal morphology. Subsequently, single-copy DNA hybridization evidence linking Dromiciops to diprotodontians and dasyurids was presented by Kirsch et al. Hershkovitz, in turn, recently suggested that Dromiciops diverged from all other extant marsupials in the Jurassic! Here we examine the phylogenetic affinities of Dromiciops using complete 12S and 16S ribosomal RNA gene sequences in combination with part of exon 1 of the nuclear interphotoreceptor retinoid binding protein gene. In addition to Dromiciops, our study included a representative(s) didelphid, bandicoot, diprotodontian, and dasyurid. Among other findings, there is no support for the Hershkovitz hypothesis.

Phylogenetic relationships of the Macropodidae: evidence from mtDNA sequences.

Angela Burk, Michael Westerman, and Mark S. Springer

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DNA sequence comparisons of the 12S rRNA and tRNA valine mitochondrial genes were used to infer phylogenetic relationships among 12 macropodid maximum parsimony and neighbor joining algorithms followed by bootstrapping. Previous anatomical and molecular studies disagree on the placement of Hypsiprymnodon, but its position relative to the potoroine subfamily has not been addressed withsequence data. Phylogenetic analyses of the sequences reported here suggest that potoroines are paraphyletic with Hypsiprymnodon a sister taxa to all other macropodids. The macropodines included in the analysis are monophyletic and separate into two well supported clades: one consisting of the New Guinean forest wallabies Dorcopsis and Dorcopsulus, and one consisting of macropodine genera Macropus, Onychogalea, Wallabia, Dendrolagus, Peradorcas, and Lagorchestes. The branching topology lends support to the hypothesis that Dorcopsis and Dorcopsulus are closely related and represent plesiomorphic macropodines.

THE DISTRIBUTION OF THE TELOMERIC SEQUENCE IN TWELVE *PETROGALE* TAXA (ROCKWALLABIES)

Cushla J. Metcalfe, Mark D.B. Eldridge, Peter G. Johnston.

School of Biological Sciences, Macquarie University, North Ryde 2019

Fluorescence *in situ* hybridization (FISH) has recently been used to demonstrate that the $(T_2AG_3)_n$ sequence is the functional vertebrate telomere. The most common reported non-telomeric site of this sequence in vertebrate chromosomes is near the margins of constitutive heterochromatin. Other non-telomeric sites of the $(T_2AG_3)_n$ sequence appear related to chromosomal rearrangements, in particular tandem and centric fusions. However, there is no consistent pattern between the presence or absence of interstitial $(T_2AG_3)_n$ sequence and known fusions.

We have studied karyotypic evolution and the distribution of the $(T_2AG_3)_n$ sequence in twelve *Petrogale* taxa. Initial work has concentrated on the large *lateralis/penicillata* group (fourteen taxa) which consists of two complexes, the eastern *penicilliata* complex (eight taxa), and a *western* lateralis complex (six taxa). Results include: 1. a signal at the site of all previously reported centric fusions; 2. a small region of telomeric sequence on chromosome 4 from species in the *penicillata* group which is absent from the *lateralis* group; 3. large amounts of telomeric sequence associated with the centromere of chromosome 7 in all species examined from the *lateralis* group, the exception is *P.l. purpureicollis*; 4. a region of telomeric sequence has just below the centromere of chromosome 1 in all species from the *lateralis/pencillata* complex.

T53

Microsatellite and chromosome evolution of parthenogenetic *Sitobion* aphids in Australia

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We present data from single-locus microsatellites in *Sitobion* aphids. These markers show very high heterozygosity and up to nine alleles per locus. However, there were only six different *S. miscanthi* genotypes in 100 diverse Australian samples. These genotypes correspond with membership of one of four chromosomal races found in the field. Chromosomal evolution in *S. miscanthi* appears rapid: the minimum numbers of changes between karyotypes corresponded roughly one-to-one with microsatellite allele changes, and we have observed a chromosomal fission in a line held in culture for nine years. Relatedness between genotypes suggests only two Australian colonization events, and we believe that founder effect largely accounts for the low genotypic diversity. There was no evidence for genetic recombination in *S. miscanthi*, and we infer that reproduction is at least nearly always parthenogenetic in Australia. Data indicate that diversification has been by mutation in parthenogenetic lineages, rather than by recombination. Mutational differences throughout the genomes of these aphid lineages seem to involve some important expressed changes, because aphids very closely-related in microsatellies have different abundances in time and space, and populations grow significantly differently under experimental conditions. Thus these aphids are a good model for evolution by mutation alone, at loci which are potentially visible to natural selection in the wild.

Seagrasses evolution: a molecular phylogenetic perspective

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Seagrasses are angiosperms which have evolved for survival in marine habitats and as such are often viewed as oddities among the flowering plants. The adaptations required by seagrasses to inhabit marine environments are significant given that the evolutionary history of the angiosperms reflects adaptation to terrestrial environments. The fossil record suggests ancient ancestry of seagrasses with little subsequent speciation. Testing evolutionary hypotheses in the seagrasses, however, has been difficult due to a general lack of pertinent data.

Seagrasses all occur in the monocotyledon subclass Alismatidae, for which a considerable body of data has been accumulated allowing a broad view of the phylogenetic relationships within this group. We have included representatives of all extant seagrass genera in the construction of a phylogeny based on *rbc*L DNA sequence data. This phylogeny indicates that there have been three independent origins of the marine habit and up to seven independent origins of hydrophily within the subclass Alismatidae.

Reproductive system evolution in seagrasses, in particular the predominance of dioecy among seagrasses is investigated. Previous researchers have suggested that the high frequency of dioecy is due to an 'outcrossing hypothesis' which assumes that dioecy has evolved as an adaptation to enhance genetic variability by means of elevated outcrossing. We compare population genetic data and phylogenetic relationships of four seagrass genera (*Amphibolis*, *Posidonia*, *Ruppia*, and *Zostera*) and seven different populations of *Posidonia australis*. Analysis reveals substantial heterogeneity in breeding system attributes indicating patterns of genetic variation in extant seagrass populations are not singly determined by either historical phylogenetic factors or sexual conditions such as dioecy.

The marsupial Y chromosome as a model mammalian Y

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Mammals have an XX:XY system of chromosomal sex determination in which a small heterochromatic Y controls male development. Comparative studies show that the Y was once homologous with the X, but has been progressively degraded, and now consists largely of repeated sequences as well as degraded copies of X linked genes. The differential region of the human and mouse Y contains the testis determining factor *SRY*, as well as several genes important for spermatogenesis. Distantly related mammal groups, the marsupials and monotremes have heteromorphic X and Y chromosomes, but their size, pairing relationship and gene content differ from those of eutherian mammals. Marsupials have a smaller basic X and a tiny Y, which do not appear to undergo homologous pairing and recombination. The marsupial Y chromosome is testis-determining and also shares three genes (*SRY, UBE1Y, RBM*) with the human/mouse Y. Marsupial and eutherian Y chromosomes are therefore likely to have a common evolutionary origin.

Using fluorescence *in situ* hybridization (FISH) we found that the tammar wallaby homeologues of these genes map together on the small short/proximal long arm of the tammar Y. We obtained other evidence that active genes are concentrated in this small region of the tammar Y chromosome which is relatively free of repetitive sequences. When we used a large (18kb Insert) *UBE1Y* clone for FISH without suppressing repetitive sequences, we observed clear and specific signals only on Yp, imlying that there are no repetitive DNA sequences within this Yp gene. In addition, we found that hybridization of used whole female DNA onto male chromosomes detected autosomal and X-linked repetitive sequences but no hybridization to Yp and proximal Yq.

We conclude, therefore, that the active genes are concentrated at Yp/proximal Yq, a region relatively free of repetitive sequences. This region may be considered a Rminimal YS, containing all the long-conserved Y genes. It may serve as a model Y for exhaustive cloning and sequencing.

Cloning the Androgen Receptor Gene in the Common Brushtail Possum, *Trichosurus vulpecula*

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The common brushtail possum, *Trichosurus vulpecula,* is considered a pest in New Zealand. Biocontrol appears to be the only affordable option for longterm possum control.

In marsupials, the majority of the sex differentiation process takes place in the first 90 days of pouch life. It is presumed that the androgen receptor plays a key role at this time. The neonatal possum during this stage lacks the ability to mount its own immune response and is dependent on maternally derived passive immunity for immunological protection. It may be possible to take advantage of this attribute and cause the mother to produce antibodies to an immunogen, such as androgen receptor to target the fertility of the male pouch young and hence a form of control for possums in New Zealand.

Androgen receptor is an X-linked gene which is required for testosterone and dihydrotestosterone to act on target tissues, with defects in the androgen receptor not permitting the masculinisation of target tissues. To isolate the androgen receptor gene in the brushtail possum (*Trichosurus vulpecula*), consensus primers based on eutherian androgen receptor sequence were used to amplify a 510bp possum product via RT-PCR. This product was then cloned, sequenced and used as a probe to screen a possum pituitary cDNA library. Putative clones were sequenced to reveal the androgen receptor sequence.

A HIGH RESOLUTION GENETIC MAP OF THE GENOMIC REGION ENCOMPASSING THE NATURAL KILLER CELL GENE COMPLEX (NKC) ON MOUSE CHROMOSOME 6.

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Natural killer (NK) cells represent a small subpopulation of $CD3^-$ lymphocytes that play important roles in controlling tumour cells and in early defence mechanisms against a number of pathogens including viruses and parasites. Recent studies of cell surface receptors expressed on mouse NK cells have identified a number of the genes that encode these molecules. Some of these molecules are encoded by the *Ly49* and *Ly55* (*mNKR-P1*) multigene families that map to the natural killer cell gene complex (NKC) on the distal region of mouse chromosome 6. The *Ly49* and *Ly55* gene products represent inhibitory and activating receptors on NK cells, respectively. Also closely mapping to the NKC region is the host resistant gene, *Cmv1,* which controls genetically determined resistance to murine cytomegalovirus (MCMV). To more precisely delineate the locations of these loci we have generated a high resolution genetic map of this region of chromosome 6. We have analysed 1250 backcross mice which comprised panels of 700 x (BALB/c x C57BL/6J)F_1 x BALB/c and 550 x (A/J x C57BL/6J)F_1 x A/J backcross progeny. A total of 23 polymorphic genes or microsatellite markers were analysed over a region of 10 cM in length spanning the interval from D6Mit134 to D6Mit59. Recombinant mice in this interval were also tested by infection with MCMV to determine their *Cmv1* phenotypes. The data obtained indicate that these functionally important NKC-associated genes represent a tightly linked cluster spanning approximately 0.4 cM.

T58

EVOLUTION OF THE ALDOLASE A GENES AND PSEUDOGENES IN THE AUSTRALIAN *RATTUS*

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The aim of this study is to compare and to contrast molecular evolution in three classes of DNA, introns, exons, and pseudogenes. For this study, we chose to look at the aldolase gene family, because in vertebrates, it consists of three isozymes (A, B and C) whose evolutionary relatedness is well characterised. Each isozyme may have one or more derived pseudogenes and/or retroposons, allowing the potential for both paralogous and orthologous comparisons. For this presentation, we will be looking specifically at Aldolase A and Aldolase A-like genes.

The Australian *Rattus* prove to be a suitable taxa to use in this study as they are well characterised in terms of a time frame for their evolution, including chromosomal and allozyme data at the species, subspecies and population levels. Preliminary data suggest that there exists in the Australian members of the genus *Rattus*, at least one pseudogene of Aldolase A derived from duplication events and at least one retroposon of Aldolase A. Standard PCR and direct sequence from PCR products has been unsatisfactory, due to the presence of the highly homologous pseudogenes. Cloning has been used in order to separate the pseudogenes from the functional genes. In order to screen large numbers of colonies for inserts of the correct size, but of variable sequence, we utilised the technique of Temperature Gradient Gel Electrophoresis (TGGE). The data will be presented and the application of TGGE in this context discussed.

T59

SPATULA : A GENE INVOLVED IN CARPEL DEVELOPMENT IN *ARABIDOPSIS THALIANA*

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Recent efforts toward understanding the genetics of flower development have contributed greatly to our knowledge of pattern formation in plants. While most of this work has concentrated on homeotic genes that specify pairs of floral organs types, genes controlling the morphogenesis of individual organ types are only now starting to be analysed.

In *Arabidopsis* recessive mutations in the *SPATULA* (*SPT*) gene specifically disrupt the structure of one type of floral organ, the gynoecium. This is the female reproductive structure of the flower, and consists of two congenitally fused carpels. Early in the development of *spt* mutant gynoecia, growth is retarded at the region of carpel fusion leading to two mature carpels that are partially unfused at the top. The amount of replum, septum and stigmatic papilla tissue is also reduced, and the pollen transmitting tract, that in the wild type differentiates within the style and the septum, is completely absent.

The organ identity gene *AGAMOUS* (*AG*) is also involved in carpel development. This is clearly shown in *apetala2* (*ap2*) mutants where ectopic expression of *AG* turns sepals into carpel-like organs. Also, when *ag* is mutant together with *ap2*, these organs now lack a style and their walls become leaf-like. However many carpel features still develop. These include ovules, stigmatic papillae, replum, septum and transmitting tract tissue. Only when *SPT* is mutant as well, as in *ap2 ag spt* triply mutant plants, do these carpel features almost completely disappear. This not only implies that *SPT* is activated independently of *AG*, but also that *SPT* is sufficient when *AG* is mutant for the production of many carpel features.

Progress towards cloning of the *SPT* gene by chromosome walking will be presented.

Two sugar kinase genes of *Arabidopsis* : *ARA1* and *GAL1*.

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Free arabinose and galactose, possibly released during the degradation or turn-over of polysaccharides, can be metabolised via "salvage" pathways. Such pathways involve the sequential action of a kinase to form a sugar-1-phosphate and a pyrophosphorylase to produce a UDP-sugar (the activated donor of polysaccharide and glycoprotein biosynthesis). The arabinose-sensitive *ara1* mutant of *Arabidopsis* has only 10% of the wild-type level of arabinose kinase activity. Thus it is likely that the *ARA1* gene encodes an arabinokinase.

The *ARA1* locus has been mapped to chromosome 4 and is closely linked to the late-flowering marker *FCA*. A 200kb chromosome walk in the region of these two genes has been completed. Fine-scale RFLP mapping of *ARA1*using *ARA1-FCA* and *ARA1-COP9* recombinants has positioned this gene within a 25kb region. This region forms part of the 2 Mb analysed during the first two years of the "European Scientists Sequencing *Arabidopsis*" project and contains sequence encoding a protein with the five conserved motifs characteristic of the galactokinase family of sugar kinases. We hypothesise that this is the *ARA1* gene and that the arabinokinase is related to the galactokinase family.

In addition, the dbEST databank contains a sequence which encodes a protein more closely related to the eucaryotic galactokinase than *ARA1*. We have obtained full-length cDNA clones associated with *ARA1* and the *GAL1*-homologue and investigated the expression of their corresponding mRNAs. We are currently assaying the activity of these gene products expressed in heterologous systems. The characterization of these genes will allow a greater understanding of the processes of polysaccharide metabolism in plants.

Cloning and Characterisation of Haemoglobins from *Arabidopsis thaliana*

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Plant haemoglobins can be divided into two families on the basis of sequence homology and expression patterns. One family is expressed only in the nodules of nitrogen fixing plants. These "Symbiotic haemoglobins" transport oxygen allowing nitrogen fixing symbioses to occur. The other family is expressed in normal plant tissues and has been identified in plants that do not fix nitrogen. These have been designated the "Non-symbiotic" haemoglobin family. The function of the Non-symbiotic haemoglobins is unclear.

In order to examine the function of Non-symbiotic plant haemoglobins we have cloned two haemoglobin genes from the model plant *Arabidopsis thaliana*. One of these genes shows strong homology to the Non-symbiotic haemoglobins. The other is more closely related to the Symbiotic haemoglobins that previously have been found only in nitrogen fixing plants. The expression of these genes has been examined in different plant tissues and after exposure to a range of environmental stress conditions. Currently we are attempting to generate transgenic plants that over or under express these haemoglobins and to express these sequences in bacteria to allow protein characterisation studies.

LUTE-1, a transposable element isolated from spontaneous mutants of the L^6 flax rust resistance gene.

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A small insert was identified in two independent mutants (Xl 17 and X3A) at separate locations in the L^6 flax rust resistance gene. The insert was amplified from both mutants, cloned and sequenced. Sequence analysis revealed the inserts were identical and displayed the physical properties of a transposable element. We have named the flax *(Linum usitatissimum)* transposable element LUTE-1. LUTE-1 is 70% AT rich, 315 bp in length and contains no open reading frame which indicates it is probably nonautonomous. It has an imperfect 14 bp terminal inverted repeat which shares some homology with inverted repeats in transposable elements from petunia, potato and maize. It contains a 28 bp internal inverted repeat adjacent to the terminal inverted repeat which has the potential to form a stem-loop structure. Insertion of LUTE-1 caused an 8 bp target site duplication in the L^6 gene in flax. Southern blot analysis indicates approximately 10-20 copies of LUTE-1 are present in the flax genome. The mutant allele X117 has reverted to wild-type, restoring the L^6 rust resistance phenotype in flax. PCR analysis of the insertion region in the mutants and the revertants confirmed that LUTE-1 had excised from the L^6 gene in the revertants. The characterisation of a native flax transposon may have implications for the isolation of other rust resistance genes in flax by transposon tagging.

Response of Two Heat Shock Genes to Selection for Knockdown Heat Resistance in *Drosophila melanogaster*

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To identify genes involved in stress resistance and heat hardening, replicate lines of *Drosophila melanogaster* were selected for increased resistance to knockdown by a 39° heat stress. Two selective regimes were used, one with and one without prior hardening. Mean knockdown times were increased from around 5 min to more than 20 min after 18 generations. Initial realised heritabilities were as high as 10% for lines selected without hardening, and crosses between lines indicated simple additive gene effects for the selected phenotypes. To survey allelic variation and correlated selection responses in two candidate stress genes, *hsr-omega* and *hsp68*, we applied denaturing gradient gel electrophoresis to amplified DNA sequences from small regions of these genes. After eight generations of selection, allele frequencies at both loci showed correlated responses for selection following hardening, but not without hardening. The hardening process itself was associated with a *hsp68* frequency change in the opposite direction to that associated with selection that followed hardening. These stress loci are closely linked on chromosome *III* and the hardening selection established a disequilibrium, suggesting an epistatic affect on resistance. The data indicate that molecular variation in both *hsr-omega* and *hsp68* contribute to natural heritable variation for hardened heat resistance.

Diazinon Resistance in *Drosophila melanogaster*.

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In an investigation studying diazinon resistance in *Drosophila melanogaster*, thirteen populations from the East coast of Australia were sampled. Between 9 and 35 lines were established for each population from single inseminated females. Each line was tested with a discriminating dose of the organophosphorous (OP) insecticide diazinon. Resistance was assayed by placing 50 adult flies aged 4-7 days old in a glass scintillation vial coated with 50 µl of diazinon. Percentage mortality was recorded after 20 hours. Results show that there is OP resistance in each of the populations sampled. The levels of resistance varied between the populations but was not associated with geographical location or mode of collection. Genetic analysis showed resistance to be polygenic in all populations investigated.

The exposure history of the East coast populations is unknown other then that there is OP use in the areas of collection. It was decided to collect a further population of *D. melanogaster* where the exposure history was known. An apple and lemon orchard at Wandin, Victoria was selected due to the high levels of OP use and the availability of a precise spray record. Levels of diazinon resistance at Wandin are similar to those seen in the East coast populations. Resistance is again under polygenic control.

Work is currently underway in the laboratory to generate monogenic OP resistance following EMS mutagenesis and selection.

CONSERVATION GENETICS : COMPARING CAPTIVE MANAGEMENT OPTIONS

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Loss of habitat, hunting, pollution and competition/predation by introduced species can reduce the census size of a species to a level where they become endangered. This has increased the importance of captive populations for breeding and long term conservation.

Finite population size will initially increase the expression of detrimental genes, along with a loss of fitness (inbreeding depression). In the longer term loss of genetic variation and fitness will reduce the capacity for adaptation and increase the likelihood of extinction.

The question arises of how best to manage these populations to minimise inbreeding and loss of genetic variation. Three management regimes are compared using *Drosophila*: minimising kinship, maximum avoidance of inbreeding, and random mating. Theoretical studies predict that minimising kinship should be the best. The difference between minimising kinship and maximum avoidance of inbreeding is predicted to occur when the founder individuals are unequally represented in the following generations.

No significant difference was found in the reproductive fitness of each regime when competitive ability, and productivity were assessed. Allozyme variation showed a non-significant trend in the expected direction. A more powerful estimator of genetic variation using microsatellites has therefore been developed.

Testing species border hypotheses using *Drosophila serrata*

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Species borders is a term first coined by Mayr in 1954 to describe the existence of distinct distributions, where species occupy a specific range. Factors affecting the distribution and abundance of species are often unknown, the environmental conditions just beyond the species border not appearing to be markedly different. Why don't populations at the edge of the species range evolve and expand into these areas? An ecological approach is used to determine relevant environmental factors and traits that constrain the species distribution, an evolutionary approach is used to determine what prevents those traits from evolving at the genetic level. The distribution of *Drosophila serrata* in Australia appears to be largely limited to the east coast, with the southern border of this species occurring around Sydney. It was previously thought that the marginal population occurred at Forster, approximately 300km north of Sydney. Further sampling has revealed a population south of Sydney, at Wollongong, during late summer and early autumn. The computer simulation program, Climex, indicates that cold winter temperatures are the most likely climatic factor limiting the distribution of *D. serrata.* This is also supported by failure to find serrata south of Forster over winter or in spring. Initial screening using isofemale lines from populations from the southern border to the centre of the range indicated a seasonal difference in cold resistance between the winter border population and more central populations. Sites from Forster to Grafton in spring and Wollongong to Grafton in autumn were sampled extensively. Using a parent-offspring regression of field caught parents and laboratory reared offspring, the field heritability of cold resistance was estimated. The genetic hypotheses proposed by Hoffmann and Parsons (1991) and Hoffmann and Blows (1993) to explain the existence of species borders are evaluated.

Identification of a plastid in Apicomplexan parasites of humans

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The discovery in malarial and toxoplasmodial parasites of genes normally occurring in the photosynthetic organelle of plants and algae has prompted speculation that these so-called protozoans might harbour a vestigial plastid. The plastid-like parasite genes occur on an extrachromosomal, maternally inherited, 35kb DNA circle with an architecture reminiscent of plastid genomes. Although the 35kb genome is distinct from the 6-7 kb linear mitochondrial genome, it has not been determined where within the parasite cells the plastid-like genome resides. To determine if a plastid is present, we used high resolution *in situ* hybridization to localise transcripts of a plastid-like 16S rRNA gene from *Toxoplasma gondii,* the causative agent of toxoplasmosis. Transcripts accumulate in a small, singular, ovoid organelle that is located anterior to the nucleus in the mid-region of the cell and is distinct from the tubulocristate mitochondria. At least two membranes (some profiles possibly indicate a third membrane) surround the organelle. Our *in situ* hybridization data thus identify a third, hitherto putative, genetic compartment (a plastid) in these parasite cells. The origin and role of the plastid in obligate intracellular parasites is completely unknown, but it is a welcome new, parasite-specific target for therapeutic agents.

T68

The Phylogeny of Eutherian Orders Based on 12S and IRBP Sequences

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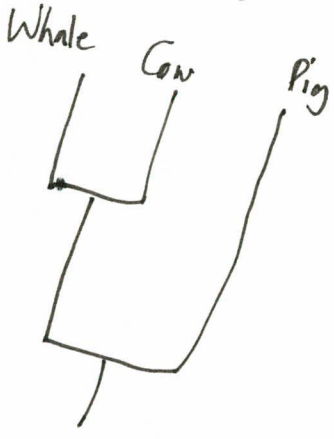
DNA sequences for the complete 12S rRNA gene and part of exon 1 of the interphotoreceptorretinoid binding protein (IRBP) gene were used to examine relationships among all extant orders of placental mammals. The strongest support for a superordinal grouping is for Paenungulata, which includes hyraxes (Order Hyracoidea), elephants (Order Proboscidea), and dugongs and manatees (Order Sirenia). Perissodactyls (e.g., horses, rhinos), which have been hypothesized as a sister-group to hyraxes based on several morphological characters, do not associate with hyraxes in any of our analyses. Sequence data also provide support for a more inclusive group that includes paenungulates, elephant shrews (Order Macroscelidea), and aardvarks (Order Tubulidentata). Notably, all five orders in this clade are of putative African origin.

? Whale more closely related to cow than cow is to pig.



—very strong statistical support for this grouping!!

The Y of human evolution: male specific DNA polymorphisms and human origins and diversity?

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The human Y chromosome comprises two distinct parts; a pseudoautosomal region that pairs with the X chromosome in male meiosis, and the remainder of the chromosome which is male specific and, therefore, not involved in recombination. It is this haploid Y chromosome that is potentially of great value in the study of human diversity as it retains a record of the paternal lineage (just as mitochondrial DNA records the maternal lineage). Unfortunately, the Y chromosome appears to be relatively deficient in sequence polymorphisms, and, further, some alleles exhibit recurrent mutation.

However, a number of Y alleles are thought to reflect unique events, and this paper examines Y-haplotype distributions in a number of populations based on some of these. The markers selected represent different types of evolutionary events, including insertion, deletion and point mutation. Europeans appear to possess considerable variation in Y haplotypes, especially when compared to Africans, but it should be noted that the number of non-Europeans examined is considerably smaller. The lower diversity among Africans compared to Europeans for these Y markers is the opposite to the findings seen in mtDNA variation. The possible role of different mate choice systems, particularly the practices of polygamy and the levirate, in acting to reduce Y-chromosome variability in some human groups must be considered in any interpretation of these observations. Y haplotypes have excellent potential as markers of European gene flow in other populations. Y-specific markers are particularly useful when it is suspected that the admixture may have been asymmetrical with respect to sex.

T70

Evolutionary divergence and conserved motifs in the gene for the intermediate-sized silk protein sp185/220

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The silk produced by larval chironomids is comprised of a number of different proteins which fall into three size classes: large size spI family (about 1000 kDa), intermediate-size class (100-220 kDA) and the small-size class (< 100 kDa). The proteins have been mostly studied in *Chironomus,* where they are largely the product of genes in the Balbiani rings, those prominent features of the salivary gland chromosomes first noted by Balbiani in 1881. The large spIs form the structural backbone of the silk fibres, but relatively little is known of the role of the small- and intermediate-size class proteins.

Some of the intermediate-size class proteins appear to be restricted to only one or a few species. However the sp185 protein appears to be present in all examined species of*Chironomus* or the related genus *Kiefferulus*. There is one exception, in *C. thummi,* where a protein with homology to sp185 has a size of 220 kDA. An investigation was therefore carried out of a number of distantly related species, in order to determine which elements of the protein are conserved. Initial studies of European species indicated a high cysteine content (about 14%), apparently associated with a cysteine-containing motif (Cys-X_{4-10}-Tyr-X_4-Cys-X-Cys-X-Cys) repeated approximately every 22-26 residues. Determination of amino acid composition of sp185 protein from Australian species of *Chironomus* and*Kiefferulus* revealed a lower Cys content (4-11%). Preliminary PCR-derived DNA sequence of a putative sp185 gene from*K. 'cornishi'* showed some reduction of Cys residues in the deduced product. However, some cysteine-containing motifs are preserved, supporting the importance of these motifs in the function of the sp185 protein.

Preliminary phylogenetic comparison of the sequences suggests that *Kiefferulus* is more closely related to the subgenus *Camptochironomus* than to the subgenus *Chironomus* of the genus *Chironomus.*

Protamine P1 Gene Sequences and the Phylogeny of Dasyurid Marsupials.

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Molecular systematic studies of dasyurid marsupials have yielded largely congruent estimates of phylogenetic structure within the family. We report the complete sequence of the nuclear protamine P1 gene for 30 species of dasyurids, including all extant species of the subfamily Dasyurinae. The protamine phylogeny is highly congruent with mtDNA sequence data sets and suggests that Dasyurinae is composed of three major phylogenetic groups: (1) a clade including *Dasyurus* and *Sarcophilus* ; (2) a clade including *Phascolosorex* and *Neophascogale* ; and (3) a basal and probably paraphyletic assemblage of the remaining dasyurine species. Genetic divergences within the latter group are remarkable in showing almost no dichotomous branching structure among species, a pattern consistent with and indicative of rapid cladogenesis near the Miocene origin of the subfamily.

A GENETIC ANALYSIS OF THE *RAS* ACTIVATING PROTEIN, *SOS1*, IN MICE.

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The Sos protein was first identified in *Drosophila* in a genetic screen for proteins necessary for development of the R7 photoreceptor. Its name relates to its requirement downstream of the sevenless protein tyrosine kinase receptor. However, this name is somewhat misleading as it is also required downstream of the *Drosophila* EGF and torso receptors. In addition, a wealth of biochemical data implicate mammalian Sos homologues, *Sos1* and *Sos2*, in signalling by many cell surface receptors.

In contrast to the situation in *Drosophila*, it has been very difficult to develop systems in mammalian cells where the functional role of Sos proteins can be investigated. This problem is a consequence of the ubiquitous expression of Sos proteins and is common to many signalling proteins. Cell lines are often not available in which a given protein can be expressed de novo in order to examine its role in signalling by various receptors. Similarly, structure-function analysis of specifically mutated proteins is hampered by the contribution of wild type protein.

Dominant negative alleles are widely used to demonstrate a functional role for individual signalling proteins, including Sos, by suppressing the activity of endogenous proteins. However, the specificity of such alleles can be unclear as they are frequently expressed at very high levels in order to compete with endogenous protein. Recently, we have taken an alternative approach to investigating the functional role of Sos proteins in mammalian cells by performing a genetic analysis of the Sos proteins in mice. We have generated mice which carry a null mutation in the *Sos1* gene and find that mice homozygous for this mutation die mid-gestation with a characteristic cardiovascular disorder. We have taken two approaches with the *Sos1* mutant mice to investigate whether *Sos1* is required for signalling by the EGF receptor (EGFR). Firstly, we have derived homozygous mutant fibroblasts to investigate EGF mediated responses. Preliminary analyses indicate that MAP kinase activation in response to EGF stimulation is severely reduced in these cells. Secondly, we have intercrossed the *Sos1* mutant mice with mice carrying a hypomorphic allele of the EGFR (*Waved2*). *Waved2* mice which are heterozygous for the null *Sos1* allele show a high penetrance eye phenotype, very similar to that observed in TGFa mutant mice. The *Sos1* mutation therefore acts as an enhancer of the *Waved2* allele, demonstrating that *Sos1* is required for EGFR signalling in the eye.

These studies underpin previous biochemical analyses of the Sos proteins in mammals and serve as a basis for future structure-function studies with the *Sos1* protein.

Cloning and characterisation of four novel cellular inhibitor of apoptosis protein (IAP) homologues.

Anthony G. Uren, Miha Pakush, Christine J. Hawkins, Kirsten L. Puls and David L. Vaux.

Walter and Eliza Hall Institute, Parkville 3052

The molecular pathways governing programmed cell death and apoptosis are known to be highly conserved between *C.elegans*, *Drosophila* and mammals. For instance, the *C.elegans* cell death genes *ced-9* and *ced-3* are structurally and functionally homologous to the respective mammalian cell death regulators *bcl-2* and Interleukin 1β converting enzyme (ICE). The baculovirus inhibitor of apoptosis protein OpIAP prevents viral induced cell death in SF9 insect cells. Previously we have shown that OpIAP is able to inhibit apoptosis induced by ICE. By searching the Genbank and EST databases we have identified four previously uncharacterised cellular homologues of baculovirus OpIAP, which we have termed MIHA, MIHB, MIHC (mammalian IAP homologues A, B and C) and DIHA (*Drosphila* IAP homologue A). Cloning and sequencing of full length cDNAs for these proteins has shown them to be highly conserved between *Drosophila* and mammals, each IAP homologue containing three BIR motifs at their N terminus and one ring finger motif at their C terminus. Using a transient transfection assay we have shown that overexpression of MIHA and MIHB (but not MIHC) in mammalian cells is able to protect against apoptosis induced by the *ced-3* homologue ICE. Thus in addition to the *ced-3*/ICE and *ced-9*/bcl-2 families of genes, cellular IAP genes also appear to have a conserved role in the regulation of apoptosis.

T74

EXPRESSION PATTERN OF THE *TRPL* CATION CHANNEL OF *DROSOPHILA MELANOGASTER*

Coral G. Warr and Leonard E. Kelly

Department of Genetics, University of Melbourne, Parkville VIC 3052 AUSTRALIA

The *transient-receptor-potential-like* (*trpl*) gene encodes a calmodulin-binding protein which shows homology to the *Drosophila* phototransduction gene *trp* and to ion channel proteins. Expression studies have proved the Trpl protein is a cation channel which is relatively non-selective for Na^+ and Ca^{2+}, and which may act at the endpoint of the phototransduction cascade.

Initially *trpl* expression was thought to be in eye only, based on Northern analysis and in-situ hybridisation with an anti-sense riboprobe. However, two anti-*trpl* antibodies raised against carboxy-terminal regions identify a membrane-bound protein of the correct size in thoraces and ovaries (not oocytes) as well as in head protein extracts. Subsequently RT-PCR has been performed on head and body RNA using primers directed to the C terminal region. The PCR products obtained from both head and body have been sequenced and found to be *trpl* in both cases. It therefore appears that the Trpl protein is not head-specific, it is highly abundant in thoraces, and also present in ovarian tissue. These results indicate that *trpl* may be involved in processes other than phototransduction.

As no mutants at the *trpl* locus are available, mutagenesis screens are being carried out to obtain *trpl* null mutants. The expression studies have raised the possibility that a *trpl* null mutant will be lethal. Accordingly methods to screen heterozygous mutagenised flies have been developed. One method combines the mobilisation of P elements which map close to *trpl* with the amplification of the DNA surrounding the P elements using an inverse PCR protocol. The PCR products can then be probed with the *trpl* cDNA and 5kb of 5' region to detect any insertions affecting the *trpl* gene. Mutagenesis strategies and results will be presented.

CHEMOSENSORY MUTANTS AND FEEDING BEHAVIOUR IN *Caenorhabditis elegans*.

Warwick Grant and Peter Hunt

Flinders University & University of Edinburgh.

The ability to modulate a behaviour in response to environmental stimuli is well understood in bacteria and some lower eukaryotes but not as well in more complex animals with nervous systems. Genetic analysis has played a major role in the elucidation of prokaryote behaviours and can be applied in much the same way to the analysis of more complex eukaryote behaviours provided that the system is sufficiently well defined. The nematode *Caenorhabditis elegans* provides such a system: the behaviours and the nervous system that mediates them are well defined and a range of sophisticated genetic and molecular techniques are available. The approach we have taken is to analyse the genetics of resistance to a class of drugs (the macrocyclic lactones) that were known to affect feeding behaviour in *C. elegans* . This resistance genetics data is then integrated with what is already known about feeding and the function of the pharynx in these and other worms.

Our initial observation was that all the loci at which we recovered ivermectin resistance alleles have been defined previously in screens for mutants with defective chemosensory behaviours or abnormal chemosensory neurones, thus establishing a link between the drug, chemoreception and feeding. We have explored this link further by constructing double mutants between our resistance alleles and mutations that impair synapse function in another chemosensory pathway. Some, but not all, alleles of the "neurotransmission" loci are able to suppress the resistances conferred by some resistance loci i.e. there is complex, allele specific suppression of ivermectin resistance by mutations that impair neurotransmission. We have not been able to decipher any consistent pattern governing this suppression/interaction between these two sets of loci. It is clear, however, that ivermectin resistance is an active process that requires a signal to be sent, presumably form the chemosensory neurones. We have constructed a model for ivermectin action and resistance based on these observations that predicts that the drug acts to reset the sensitivity of the pharynx to chemosensory feeding stimuli from the environment. The existence of this pathway has been postulated to explain the relationship between the presence of food and pharynx pumping rates: this work provides the first evidence that such a pathway exists and defines some of the genes involved. We are currently seeking to define more components through a combination of screens for new suppressors and construction of more double mutants.

T76

The contribution of neurogenic loci to bristle number variation in natural populations of *Drosophila*

Charles H. Langley

University of California, Davis, CA 95616

Most characters of interest to those who study natural populations of plants, animals or humans are continuously distributed. Because the effects of segregating alleles on these traits are often too small to be individually detected, the statistical "infinitesimal" model has remained the dominant paradigm since Fisher's reconciliation of Mendelian inheritance with correlations among relatives. This model assumes that genetic variation in the quantitative trait is determined by small allelic contributions from a large number of loci. While empirical and practical applications of this model (e.g., correlation among relatives and predictions of selection response) are demonstrably robust, population genetic models accounting for the maintenance of variation and long-term selection response often rely critically on the assumption of many loci each with small effects.

The numbers of various bristles of *Drosophila melanogaster* are characters that have been canonical quantitative traits for many decades. Quite coincidentally rapid progress in elucidating the molecular/developmental genetics of the Peripheral Nervous System (including bristles) of adult Drosophila has provided a set of candidate loci that can be surveyed with both classical genetic mapping and potentially more powerful (if indirect) molecular population genetics to determine the contributions of these loci to naturally occurring bristle variation. Mapping of differences between short-term selection lines using *in situ* hybridization to salivary gland chromosomes of probes for naturally occurring polymorphic transposon insertions localized several factors near candidate loci (e.g., *ASC*, *bb*, *Dl*, *emc*, *h*, *H* and *E(spl)*). Quantitative genetic complementation assays further support the involvement of these loci. Although bristles are often regarded as a classic additively determined trait, we have found sex-specific and epistatic interactions of magnitudes comparable to the average allelic effects.

The identification of strong linkage disequilibrium between naturally occurring DNA sequence polymorphisms and factors determining measurable phenotypic effects among large random samples of chromosomes (or gene regions) may unambiguously confirm and afford some analysis of the contributions of candidate loci to standing quantitative genetic variation. Surveys of the first three candidate loci (*ASC*, *scabrous* and *Dl*) indicate that much of the quantitative genetic variation in bristle number ($\approx$ 5% per locus) may be due to alleles of relatively large affect ($\approx$ 0.5 phenotypic standard deviations), some at intermediate frequencies. If these initial conclusions are confirmed in the completed survey of the candidate loci, the predictions of several popular models for the maintenance of standing quantitative genetic variation will be untenable and it may emerge that much of the genetic variation within populations is not the raw material for long-term evolutionary divergence in quantitative traits between populations or species.

T77

MAMMALS THAT BREAK THE RULES: GENETICS OF MARSUPIALS AND MONOTREMES.

Jennifer A. Marshall Graves

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Marsupials and monotremes, the mammals most distantly related to human and mouse, share essentially the same genome, but show major variations in chromosome organization and function. Rules established for the mammalian genome by studies in human and mouse do not always apply, and we must make new and more general laws. Some examples are:

• Sex differences in marsupial recombination rates favour males rather than females, making a universal selective factor unlikely.

• Conservation of gene arrangements among other vertebrates, suggests that eutherians, not marsupials, break a more general rule of genome conservation.

• Marsupials have a small (original?) X and Y chromosome and no pseudoautosomal region. This casts doubt on the universal importance of a PAR for pairing and sex chromosome segregation, and eliminates divergence of the PAR as a general explanation of Haldane's Rule.

• Marsupials and monotremes break Ohno's Law of conservation of the X, and conservation in the absence of inactivation implies that inactivation cannot wholly account for conservation of the X.

• A subset of conserved markers on the X and Y in all three mammal groups defines a smaller ancestral X and Y which were once homologous, and a recently added region. Faulty heterospecific interactions between X and Y-borne paralogues may explain Haldane's Rule.

• The eutherian and marsupial Y have a common origin, but the eutherian Y has received at least three additions. The tiny marsupial Y is a good model Y.

• Marsupial X inactivation is different from eutherian in many respects. Features are shared by eutherian extraembryonic membranes, implying that it is the random, hyperstable eutherian system which is exceptional.

• A "marsupial test" can be used to assess candidate sex determining and spermatogenesis genes. Thus *SRY,* but not *ZFY*, and *RBM1,* but not *DAZ* are also included on the marsupial Y and are the more promising candidates.

• *SRY* is not subject to directional selection in a rapidly and recently radiating marsupial species complex, so is unlikely to drive speciation.

• The Y is not paramount in marsupial sex determination. Some sexual dimorphisms are determined, not by the presence or absence of a Y, but by dosage or parental origin of a putative "switch gene" on the X.

Thus it is not always the marsupials and monotremes - usually considered weird mammals - that are exceptional. In many of these features, it appears that the weird mammals are humans, and particularly mouse, which break more general mammalian, or even vertebrate rules.

J.A.M. Graves (1996). Mammals that break the rules: genetics of marsupials and monotremes. Ann. Rev. Genet. (in press).

INTERPATH SERVICES

Product Showcase

New Autoclavable Range

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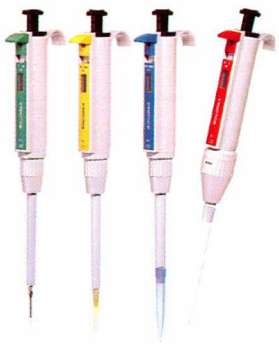
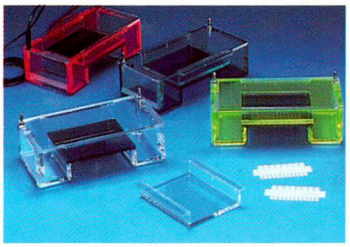


Interpath Services has released a new range of Micropipettes from Socorex. This range incorporates the unsurpassed digital accuracy of Swiss solid calibration. Research has resulted in a much lighter and ergonomically designed pipette that is simple and easy to use whilst incorporating precision and reliability.

Other features of the range include:

- ***fully assembled autoclavability*** for repeatable in-house decontamination
- ***colour coded sizes*** provide instant volume identification thus eliminating potential selection errors
- ***instant digital volume setting*** and read out eliminating tedious windings and accidental volume adjustments and
- ***in-house calibration*** easily performed by means of key and data supplied.

Mini-Horizontal Electrophoresis

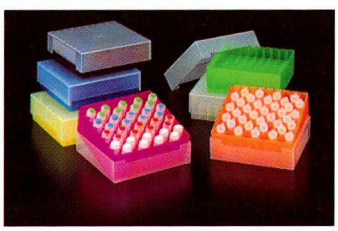


Constructed of durable, leak proof acrylic Molecular Bio-Products new Mini-Horizontal Electrophoresis Gel System incorporates a smoke grey stage making sample loading effortless, even with the smallest wells. **This feature contrasts the gel so that the sample wells are clearly defined, virtually eliminating loading errors.**

The UV transparent gel tray allows for easy viewing and gel transport without having to handle the gel. Perfect for working with low melt agarose gels, the gel tray eliminates the risk of tearing or dropping your precious gel. The gel tray also has comb slots to ensure straight, parallel wells every time.

This electrophoresis system utilises platinum wire for current generation guaranteeing a consistent current through the gel. Its construction also allows the researcher to replace the electrode set, extending the life of the system.

Cryogenic Rack



The TR81 cryogenic storage rack is constructed of durable polypropylene and is a colourful economic alternative to the 'disposable' cardboard freezer box.

The Rack features include: rating to -90°C; fits existing standard freezer storage modules; accommodation for all 1.5/2.0mL microcentrifuge tubes and standard cryovials; alphanumerically hotstamped for fast and accurate identification of each tube; a clear lid that features a frosted writing panel on two sides for further identification options; and is available in varying colours for indexing of specific experiments and/or those of individual researchers.

This rack is only one of many racks produced by Scientific Specialties Inc for Interpath Services. **Other versions include small to large configuration Flipper racks, Flipper freezer storage racks, microtube racks with containers, Floater microtube racks, B-Radiation Shielding Racks and a 4-Way Flipper rack to suit four different sized tubes.**

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The Socorex range of bottle top safety dispensers provides end-users with a safe, convenient, trouble-free method of dispensing both general solutions plus potentially hazardous acids, solvents etc. Available in various sizes ranging from 0.2-109mL these dispensers give users absolute precision with supreme reproducibility. The patented volume selection hand piece is not only fast and simple to operate but, combined with the easy-to-read digital volume display, virtually eliminates the risk of incorrect volume settings. As added security the volume selector securely locks into position whilst the dispenser is in use.

Other features include: full autoclavability; fixed volume selector steps are permanently pre-calibrated during manufacture; chemical resistant with safety poly-coated glass barrel; teflon coated plunger which prevents 'plunger freeze' when using alkaline solutions; screws directly onto original bottle. A full range of adaptors are also available for non-standard bottles.

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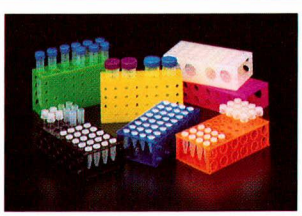

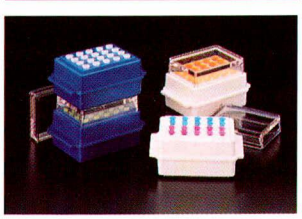

ISO Freeze Temperature Maintenance Systems are available in two temperature ranges for keeping PCR reagents, cells, enzymes or other temperature sensitive reagents chilled and stable when removed from freezer to bench top. Model CR-20-0 will maintain a temperature of 0°C on the bench top for a minimum of 3h and Model CR-20-20 between -15°C and 20°C for the same period. Made from polycarbonate they are virtually unbreakable.

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Available in a variety of various colours, plus natural and black, these racks can be autoclaved and are ideal for freezer storage.

ART for PCR

Interpath Services announce their appointment as the exclusive Australian agent for Molecular Bio-Products range of aerosol resistant tips (ART). ART brand tips are impenetrable to aerosols, and the self-sealing filter also eliminates any dangerous liquids being drawn into the pipettor.

The ART range includes 19 different pipette tips covering all leading brands. Also available:

- tips for electrophoresis gel loading
- genomic tips for optimal cell recovery
- multichannel tips
- ART Reach, an elongated tip used when retrieving samples from small vials and the
- ART XLP tip for those hard to reach places.

These sterile tips are perfect for PCR, pipetting radio-active samples and loading horizontal gels. The ART range is certified DNase, RNase and Pyrogen free. Molecular Bio-Products also offer customers Solvent Safe Tips, RNase and DNA Away; plus their new Mini-Horizontal electrophoresis Gel System.

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HYBAID

One step ahead in molecular biology

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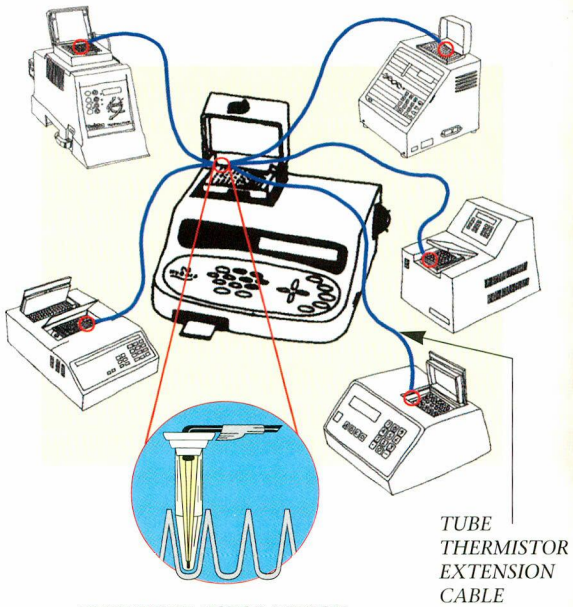

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Temperature			
Temperature Range	Ambient +10°C -99°C Ambient +15°C -99°C with 96 well plates	Ambient +10°C -99°C Ambient +15°C -99°C with 96 well plates	Ambient +10°C -99°C
Block Heating Rate	up to 2°C/sec	up to 2°C/sec	up to 2°C/sec
Block Cooling Rate	up to 2°C/sec	up to 2°C/sec	up to 2°C/sec
Temperature Control Precision	±1°C	±1°C	±1°C
Display Resolution	0.1°C	0.1°C	0.1°C
Block Uniformity	±0.5°C within 25 secs	±0.5°C within 25 secs	±1.0°C within 30 secs
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Block Capacity	48 x 0.5ml tubes 96 x 0.2ml tubes 1 x 96 well plate	96 x 0.2ml tubes 1 OmniPlate 96	4 microscope slides (76 x 26 x 1mm)
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HYBAID

One step ahead in molecular biology

Genetic variability in a translocated population of Swamp Antechinus (*Antechinus minimus maritimus*) as assessed by microsatellite variation.

David J. Edwards and Barbara A. Wilson

School of Biological and Chemical Sciences, Faculty of Science and Technology, Deakin University, Geelong, 3217.

When a wild population is small in size, the genetic diversity may well be reduced compared to larger populations. This in turn could compromise the ability of the population to respond to environmental challenges. The marsupial Swamp Antechinus (*Antechinus minimus*) is rare in Victoria, where it has a disjunct distribution and occurs in small patches of suitable habitat. The species is prone to random events such as fire which can eliminate suitable habitat and result in the loss of entire populations. Such an event occurred to populations of *A. minimus* when the 1983 "Ash Wednesday" fires eliminated the species from previous known locations in the eastern Otway ranges. Between 1992 and 1994 a series of translocation experiments were undertaken in an attempt to re-establish the species' previous distribution in the area. Thirty individuals from one population were introduced as founders over the this period.

The aim of this study was to determine and compare genetic variability in the founder and translocated populations of the Swamp Antechinus. Measuring and monitoring of genetic variation in marsupial species is best accomplished by targeting at the DNA level, with microsatellites providing the most logical method. Preliminary results of microsatellite variation within the translocated and founder populations will be presented and implications of the results discussed.

Multilocus DNA Fingerprinting of Orange-Bellied Parrot (*Neophema chrysogaster*)

J.A.Baril, M. T. Ivanyi and N.D. Murray

School of Genetics and Human Variation and Centre for Conservation Genetics, La Trobe University Bundoora, Victoria 3083

The Orange-Bellied Parrot (*Neophema chrysogaster*) is listed as a critically endangered species by the Federal Government and the State Governments of South Australia, Tasmania and Victoria (Menkhorst 1996). With a population of less than 250 adults left in the wild, a captive population has been set up in Healesville Sanctuary and Tasmania to help in the conservation of this species.

To characterize both the wild and captive populations, allelic variation has been identified by multilocus DNA fingerprinting using Per probe pSP 64.2.5E1 with Hinf I and Hae III digestion enzymes. Results showed that there has been non-representative sampling of the wild population at the time of the establishment of the founders in the captive colony or that there has been non-representative breeding amongst captive individuals.

Microsatellite markers are being developed to answer further questions concerning the structuring in the wild population and to monitor variation in the captive colony.

Menkhorst, P. 1996. Wingspan . p.10

Kinship and alliance formation in male dolphins at Shark Bay, WA

M. Kruetzen[1], W. Sherwin[1], R. Connor[2], R. Smolker[2], N. Gales[3], R. Shepherd[4]

[1]University of New South Wales, School of Biological Science, Sydney 2052
[2]Museum of Zoology, University of Michigan, Ann Arbor, USA
[3]New Zealand Department of Conservation, Wellington
[4]Western Australia Department of Conservation and Land Management, Denham

Alliance formation for the purpose of competing with other members of the same social group is rare in mammals, especially males. An elaborate and extraordinarily well-documented case occurs in bottlenose dolphins in Shark Bay, WA, where males form two levels of alliances in order to control individual females. Evolutionary explanations of this behaviour depend critically on the genetic relatedness of cooperating males and their reproductive success, both of which will be investigated by molecular techniques in animals with known behaviour.

We present the results of cloning microsatellite markers from this species, and preliminary screening for variants.

Molecular and Ecological Studies of Family and Population Structure in the Platypus

Shiro Akiyama, Tom R. Grant*, Neil J. Gemmell, Jaclyn M. Watson, Jennifer A. Marshall Graves and Neil D. Murray

Department of Genetics and Human Variation, La Trobe University, Bundoora, VIC 3083

**School of Biological Science, University of New South Wales, Kensington, NSW 2033*

One of only three extant monotreme species of egg-laying mammals, the platypus (*Ornithorhynchus anatinus*), is uniquely Australian. It is widely distributed in the coastal rivers, creeks and lakes of eastern side of the Great Dividing Range from north of Cooktown to south of Tasmania. This species has been well studied anatomically because of its unique features and important position in mammal phylogeny. Its mating system, however, is entirely unknown because of its secretive life style (they are mainly nocturnal and spend most of their time for foraging under the water and resting in the underground burrow) and the difficulties of breeding in captivity.

To investigate the genetic relationships of such secretive animals, molecular genetic methods employing DNA analysis are very effective, and especially for population studies, microsatellite variation has been shown to be the most effective.

Several microsatellite loci have already been detected and sequenced and PCR primers have been constructed and used to identify several polymorphic microsatellite variants. From our microsatellite analysis data, heterozygosity differences and allele frequency differences were recognised between sexes in resident adults and among populations respectively. However, in the main breeding population, an allele not found among resident adults was detected in female juveniles which emerged in the same breeding year.

We therefore suggest the mating system in the platypus is more complicated than the simple polygyny which most platypus researchers expect.

GENETIC DIFFERENTIATION WITHIN AND AMONG SPECIES OF *EUASTACUS* (PARASTACIDAE: DECAPODA) FROM QUEENSLAND

Mark Ponniah and Jane Hughes

CRC for Tropical Rainforest Ecology and Management, Faculty of Environmental Sciences, Griffith University, Nathan, Queensland, 4111.

Euastacus Clark is a genus of endemic, freshwater crayfish. There are fifteen species described from Queensland. They occur on isolated, rainforested mountain tops along the east coast. Their distribution seems to be limited by temperature - the further south a species occurs, the lower altitude it occurs at.

This project is examining the phylogenetic relationships among species and the level of isolation of populations within species. Within species variation is being examined at two levels: 1) the differentiation between mountains, and 2) the differentiation between streams from different catchments on the same mountain. This poster presents preliminary allozyme results for within species variation for *E.robertsi* and *E.fleckeri.* Phylogenetic relationships among species, inferred from sequencing the 16s mtDNA region, will also be presented.

T6

Genetic variation and social structure in the quokka, *Setonix brachyurus* (Marsupialia: Macropodidae): two island populations.

Elizabeth A. Sinclair

Department of Zoology, University of Western Australia Nedlands W.A. 6907

Genetic differentiation among geographic populations depends upon physical or ecological barriers which may inhibit migration. Many studies have focused on the macrogeographic scale to identify significant heterogeneity within species. However, few if any studies of marsupials, have examined the microgeographic level, where behavioural traits such as territoriality and sedentary behaviour may also give rise to genetic subdivision. A number of previous studies have examined the social structure within *S. brachyurus* on Rottnest Island. Five relatively discrete populations were identified. These populations were further subdivided into local group territories of between 25-150 animals. A dominance hierarchy was observed within these groups, in which males were dominant to females and juveniles. No evidence of a dispersal phase in the young has ever been observed. It is proposed that if this structure is rigidly adhered to and there is little or no movement of animals between group territories and populations, then there should be observable genetic structure.

Allozyme electrophoresis was used to examine variation in *S. brachyurus* on Rottnest and Bald Islands. Only two presumptive loci were found to be polymorphic. Conflicting evidence was identified. There was little variation in the allele frequencies between four of the five populations on Rottnest Island. However, Bald Island animals showed significant structuring over distances smaller than those on Rottnest ($F_{st} = 0.364$). It is possible that this structure also exists on Rottnest but was not detected using the two loci. The identification of structuring on Bald Island indicates that microgeographic structure can occur. MtDNA techniques are being used to further examine population structure at this level.

P7

LOSS OF GENETIC DIVERSITY IN SMALL POPULATIONS OF THE GREATER STICK-NEST RAT (*Leporillus conditor*)

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The greater stick-nest rat is a native Australian rodent that diverged from true rats (members of the genus *Rattus*) around 25 million years ago. It has suffered severe population decline and range reduction since European settlement and until recently was present on only two islands linked by a sandbar at low tide (East and West Franklin Island). In 1988 action began to increase the species chance of survival. Stick-nest rats were taken into captivity and a breeding program was established. Several releases of captive bred rats have occurred, the first in 1990. Throughout the implementation of the recovery program small tissue samples for genetic analysis have been collected from many of the rats.

Current theory suggests that a significant amount of genetic diversity should be lost in small populations. This loss of diversity will be of two forms, a reduction in population heterozygosity, and a loss of rare alleles. Microsatellite markers represent an ideal method of monitoring such changes. They are highly variable, easily detectable and allow direct analysis of heterozygosity. However, the process of cloning and developing microsatellite markers for a species is both time consuming and expensive. For this reason we are developing microsatellite analysis based on commercially available rat (*Rattus norvegicus*) and mouse (*Mus musculus*) primers in the stick-nest rats.

P8

Microsatellite differentiation between Phillip Island and mainland Australian populations of the red fox *Vulpes vulpes*

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Predation by the red fox *Vulpes vulpes* is believed to be threatening the little penguin *Eudyptula minor* on Phillip Island in Victoria. Polymorphism at seven microsatellite loci was examined to estimate the extent of differentiation between Phillip Island and mainland populations of *V. vulpes*. Loss of alleles has occurred on Phillip Island where foxes first appeared approximately 88 years ago compared with mainland populations. Genetic differentiation between the Phillip Island and mainland populations was high. The relatively high differentiation found between the two populations could be due to either low migration rates, the effect of the composition of founder animals or both effects. Further ecological and historical information about the population is needed to explore the likely significance of these effects.

Stasis and Symmetry in a Magpie Hybrid zone.

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One of the most noticeable hybrid zones in southern Australia occurs between the Australian Black-backed Magpie (*Gymnorhina tibicen tibicen*) and the White-backed Magpie (*G.t. leuconota*). The white-backed magpie is found in Tasmania and Victoria while the black-backed magpie first appears in its pure form just north of Canberra. A hybrid zone between the two forms, extending between Cooma and Canberra was described by Burton and Martin (1975). In this region a range of intermediate forms exist, which can easily be identified and classified on a scale of 0 - 5 on the basis of appearance.

Hughes (1982) discussed the apparent asymmetry of the zone (based on back pattern) and put forward an hypothesis to explain this. Her hypothesis involves the presence of 2 genes, each of which has at least two forms (one for black, the other for white), of which black is dominant in each case.

We have collected back pattern data from a transect across the zone extending from Cooma to Canberra. A statistical analysis of these data along with transect data from previous years, including Burton and Martin's, indicates that the hybrid zone has remained stable for the last 25 years. Furthermore, the suggestion that the zone is asymmetrical is unsupported. This implies that the genetic basis for back pattern variation involves several loci with codominant alternative alleles.

A study of mating preference between the various forms suggests that the zone may be maintained through assortative mating.

P10

Towards conservation priorities for the endangered stream-dwelling frog*Taudactylus eungellensis* using mtDNA data from it and a secure species, *Litoria lesueri*.

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The behaviour of these stream dwelling frogs is little-known, except that they go to the water to breed between November and May. Of the six species of *Taudactylus*, three have gone "missing"; genetic data are sought to assess the degree of divergence between surviving populations as an aid to determining conservation priorities for them.

T. eungellensis from Eungella National Park, North Queensland, still has a few numbers in a few creek systems within the park. This species however was not seen for about 7 years, the numbers are only now starting to swell again. It is thought it went through a bottle neck, and genetic analysis could give evidence for this, also indicating whether the current populations have come from a single refuge or multiple refugia.

Mitochondrial variation will be surveyed using RFLPs and possibly COI gene sequence.The expected results would be that within the individual populations of *T.eungellensis* there would not be much variation (chi square, Fst) if the populations have been through a bottle neck. There would, however, be large variation among the seperate populations. The opposite story would be expected with the populations of the more common species, *L. leseueri*; that within the populations there would be much variation and among the seperate populations, possibly not much variation.

Dispersal and parentage in a NSW koala population.

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Throughout Australia koala populations are being fragmented due to loss of habitat. This fragmentation has lead to the isolation of local populations, making them vulnerable to extinction. To assess the risk of extinction, and hopefully prevent it, these isolated populations need to be properly managed. Management strategies require both ecological and genetic information. Habitat quality and utilization data are needed for short-term predictions of survival, whereas long-term predictions rely on genetic information such as the level of genetic variation and amount of gene flow within the population.

The aim of this study is to investigate the genetic population structure of a single free-ranging koala population in NSW. We have used hypervariable microsatellite markers to provide information on family relationships, levels of variation and the degree of dispersal within the population. Preliminary results are presented from the analysis of 250 samples, using six microsatellite loci.

This study complements ecological work done by others and forms the genetic component of a larger comprehensive project to assess the long-term viability of this koala population. The methods established will be applied to the management of other koala populations.

P12

Helping behaviour in the bell miner.

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The bell miner, *Manorina melanophrys*, is a cooperatively breeding honeyeater with a complex social system atypical of most cooperative breeders. Nestlings are fed by a number of helpers, some of which may be breeders themselves that are also provisioning their own offspring. A helper may provision young at several different nests belonging to different breeding pairs within its social group. We have developed microsatellites for the bell miner and these, together with behavioural observations of provisioning birds, provides an ideal system for evaluating two hypotheses proposed for this behaviour:

- kin selection: helpers are increasing their indirect fitness by helping to raise kin, or
- helping as an unselected byproduct of selection for effective parental behaviour.

Behavioural and genetic genealogies are also compared and do not always agree, indicating that the combination of molecular and behavioural data is essential to understanding both the evolution and maintenance of such extensive helping behaviour in the bell miner.

Factors Influencing the Rate of Molecular Evolution, and Compositional Bias in the Hymenoptera

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An increase in the rate of evolution and AT-content of hymenopteran mitochondrial genes has been previously reported (e.g. Crozier et al., 1989). My investigations indicated that both phenomena arose at the same time as the parasitic lifestyle (Dowton and Austin, 1995). Descendants of parasitic wasps have diverged much more (2-3 fold) from dipteran outgroups and have significantly higher AT-contents than wasps that diverged prior to the appearance of parasitism. However, the increased genetic diversity in the parasitic wasps may be related more to their capacity for adaptive radiation than to the parasitic lifestyle *per se*. They are the third-most speciose insect group (after the Lepidoptera and Diptera), and have invaded most orders of the Insecta, as well as some spiders. Further, the parasitic clade that demonstrates the greatest degree of adaptive radiation (the Chalcidoidea) also has the highest level of sequence divergence. The parthenogenetic mode of reproduction, together with a tendency to sib-mate, may mean that founder events occur relatively frequently in parasitic wasps, resulting in a high rate of molecular evolution.

The finding of an increased AT-content in the descendants of the parasitic wasps generally infers a strand-nonspecific compositional bias. However, phylogenetic mapping of individual nucleotide contents clearly indicated that fluctuations in AT-content have occurred entirely through H-strand-specific fluctuations in A-content; the T-content has not varied during the evolution of the Hymenoptera. Thus, the assessment of individual nucleotide contents, rather than pairs of nucleotides, may more accurately describe the precise variant of compositional bias.

Crozier, R.H., Y.C. Crozier, and A.G. Mackinlay. 1989. Mol. Biol. Evol. 6:399-411.

Dowton, M., and A.D. Austin. 1995. J. Mol. Evol. 41:958-965.

The Evolution of Parasitism in the Hymenoptera

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We present a synopsis of various investigations (e.g.Dowton and Austin, 1994) into the evolution of parasitism in the Hymenoptera, based on parsimony analysis of the 16S rRNA and cytochrome oxidase-I mitochondrial genes. Analysis of both genes confirms that the non-parasitic wasps (Symphyta) evolved prior to the parasitic wasps (Apocrita), and that ectoparasites evolved prior to endoparasites. However, contrary to anatomical phylogenies, molecular evidence suggests that the ectoparasitic lifestyle had at least two origins. Descendants of those groups close to the origin of the parasitic lifestyle are either wood-boring themselves or parasitize other wood-boring insects, suggesting that adaptations that accompanied the movement of wasps into a confined, wood-boring habitat may have pre-adapted them to becoming ectoparasitic.

More recently diverged parasitic wasps are predominantly endoparasitic, and have invaded almost every order of the Insecta, as well as some spiders. Character-mapping indicates that the endoparasitic lifestyle has evolved from ectoparasitic ancestors at least seven times. The social Hymenoptera (ants, bees) appear also to be derived from ectoparasitic ancestors.

Dowton, M., and A.D. Austin. 1994. Proc. Natl. Acad. Sci. USA 91:9911-9915.

P15

Reproductive dominance in honeybees, *Apis mellifera*, detected by microsatellite analysis

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Polyandry in honeybee queens leads to the presence of between 6 and 21 subfamilies among the workers of a colony. While all workers share the (diploid) queen as mother, workers which share a (haploid) drone father (super sisters) are three times more closely related to one another (r = 3/4) than workers which do not (half sisters; r = 1/4). When a queen dies, nurse bees choose worker eggs or young larvae to raise as new queens. According to kin selection theory, the disparity of relatedness among workers leads to the possibility of competition among subfamilies, potentially leading to some subfamilies being more successful at having their larvae raised as queens than other subfamilies. If some subfamilies are preferentially reared as queens at the expense of others (reproductive dominance), then this phenomenon may be the result of nepotism (bees acting to promote the welfare of supersisters at the expense of half sisters), or larval competition (worker larvae of some subfamilies being more attractive for rearing as queens to workers of all subfamilies). We dequeened three colonies, and used microsatellite markers to determine the subfamily of larvae chosen to be reared as queens. Subfamily relative frequency differed significantly between queens and workers in all three colonies (P = 0.024, P = 0.008, P = 0.001, G -tests of heterogeneity for hives 1-3 respectively), conclusively demonstrating selection of larvae for queen rearing is non-random in queenless honey bee colonies, and that the biases which occur have a genetic basis. These data finally provide unequivocal proof that reproductive dominance does indeed occur in honey bees.

P16

Identification of a major gene affecting heat hardening in *Drosophila melanogaster*

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The heat shock genes have been the focus of our search for determinants of heritable variation in heat resistance and acclimation ability in *Drosophila melanogaster*. Frequencies of alleles of the *hsr-omega* gene, which is constitutively expressed and induced by numerous stresses, were associated with increased knockdown heat resistance in replicate lines that were heat selected each generation after a mild hardening heat shock. This gene produces two RNA transcripts but no protein product. We examined the constitutive levels of the two transcripts, *omega-n* found in the nucleus and *omega-c* in the cytoplasm, in the heat resistant and control lines using competitive reverse transcriptase PCR (RT-PCR). Selection for resistance to knockdown by a 39°C heat stress after hardening by a mild heat shock (37°C) each generation involved a decrease in *omega-n* levels and a simultaneous **increase** in levels of *omega-c*, both relative to the control lines. In contrast, selection for resistance to knockdown by the same heat stress but without the mild hardening each generation involved no change to levels of *omega-n* and a **decrease** in *omega-c* levels. These distinct regulatory responses to the different selection regimes suggest divergent adaptive strategies. However, a large, similar, change in frequency of a length polymorphism detected in the *omega-c* transcript using RT-PCR, was a common response to both selection regimes. These *hsr-omega* structural and regulatory changes of considerable magnitude and high statistical significance argue for the central role of this gene in the mechanism of heat hardening.

Population genetic structure of *Sporobolus virginicus* (L.) Kunth. within a single saltmarsh.

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Sporobolus virginicus (L.) Kunth is a halophytic grass species with a wide series of polyploid races. One particular race is a major component of the saltmarsh flora in eastern Australia where it displays a wide ecological amplitude and is important for sediment stabilisation. Thirty-eight RAPD markers derived from four primers were used to investigate the population structure of *S. virginicus* in a single saltmarsh. Individuals were collected from six plant communities in order to assess population genetic structure in relation to habitat. Plant communities were ordered into a hierarchical scheme of zones for use with the Analysis of Molecular Variance (AMOVA). Overall, statistically significant genetic divergence was observed between plant communities but pairwise comparisons revealed that the differentiation was limited to the upper regions of the saltmarsh. The data is consistent the prediction that the lower marsh, as the site of colonisation, would not show significant genetic sub-structure. However, in contrast to this prediction, the magnitude of genetic diversity in upper marsh communities was similar to that in the lower marsh. When communities were grouped into zones no differentiation was observed between the upper, mid and lower marsh zones. A combination of secondary colonisation of the upper marsh, differences between the upper and lower marsh environments, and the use of phytosociological classifications as surrogate measures of environmental variation contribute to patterns of population structure in the species.

Does mutational meltdown threaten the survival of finite sexually reproducing populations?

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Lande (1994) and Lynch et al. (1995) have predicted that the accumulation of deleterious mutations constitutes a significant threat to the survival of finite sexually reproducing populations. Their models are both untested and controversial. The relevance of mutation accumulation in time spans appropriate for conservation management programs was tested empirically using populations of *Drosophila melanogaster* maintained at effective population sizes of 25, 50, 100, 250 and 500 for 45 generations and then pooled with other replicates of the same size. Their base population and a wild caught population were used as controls. The effects of accumulated mutations were observed as fitness differentials between inbred and outbred lines of these finite populations. Contrary to predictions, smaller populations did not suffer greater inbreeding depression than larger ones when measured under either benign captive or crowded competitive conditions. Inbreeding depression was not more severe in captive than in wild populations. Further, frequencies of mildly deleterious and lethal alleles on chromosome II exhibited no relationship with population size. The accumulation of detrimental mutations does not appear to pose a significant threat to the survival of finite sized sexual populations over time-scales of relevance to conservation programs.

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P19

Genetic Population Structure in the Catadromous Australian Barramundi, *Lates calcarifer* : evidence from mitochondrial DNA control region variation

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The Barramundi, (*Lates calcarifer*) a catadromous sequential hermaphrodite currently supports large commercial and recreational fisheries throughout Australia's North. Previous allozyme-based investigations of genetic population structure reported significant heterogeneity of allele frequencies among geographic locales - a marked contrast to other catadromous fishes. The pattern of differentiation displayed, interpreted as a stepping-stone model of genetic population structure, suggests greater gene flow between neighbouring estuaries than between more distant ones. One potential pitfall of using allele frequencies for gene flow estimation is the inability to separate the effects of historical and contemporary migration/isolating events. The employment of genealogical analyses in concert with frequency-based approaches can greatly assist in clarifying issues of past and present gene flow by adding an historical context to the data.

This study used an hierarchical sampling design and mtDNA control region variation (haplotype frequencies and genealogical analyses) to investigate the influence of historical geneflow and intermittent geological barriers on current-day *L.calcarifer* genetic population structure.

A total of 65 haplotypes were resolved from 270 individuals from 9 river systems within 3 geographic regions spanning the north Australian coastline. Control region haplotype frequencies differed significantly among sites within geographic regions and also among geographic regions. There was a large degree of both regional and site-specific haplotype endemism. An overall lower level of genetic diversity was detected in samples from the east coast of Australia. Relative gene flow estimates were lower among regions than among sites within regions. Preliminary genealogical analyses are also presented and discussed.

P20

Genetic diversity and population genetic structure of *Geleznowia verrucosa* (Rutaceae) - an endemic monotypic genus.

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Geleznowia verrucosa Turcz. (Rutaceae) is a woody shrub of significant horticultural value endemic to south west Australia. It is restricted to sand plain country north and east of Perth, occurring in small, disjunct populations. Despite being described as monotypic, the genus displays a great diversity in form with at least two distinct groups - a shorter small leaved, small flowered form and a larger, large leaved, large flowered form being separated by a somewhat intermediate group. While this variation may reflect ecological conditions due to the wide distribution, it is possible the groups are representative of different taxa.

Population genetic structure and patterns of differentiation between populations, based on 20 allozyme loci, were examined in 19 widely distributed populations representing the various morphological forms. Genetic diversity within populations was extremely low while differentiation between populations was unexpectedly high. In some populations additional isozyme loci were observed for three enzyme systems: aspartate aminotransferase, malate dehydrogenase and 6 phosphogluconate dehydrogenase. Chromosome studies showed that these populations were not polyploid suggesting that these additional loci may be due to gene duplication. Phylogenetic studies demonstrated the existence of three distinct groups which correspond reasonably well with a morphological analysis of the same populations.

PCR-BASED MICROSATELLITE ISOLATION USING 5'-ANCHORED PRIMERS

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Microsatellite loci are informative genetic markers because they are co-dominant, easily scored and highly polymorphic. Because of these attributes, microsatellites have become widely used for fingerprinting, linkage analysis, paternity analysis and population genetic studies. A major drawback of single locus microsatellite markers is the time and cost required to characterise each locus; a process typically involving library construction and screening, DNA sequencing, PCR primer design and PCR optimisation. To capture some of the polymorphism associated with microsatellite loci without the costs of single locus isolation, a range of DNA profiling techniques have been developed which target microsatellite regions in the genome. Some approaches use PCR primers containing microsatellite repeats at their 3' ends and several "anchoring" bases at the 5' end, allowing amplification across entire adjacent microsatellites. However the repeat length polymorphism of individual microsatellite loci is typically not assayed. The technique described in this poster produces such microsatellite-rich profiles which maintain their original repeat length polymorphisms. Cloning of these profiles yields a library highly enriched for single locus microsatellites which segregate normally when amplified using one locus-specific primer. This PCR-based enrichment procedure is easy and quick, and because only one specific primer is required for many loci, the cost of microsatellite characterisation is reduced considerably.

Genetic Variation and Origin of Siam Weed (*Chromolaena odorata*)

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Asynchronous flowering has been noted for *Chromolaena odorata* (L.) In northern Australia, raising questions concerning the genetic diversity and origin of this infestation. ITS1 sequencing data was obtained for *C. odorata* from North Queensland and overseas (Indonesia, Thailand, South Africa, Ivory Coast, Brazil, Columbia and USA), demonstrating that there are two distinct genotypes present in the Australian infestation. This distinction accounts for the different flowering times. The dominant genotype in North Queensland is widespread elsewhere, occurring in both the native origin and throughout the introduced range in Asia and Africa. The second genotype is only reported in southern Brazil and North Queensland. Brazil is the most likely source of the infestation in Australia.

Group I introns in 26S rRNA genes of *Gaeumannomyces graminis* as indicators of host range of *G. graminis* varieties.

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The 26S rRNA genes of *Gaeumannomyces graminis* exhibit length polymorphisms due to the presence of introns. Three group I introns have been discovered and their distribution correlates with the 3 varieties of *G. graminis.* No intron is found in var. *graminis* and *Phialophora sp.* (lobed hyphopodia) which are generally not pathogenic to wheat, oats and barley. *G.g.* var. *avenae* isolate, 91/56 and var. *tritici* isolate, 90/921 have a common intron (intron AT) and an unique intron each (intron A and intron T respectively). The 3 introns are inserted at different sites in the 26S rRNA genes. Intron T is self-splicing. The catalytic core elements and stem P8 of intron T are extremely similar to corresponding regions of the group I intron in the 26S rRNA gene of *Candida albicans.* Intron T has so far been found only in var. *tritici.* The sequence of intron A is more similar to the intron I of the 26S rRNA gene from human-derived *Pneumocystis carinii* isolate, Pc3 and of the 1 8S rRNA of *Ustilago maydis* than to the other 2 *G. graminis* group I introns. Intron A is found also in var. *tritici.* Intron AT has a unique sequence. *Rsa I* analysis gave no site differences between intron AT in var. *avenae* and var. *tritici.* However, *Msp I* analysis gave 2 restriction profiles, segregating intron AT into those of var. *avenae* and var. *tritici.* Each of the 3 introns appeared to have a different origin.

P24

The roles of phytochelatins and metallothioneins in heavy metal detoxification in the plant *Arabidopsis*

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In animals, metallothioneins (MTs), small MW cysteine-rich, gene-encoded, heavy metal-binding proteins are the major mechanism for the detoxification of heavy metals. In contrast, in response to heavy metal exposure plants express a class of cysteine-rich, heavy metal-binding peptides called phytochelatins (PCs) which, unlike MTs, are synthesized by an enzymatic process from the tripeptide, glutathione (GSH) and consist of repeated γ-glutamylcysteinyl groups with a C-terminal glycine [$(\gamma EC)_nG$]. In addition, plants also express MTs and Arabidopsis has at least five functional MT genes.

The cadmium-sensitive, *cad1-3*, mutant of *Arabidopsis* is deficient in PC biosynthesis and can be used to assess the role of PCs in the detoxification of various heavy metals. Our results show that PCs play a significant role in vivo in the detoxification of cadmium, arsenate and silver ions but only a minor role in the detoxification of mercury and copper but have no apparent role in the detoxification of nickel, selenite or zinc. Interestingly, many of these metal ions are effective inducers of PC biosynthesis and can be bound in vitro in PC-metal complexes.

To assess the role of MTs in heavy metal detoxification we have used transgenic lines expressing antisense copies of the MT1a and MT2a genes and which have the *cad1-3* mutation. This allows the effects of MTs on heavy metal detoxification to be studied in the absence of PCs.

P25

AINTEGUMENTA, AN *APETALA2*-LIKE GENE OF ARABIDOPSIS WITH PLEIOTROPIC ROLES IN OVULE DEVELOPMENT AND FLORAL ORGAN GROWTH

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To better understand the role of genes in controlling ovule development, a female-sterile mutant, *aintegumenta* (*ant*), was isolated from Arabidopsis. In ovules of this mutant, integuments do not develop and megasporogenesis is blocked at the tetrad stage. As a pleiotropic effect, narrower floral organs arise in reduced numbers. More complete loss of floral organs occurs when the *ant* mutant is combined with the floral homeotic mutant *apetala2*, suggesting that the two genes share functions in initiating floral organ development. The *ANT* gene was cloned by transposon tagging, and sequence analysis shows that it is a member of the *APETALA2*-like family of transcription factor genes. The expression pattern of *ANT* in floral and vegetative tissues indicates that it is involved not only in the initiation of integuments, but also in the initiation and early growth of all primordia, except for roots.

P26

Molecular Organisation of a Plant B Chromosome.

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Supernumerary B chromosomes are found in all major taxonomic groups of organisms, particularly plants. Although the B chromosomes significantly increase the nuclear DNA amount, no active genes have yet been unequivocally localised on these chromosomes by genetic or molecular means. The presence of B chromosomes is, however, often associated with changes in chromosome behaviour, such as meiotic pairing and recombination, and it is therefore thought that they play a significant biological role. The mechanisms which are responsible for these effects remain to be determined.

Brachycome dichromosomatica is an Australian native ephemeral plant of the arid regions of South Eastern Australia. This species contains only two pairs of chromosomes in the normal complement (A chromosomes) and 0 - 3 B chromosomes. A repetitious and tandemly repeated B chromosome specific sequence Bd49[1] is localised at the centromere of the B chromosome[2]. The presence of this sequence at the centromere may have some influence on the abnormal separation (nondisjunction) of B chromosomes which is observed at pollen grain mitosis in this, and many other, species and is thought to be responsible for the maintenance of B chromosomes in plant populations. Such drive mechanisms are thought to counteract the generally negative selection imposed by the presence of B chromosomes.

The localisation of rRNA genes to the secondary constriction of the B chromosome of *B. dichromosomatica* and apparent identity between these regions on the A and B chromosomes[3] suggested that the rDNA on the B chromosome has the potential to be an active NOR. An attempt was made to find differences in this region between A and B chromosomes that could be used to look for B chromosome specific rRNA transcripts. PCR was carried out using primers designed to amplify the internal transcribed spacer regions of the rRNA repeat unit from 0B genomic DNA and DNA from microdissected B chromosomes. Consistent sequence differences between A and B chromosome ITS2 regions were used to look for B specific rRNA transcripts.

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LOCALISATION OF THE PROTEIN PRODUCTS OF THE L6 GENE

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L^6 confers resistance, in a gene for gene dependent manner, to isolates of the flax rust fungus *Melarnpsora lini* possessing the avirulence gene AL^6. Analysis of L^6 message has established the existence of 2 transcripts which are differentially spliced with respect to intron 3. These transcripts predict two protein products of 1294 amino acids (intron 3 spliced) and 705 amino acids (intron 3 retained). Both proteins encode a putative signal peptide, however, their cellular location(s) are unknown. It is our aim to determine them.

We anticipate that native levels of L^6 protein are low. This assumption is consistent both with the low abundance of transcripts observed from the native gene and with its predicted function as a receptor for the product of AL^6.

To increase the likelihood of determining L^6 localisation, we require an *in vivo* system in which protein is maximally expressed. With a view to achieving this purpose, molecular characterisation was completed on six lines of tobacco independently transformed with a 35S L^6 cDNA construct. Intron 3 of the L^6 gene was retained in this cDNA, potentially enabling high level expression of the full length and truncated proteins.

By Southern analysis, it was established that all 6 lines contained at least one copy of the L^6 gene. Surprisingly however, during Northern analysis and subsequent RT-PCR analysis, no expression of the L^6 transcript was detectable in any of the transgenic lines.

We propose that introduction of functional L^6 into a heterologous system, lacking the appropriate regulation for its product(s), can result in the activation of a conserved resistance signal transduction pathway and therefore cell death. We are currently testing this hypothesis by transiently expressing L^6 in tobacco.

The search for a more suitable source of plant material in which to localise L^6 is ongoing.

ORGANISATION OF RIBOSOMAL DNA (rDNA) OF THE BLACKLEG FUNGUS (*LEPTOSPHAERIA MACULANS*).

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Leptosphaeria maculans is a haploid ascomycete which causes blackleg disease of oilseed Brassicas. Australian field isolates of *L. maculans* have large chromosomal length polymorphisms. In other fungi chromosomal length polymorphisms are often due to the presence or absence of ribosomal DNA (rDNA). Ribosomal DNA, in most organisms, comprises large blocks of tandem repeats of genes encoding the small subunit, the 5.8S subunit and the large subunit of the ribosome. These subunits are transcribed as a single operon, with two internal transcribed spacer (ITS) regions separating them. A non-transcribed intergenic spacer (IGS) separates each ribosomal repeat.

We have examined rDNA organisation in a tetrad (four sets of twins) of *L. maculans* where a chromosome varies in length by up to 50% depending on the presence or absence of rDNA. In one isolate of this tetrad rDNA is found on two chromosomes whilst in another it is present on only one chromosome. Using primers to conserved sequences we have amplified individual components of the rDNA repeat, including all subunits and the ITS and IGS. These results and Southern analyses have shown that, in contrast to other haploid fungi, there are several different sized rDNA repeats within a single isolate of *L. maculans* and this variation is found within the IGS region. We are examining the inheritance of the blocks of rDNA and of the different sized repeats in a cross between these isolates.

The *facB88* Translocation: A Fusion of Two Regulatory Genes Creating a Transcriptional Superactivator.

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The expression of the *amdS* (acetamidase) gene of *Aspergillus nidulans* is regulated by a number of transcription factors such as *facB*, which encodes a regulatory protein with a Zn(II)2Cys6 DNA binding domain. The *facB88* reciprocal translocation results in very high level, constitutive expression of *amdS*. The translocation breakpoints lie within the *facB* gene and a previously unidentified gene, designated *amdX*. This creates two fusion genes; *facB-amdX* contains the 5' half of *facB* fused to the 3' portion of *amdX* and *amdX-facB* contains the 5' portion of *amdX* fused to the 3' half of *facB*. Only *facB-amdX* is required for superactivation of *amdS*. The sequence of *facB-amdX* revealed that the DNA binding domain of *facB* is fused to another DNA binding motif, two C2H2 zinc fingers, encoded by *amdX*. *In vitro* DNA binding studies demonstrated that AmdX binds to sequences 5' of *amdS* that are known binding sites for the CreA and AmdA proteins. Mutational and transformation analysis of *facB-amdX* has shown that the FacB and AmdX DNA binding domains, and the FacB and AmdX binding sites 5' of *amdS* are essential for maximal function of *facB-amdX*. The native *amdX* gene was cloned and sequenced. Creation of an *amdX*$^-$ strain indicated that *amdX* is a non-essential gene. The phenotypes of this loss of function mutant and *amdX* multicopy strains are consistent with a minor positive role for native *amdX* in the regulation of *amdS* expression.

The *facB88* regulatory mutation provides a unique example of a mechanism for increasing gene expression. Analysis of *facB88* has also uncovered a new native regulator of *amdS* expression, *amdX*, which has not been identified in previous or subsequent mutant screens.

The Extracellular Proteases of *Aspergillus nidulans*.

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In response to growth conditions limiting for carbon, nitrogen, or sulphur *Aspergillus nidulans* secretes extracellular proteases, making this a useful model system for multi factorial gene regulation. The present study has used PAGE in conjunction with a variety of protease inhibitors to show that at least three different proteolytic enzymes are secreted. To date, two protease structural genes from *A. nidulans* have been cloned, *prtA* which encodes a serine protease and *prtB* which encodes an aspartic protease.

Transformation experiments were used to obtain *prtA* gene-replacement mutants in which *prtA* had been disrupted. Transformants were initially screened using PCR. Southern blot analysis was then used to confirm our results. Haploidisation analysis has been used to determine the chromosomal location of *prtA*. Plate tests and assays for protease production have been employed to analyse the phenotype of the *prtA*$^-$ transformants. No reduction in viability was observed, indicating that *prtA* is a non-essential gene. Protease production on plate tests did not appear to be affected in the *prtA*$^-$ transformants, but the protease activity detected in assays was reduced in comparison to wild type strains. The difference between the level of protease activity produced by the *prtA*$^-$ transformants compared to the level of protease activity produced by wild type strains decreased with prolonged exposure to nutrient limiting conditions. This result could suggest that prolonged starvation results in the production of additional proteases, or that accumulation of less abundant proteases occurs with time. Interestingly the *prtA* protein product is secreted (and active) at low pH (pH 4.5).

P31

Interspecies sequence and functional comparisons of the acetamidase gene in *Aspergillus* species and the discovery of an orthologous amidase.

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The acetamidase gene of *Aspergillus nidulans* (*amdS*) encodes an acetamidase enzyme involved in both carbon and nitrogen metabolism. The production of the acetamidase is regulated at the transcriptional level by at least seven independent regulatory proteins to control expression under a variety of substrate and inducer conditions. This elaborate display of regulatory control makes this system an excellent model for the study of gene regulation. Sequences with homology to the *amdS* gene in *A. nidulans* have been cloned and sequenced from a variety of *Aspergillus* species in an attempt to identify conserved promoter sequences to elucidate specific DNA binding sites for the known regulatory proteins and also to identify potential unknown binding sites conserved over evolutionary time. *Aspergillus* species with a close evolutionary relationship to *A. nidulans* have revealed blocks of similar promoter sequences indicating potentially important regulatory sequences, while more divergent *Aspergillus* species have revealed the existence of another amidase gene with altered substrate specificity and different regulatory control.

P32

Isolation and promoter analysis of acetamidase genes in *Aspergillus ustus*

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Evolutionary studies are powerful tools which can be used to identify functionally important sequences. Over time, random mutations build up within the genome of any organism. Changes within the DNA which are detrimental to that organism are likely to be lost through the process of selection, whereas beneficial mutations are likely to be retained and perhaps shared between closely related species. It has been noted that regions of DNA which are of functional significance such as DNA binding domains of proteins or areas of DNA to which regulatory proteins bind are often conserved accross species.

The acetamidase gene of *Aspergillus nidulans* (*amdS*) metabolises acetamide to acetate and ammonium which can act as a carbon and nitrogen source for the organism. Studies have shown that the expression of *amdS* is regulated by a variety of different regulatory systems. We are using the *amdS* gene to locate equivalent genes from other closely related *Aspergillus* subspecies, using homology to the original gene to locate candidate genes.

Specifically, *Aspergillus ustus* is being studied. Unlike most *Aspergillus* species, *A. ustus* grows well on acetamide. At this stage, it appears that there are more than one putative amidase genes present in this organism based on homologous probing to the *A. nidulans* gene. These genes will be sequenced to determine function and identify conserved sequences, especially within the promoter regions.

Characterisation of *creA* alleles in *Aspergillus nidulans*.

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Carbon catabolite repression is a regulatory process which acts to repress the expression of enzymes involved in the breakdown of alternative carbon sources in the presence of a readily metabolised carbon source, such as glucose. Genetical analysis has shown that the gene *creA* plays a central role in the process of carbon catabolite repression in *Aspergillus nidulans*. The dominance properties, derepressed phenotype and the non-hierarchical heterogeneity demonstrated by *creA* alleles suggest that this gene codes for a negatively acting repressor protein. The *creA* gene has been cloned. Theoretical translation of the DNA sequence identified a number of features, for example a Cys_2-His_2 DNA binding motif, consistent with CREA being a transcriptional repressor (Dowzer and Kelly, 1991). In addition, sequence comparisons between the *creA* genes from *A. nidulans* and *A. niger* have shown some highly conserved regions within the *creA* gene. One of these conserved sequences is a stretch of 42 amino acids which has a significant degree of similarity to a region found in RGR1 - a protein implicated in carbon catabolite repression in *Saccharomyces cerevisiae* (Drysdale *et al.* 1993).

Using a PCR-SSCP approach, sequencing and phenotypic testing we have analysed a large number of *creA* alleles. The characterisation of these alleles will be presented in this poster. The majority of alleles characterised have point mutations within the Cys_2-His_2 zinc finger binding domain or are the result of frameshift or nonsense mutations leading to various truncations of the CREA polypeptide. Two of the alleles studied result in severe truncation of the CREA polypeptide and are potential candidates as null alleles. Phenotypic analysis of these two alleles also supports this suggestion.

1. Dowzer, C. and Kelly, J. (1991) Mol. Cell. Biol. 11, 5701-5709.
2. Drysdale, M. *et al.* (1993) Gene 130, 241-245.

P34

The role of the *facC* gene of *Aspergillus nidulans* in acetate metabolism

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The study of acetate metabolism in fungi provides an elegant model for the coordinate regulation of multiple genes involved in a complex process. The *facC* gene of *Aspergillus nidulans* was first identified by recessive mutations that provided resistance to fluoroacetate toxicity, and resulted in loss of growth on acetate. The *facA* and *facB* genes, isolated in the same mutant screen, have been shown to encode acetyl-CoA synthetase and a positive acting transcriptional regulator of genes involved in acetate metabolism, respectively. Until this study, no function had been described for the *facC* gene.

The *facC* gene was cloned using a chromosome walking strategy, utilising a chromosome VIII specific cosmid library. A single cosmid was identified that complemented *facC* mutants at high frequency for growth on acetate. Subcloning and sequencing of this cosmid revealed an ORF in the region of complementation with a high level of similarity to several carnitine acetyltransferases, primarily the *Saccharomyces cerevisiae YCAT* gene.

By analogy to the situation in *S. cerevisiae* we propose that the carnitine acetyltransferase activity encoded for by *facC* is necessary for transportation of acetyl groups into the peroxisome and/or mitochondria. Hence, *facC* is necessary for growth on acetate, where the peroxisome utilises acetyl groups to form more complex carbon molecules via the glyoxylate bypass.

Work is in progress to test this hypothesis, including assaying carnitine acetyltransferase activity in *facC* mutants, and analysing the expression pattern of *facC* by Northern blot analysis.

CONVERSION EVENTS AT THE *AM* LOCUS IN *NEUROSPORA CRASSA* RARELY HAVE AN ASSOCIATED CROSSOVER.

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The incidence of crossing over between flanking markers during meiosis is enhanced when gene conversion is observed at an intervening locus. This is taken as evidence that gene conversion and crossing over are intimately associated. Estimates of the level of association between conversion and crossing over in Neurospora based on closely flanking genetic markers are similar to that for yeast (r ~ 0.33) (Perkins *et al* 1993). The Neurospora *am* locus is not extraordinary in this respect (r = 0.26).

We have used restriction site and sequence polymorphisms at the *am* locus to re-examine recombination events in Neurospora at a resolution higher than is possible using conventional genetic flanking markers. We find a much lower association of conversion and crossing over (r = 0.07). Only 7% of *am* conversions enjoy a crossover nearby (Bowring and Catcheside, 1996a).

The spectrum of recombination events at *am* is similar to that seen at a number of loci in yeast, suggesting that *am* is not atypical and adding weight to the argument that the level of association between conversion and crossing over may have been generally overestimated. While this could be explained by a mechanism that biases resolution of a recombination intermediate, common to both conversion and crossing over, in favour of preserving the parental combination of flanking markers, the pattern of segregation of molecular markers among *am* recombinants argues against such a bias (Bowring and Catcheside, 1996b). The existence of more than one pathway for meiotic recombination seems a more attractive proposition.

Bowring FJ and DEA Catcheside (1996a) Gene conversion alone accounts for more than 90% of recombination events at the *am* locus of *Neurospora crassa*. Genetics, *in press*.

Bowring FJ and DEA Catcheside (1996b) Physical analysis of recombination events at the *am* locus of *Neurospora crassa. Submitted.*

Perkins DD, R LANDE and FW STAHL (1993) Estimates of the proportion of recombination intermediates that are resolved with crossing over in *Neurospora crassa*. Genetics, **133:** 690-691

Functional dissection of transcriptional activation domains in *Aspergillus nidulans*.

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Transcriptional activation domains are subdivided into several broad classes based on amino acid composition of these regions. The most common classes of activation domains are the acidic-, glutamine- and proline-rich motifs. These activation motifs interact either directly with transcriptional machinery components (TFIIs) or indirectly with the transcriptional machinery via transcriptional activation factors (TAFs). Analysis of representatives of each of these classes has shown that all three major groups function in mammalian cells. At the other end of the spectrum, acidic activation domains function in the yeast S*accharomyces cerevisiae* while proline rich regions only function when the binding site of the DNA binding protein bearing this motif is close to the transcriptional start site. Glutamine rich activation domains appear to be totally non-functional in *S.cerevisiae*. Therefore there are clear differences in the transcriptional machinery components among organisms. Furthermore, essential determinants for a functional activation domain are obscure and recent studies have cast doubts about the classification of activation domains based on the predominant amino acid.

The *amdS* system of *Aspergillus nidulans* is a very well characterised regulatory system where the genetic controls and molecular mechanism are clearly understood. Multiple independent mechanisms control *amdS* expression and five of the regulatory genes have been cloned. Using this system and a number of approaches we have begun to identify and analyse transcriptional activation domains in regulatory factors controlling *amdS* expression. Firstly, using a series of gain of function mutations in the transcriptional activator gene *amdA* we have shown that these mutations map to the putative acidic activation domain. Interestingly a number of these do not increase the acidic nature of this region. However, two of the mutations do result in substitution of non acidic for acidic amino acids. Secondly, using a heterologous system, we have mapped regions capable of functioning as transcriptional activation domains in a number of other regulatory genes from *A. nidulans* controlling *amdS* expression. Thirdly, the capacity of the mammalian Sp1-derived glutamine-rich activation domain to activate transcription from the *amdS* promoter in *A. nidulans* was tested. These data will be discussed with respect to transcriptional activator requirements and organism specificity.

Transcriptional control elements of the yeast mid-sporulation gene *SPR3*.

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The *SPR3* gene encodes a sporulation specific homologue of the yeast CDC3/10/11/12 family of bud neck filament genes. *SPR3* is expressed specifically during meiosis and sporulation in *Saccharomyces cerevisiae*. Mutants homozygous for a disruption of *SPR3* sporulate but at a lower efficiency compared to wild type (Kao *et al* (1989), MGG 215:490-500). 5'-deletion analysis and site-directed mutagenesis of SPR3::lacZ fusion constructs has shown that transcription of *SPR3* is controlled by a complex interplay of repression and activation events: During non-sporulation conditions the gene is repressed and at the appropriate stage of sporulation the gene is activated. A 9 bp sequence (termed mid-sporulation element, MSE) was shown to be essential to the developemental regulation of *SPR3* and is the only element directly activating this gene at the correct time during sporulation. The MSE sequence has also been identified in a region associated with regulation of the sporulation-specific gene *SPS4* (Hepworth *et al* (1995), MCB 15:3934-3944). An ABF1 element directly upstream of the MSE has been shown to indirectly activate the *SPR3* gene as a result of repression of a putative distal repression element. Under vegetative conditions both the MSE and ABF1 regions are required for repression of *SPR3* expession. An association between MSE and ABF1 sites has been found upstream of a number of genes specifically activated during the middle stages of sporulation in *S. cerevisiae*.

Genetic Variation in *Aspergillus fumigatus*: Is Australia Home to a Unique New Species of *Aspergillus*?

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Aspergillus fumigatus is the organism most frequently associated with aspergillosis in humans, birds and other animals. Because of its role as an opportunistic human pathogen, a number of studies have sought to develop methods to type *A. fumigatus* strains for epidemiological analysis of aspergillosis outbreaks. To study aspergillosis in ostriches, we developed a method to detect DNA from *A. fumigatus* using PCR amplification of a fragment from the *A. fumigatus* alkaline protease gene. One isolate (NSW3) consistently failed to yield an amplification product with the first set of primers that was tested. The DNA sequences of 1.2 kb from the alkaline protease gene of NSW3 and one other ostrich isolate (QLD1) were determined. The results show that the sequence of the QLD1 alkaline protease gene is virtually identical to two published sequences from human clinical isolates obtained in the USA and France, but the sequence of NSW3 differs by 5.2% in the coding region and 12% in the non-coding region of the gene. Further evidence for a new species comes from a recent study of *A. fumigatus* strains from 6 continents, in which the isoenzyme, RAPD and RFLP pattern of one isolate from NSW differed so significantly from the remaining 61 isolates that the authors were prompted to propose that the strain may represent a new species[1].

[1]Rinyu, ER *et al.* (1995) J Clin Microbiol 33:2567-2575.

Characterisation of a novel cell cycle gene in *Drosophila melanogaster:* a role in chromosome condensation?

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The P element-induced *Drosophila* mutation, *l(2)3300,* exhibits a phenotype strikingly similar to mutations in the cyclin A gene, i.e. an apparent cell cycle arrest in G2 phase of embryonic cycle 16. Contrary to expectations, however, cyclins A and B were shown to accumulate and degrade as if the 16th mitosis had occurred, indicating that the cells were not in a true cell cycle-arrest state. We conclude, therefore, that the primary defect is in chromosome condensation. Using P insert plasmid rescue, genomic and cDNA clones covering the site of P element insert were isolated. Sequence analysis showed that the P element was inserted within the cDNA. The introduction of P transposase activity led to reversion of the mutant phenotype in association with loss of the P element, indicating that the P insertion was the cause of the mutant phenotype. We conclude that the cDNA identified corresponds to the *l(2)3300* gene. Sequence analysis revealed that this cDNA encodes a novel, highly basic protein. *In situ* hybridisation revealed that the product is expressed ubiquitously and not in a cell cycle fashion.

The lack of chromosome condensation in the mutant, the ubiquitous expression of the gene and the highly basic nature of the encoded protein suggest that *l(2)3300* may encode a ubiquitous chromosomal protein that is required for chromosome condensation. We are currently attempting to generate specific antibodies to test this hypothesis.

Subcellular localisation of the *Drosophila melanogaster stoned* gene products

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The *Drosophila melanogaster stoned (stn)* gene has a novel dicistronic structure, of two tandemly arranged open reading frames (ORF1 and 2). ORF1 and ORF2 are separated by 55 basepairs, which contain a total of five stop codons in all three reading frames. This intercistronic region has been sequenced from four independently isolated cDNA clones, as well as genomic clones and genomic PCR products. RT-PCR of this region gives a product of equal size to that obtained from cDNA[1].

Western blot analysis indicates that ORF1 and ORF2 encode two separate proteins, stnA and stnB respectively. Genetic interactions and sequence homology data suggest that the *stn* gene products may be involved in synaptic vesicle cycling in synaptic terminals. Western blots of synaptosomal preparations have localised both stnA and stnB to the synaptic fractions. Differential centrifugation has indicated that the stnA protein is most abundant in the plasma membrane fraction. However in the absence of Ca^{2+}, a portion stnA co-sediments with synaptic vesicle proteins, suggesting that stnA could be associated with these organelles. These data are consistant with the *stn* gene products being involved in a synaptic vesicle cycling pathway.

1. Andrew, J., Smith, M., Merakovsky, J., Coulson, M. and Kelly, L. (1996) *Genetics* (in press)

The Suppressor of Stoned.

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Viable *stoned* mutants of *Drosophila melanogaster* exhibit neurological abnormalities which have been attributed to defects in the synaptic vesicle recycling pathway, *stoned/shibire* double mutants being synthetic lethals. Complementation analysis of mutants (Petrovich et al., 1993) and antibody /antigen crossreactivity (Andrews et al., 1996) is consistent with the *stoned* locus encoding two proteins, Stoned A and Stoned B. The sub-cellular localization and function of these proteins is currently being investigated [1]. Temperature sensitive mutations in the open reading frame encoding the Stoned B protein are suppressed by the *Suppressor of Stoned* mutation, a dominant modifier that arose spontaneously in laboratory stocks (Petrovich et al.,1993). One hypothesis to explain this gain of function suppression is that the Suppressor of Stoned protein interacts with the Stoned B protein and that the changes in the double mutant allow more normal synaptic function .
Our overall aim is to clone the *Suppressor of Stoned* gene, determine its function and the nature of interaction between the Suppressor protein and the Stoned B protein. We are taking two approaches
(1) an analysis of clones (100kb of genomic DNA) mapped to the region of the Suppressor of Stoned locus
(2) the identification of the protein domain mutated in the *stoned* ^{ts2} mutant. This protein domain will then be used as "bait" to identify interacting proteins in both expression libraries and in fly protein extracts.
This report discusses the identification of the *stoned* ^{ts2} mutation using a combination of SSCP (single strand conformation polymorphism), PCR, and sequence analysis.

1. Smith and Kelly. This meeting.

Petrovich et al.,(1993) Genetics133,955-965.

Andrews et al.,(1996) Genetics (Accepted for publication)

A Search for genetic markers for resistance to *Trichostrongylus colubriformis* in sheep

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We are undertaking a large linkage study to identify chromosome segments that carry genes that affect the level of resistance of sheep to the parasitic intestinal worm *Trichostrongylus colubriformis*.

Almost one thousand lambs have been bred in six large half-sib families and then challenged with infective *T. colubriformis* larvae to determine their level of resistance to the parasite. The rams used to sire the half-sib families were bred by crossing sheep from the resistant and susceptible lines of the CSIRO *T. colubriformis* selection flock.

We selected the 20% of lambs with the highest level of parasite resistance and the 20% least resistant lambs for genotyping. The lambs were genotyped with 120 microsatellite markers, approximately half of which were developed in this laboratory, while the remainder were published markers selected from the sheep gene map.

We will present a summary of the results of this linkage analysis study.

A COMPARISON OF THE NON-CODING REGIONS OF THE FGF 1 GENE IN SUPER FINE WOOL MERINO AND BORDER LEICESTER SHEEP.

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In a previous study, a restriction fragment length variant was identified in sheep using an acidic fibroblast growth factor (FGF 1) clone as a labelled probe. This variant was found to be associated with finer wool fibre and lighter fleece weight in cross bred half sibling families of sheep. The flock was initiated by mating two super fine wool Merino rams with Border Leicester and strong wool Merino ewes. Five ram progeny were backcrossed to strong wool Merino ewes, creating five half - sibling families, each with over one hundred offspring. In an attempt to develop a PCR based test, for the detection of variation associated with reduced fibre diameter and lighter fleece weight, ovine FGF 1 cDNA was cloned and sequenced. However, no differences were detected between Border Leicester (strong wool) and super fine wool Merino sequences. Therefore, we believe that sequence polymorphism may actually be located outside the protein coding region. We report here the cloning of long PCR products and the comparison of the sequence of FGF 1 intron DNA between the Border Leicester and Merino breeds. These comparisons will be used to develop a PCR based test for selecting animals carrying fine wool FGF 1 variants.

PARENTAGE STUDIES IN SHEEP

B.L. Fricke[1], Y.M. Parsons[1], M. Fleet[2], I. Franklin[3] and D.W. Cooper[1].

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Sheep breeding programs call for accurate parentage records to be maintained. It is possible for this to be done on the sheep station itself, but this process is labour intensive and subject to error, especially if lambing is not closely monitored. Modern molecular genetic techniques makes DNA pedigreeing of sheep flocks a viable option. PCR based techniques and sheep gene mapping research has generated a large number of DNA markers that are highly polymorphic in sheep. A system whereby pedigree relationships within flocks can be de termined using microsatellites is now possible. As part of a research program to identify pigmentation loci in sheep a commercial based flock has been utilised. No pedigree data was recorded for this flock therefore parentage needs to be established before linkage analysis can be performed. Eleven microsatellite markers, located on ovine chromosomes 1, 3, 6, 13 and 26 have been utilised to genotype the pigmentation flock and computer based pedigree analysis has been carried out.

Replaying the evolutionary tape - the insecticide resistance story

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Stephen J. Gould asked whether the biological diversity that now surrounds us would be different 'if the tape were played twice?' His question elegantly encapsulates a major pursuit of evolutionary biology - to discover the extent to which options used in molecular evolution result from the channelling of variation by natural selection or from stochastic processes. For most experimental systems the notion of 'replaying the tape' represents pure fantasy. However with insecticide resistance it is possible to mimic the evolutionary processes of mutation and selection in the laboratory. We are using the organophosphorous insecticide (OP) resistance system to address the question of evolutionary flexibility, i.e. how many genetic, biochemical and molecular options does a species have in responding to changes in the environment, in coping with environmental stress?

In insects there are a number of candidate genes which could be mutated to provide OP resistance - carboxylesterase, acetylcholinesterase (AChE), glutathione-s-transferase (GST) and mixed function oxidase (MFO) encoding genes. To identify which of these options can be used, we have subjected susceptible strains of the Australian sheep blowfly, *Lucilia cuprina*, to EMS mutagenesis and selection with lethal doses of OP. Each of six mutations recovered are alleles of the field resistance gene, *Rop-1*. Indeed the amino acid replacements in the laboratory mutants are the same as those identified in field resistant strains. Thus, in 'rewinding the evolutionary tape', the same outcome has been repeatedly achieved. Factors which may act to limit evolutionary flexibility with repect to OP resistance will be discussed.

The sequence of the Acetylcholinesterase gene in the Australian sheep blowfly - consequences for insecticide resistance.

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Insect species utilise different mechanisms to adapt to the selective pressure imposed by the same insecticide. In some *Drosophila melanogaster (Dm)* and *Musca domestica (Md)* populations organophosphorous (OP) insecticide resistance is due to a change in substrate specificity of acetylcholinesterase (AChE), the molecular target of OPs. In *Lucilia cuprina (Lc)* field resistance is associated with mutations in the *Rop*-1 gene, which encodes a carboxylesterase E3. EMS mutagenesis and OP selection on suceptible strains of *Lc* in the laboratory produced six *Rop-1* mutants. No *Ace* mutants were recovered. One explanation for this result is that OP resistance cannot occur in a single mutational step via the *Lc Ace* gene due to differences in codon usage. This hypothesis was tested by sequencing the *Lc Ace* gene.

A 2651 bp *Lc Ace* cDNA was isolated. It conceptually encodes a protein of 617 amino acids. The *Lc* and *Dm Ace* genes share 83.7% overall amino acid identity, but there is a marked divergence in N- and C-terminals of the proteins. Sequence comparison revealed that each of the five point mutations associated with OP resistance in *Dm Ace* could occur in *Lc Ace* via a single nucleotide substitution. Other hypotheses framed to explain the preference for *Rop-1* mediated resistance in *Lc* are currently under investigation.

Gene structure of the *Notch* homologue from the Australian sheep blowfly.

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The lineages which gave rise to *Drosophila melanogaster* and the Australian sheep blowfly, *Lucilia cuprina*, diverged approximately 100 MY ago. Previous work has shown that the *Scalloped wings* (*Scl*) gene of *L. cuprina* is homologous to the *Notch* (*N*) gene of *D. melanogaster*. *Scl* and *N* mutants share phenotypic similarity for a number of traits including dominant wing notching and recessive embryonic lethality due to the abnormal expansion of the nervous system. In the coding region 65.1% of nucleotides and 73.7% of amino acids are identical in the *Scl* and *N* genes. The amino acid sequence identity varies from 25% in the signal peptide to 100% in the cdc/ankyrin repeats 2-6.

Here the analysis of the gene structure of *Scl* will be described. 2.5 kbp of DNA 5' to the transcriptional startpoint has been cloned and sequencing is in progress. Several of the introns have also been characterised. Intron-exon boundaries have been conserved between the *Scl* and *N* genes, however the *Scl* introns are in most cases larger. A polymorphism for the size of the C to C' intron has been detected with at least four alleles varying in size by 50-100bp. Sequence analysis and Southern blotting indicate that these differences are not due to repetitive DNA. Insertion/deletion polymorphisms have also been found in the OPA (glutamine) repeats. These polymorphims are being used to test the hypothesis that the asymmetry/fitness modifier, which arose in *L. cuprina* populations, is an allele of the *Scl* gene.

Predicting resistance and managing susceptibility to cyromazine in the Australian sheep blowfly, *Lucilia cuprina*

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Four cyromazine resistant variants of *Lucilia cuprina* were selected after ethyl methanesulfonate mutagenesis and screening above the concentration of cyromazine lethal to susceptibles. Resistance is controlled by a single gene in each variant. Two resistance loci have been identified, one (*Cyr 4*) closely linked to the marker *reduced eye* on chromosome IV, the other (*Cyr 5*) closely linked to the *stubby bristles* marker on chromosome V.

Concentration-mortality line analysis shows resistance ratios are low (1.5 - 3X). One variant [*Cyr 4(2)*] is viable as a homozygote, the others are lethal [*Cyr 4(1)*] or, at best sub-vital [*Cyr 5(1)* and *Cyr 5(2)*]. Competition experiments between resistant heterozygotes and susceptibles show that resistance to cyromazine is selected for over a limited range of concentrations.

The Isolation and Genetic Characterisation of Cyromazine Resistant Mutants of *Drosophila melanogaster*

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Cyromazine is an insecticide used in the control of a number of insect pests including the Australian Sheep Blowfly (*Lucilia cuprina*) and the housefly (*Musca domestica*). The nature of cyomazine's toxicity is unknown, as are the biochemical and molecular bases of potential cyromazine resistance mechanisms.

Drosophila melanogaster, a model organism for insecticide resistance studies, is being used to investigate the genetic and molecular bases of cyromazine resistance and mode of cyromazine action. Following a mutagenesis and selection strategy, a number of *D. melanogaster* strains resistant to cyromazine have been generated. Resistance in each strain is monogenic. At least three genetic loci that confer cyromazine resistance have been identified, with cyromazine resistance loci being mapped to each major *D. melanogaster* chromosome. Genetic and toxicological analysis of each mutant will be presented.

Cytogenetic mapping of cyromazine resistance genes is being conducted, as a precursor to their isolation via a positional cloning strategy.

P50

ISOLATION AND CHARACTERISATION OF REPETITIVE SEQUENCES SPECIFIC FOR THE ERIANTHUS GENUS (SACCHARINAE - ANDROPOGONEAE).

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As part of sugarcane breeding programs in Australia, introgression between *Saccharum* L. species and other related genera within the Saccharinae subtribe, such as *Erianthus* sect. *Ripidium* Henrard, is being attempted. *Erianthus* species, and *E. arundinaceus* (Retz.) Jeswiet in particular, represent a promising source of improved ratooning and vigour, drought and flooding tolerance, and *Pachymetra* resistance. Markers are needed to follow chromosomes during successive introgression generations. Specific repetitive sequences would be useful to tag specific or multiple chromosomes and to assess the occurrence of chromosome loss. Four repetitive sequences specific for the *Erianthus* genus were isolated from a genomic library of *E. arundinaceus*. The distribution of these sequences in a collection of 65 *Erianthus* individuals (9 species) was investigated. We demonstrated that these sequences are unique and correspond to non coding repetitive DNA. The chromosome location of these sequences using *in situ* hybridisation technology is being investigated. The usefulness of these sequences to follow introgression of *Erianthus* chromosomes in intergeneric hybrids is discussed.

The Homer transposable element of *Bactrocera tryoni* is a member of the hAT family of transposable elements.

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The Homer transposable element of *Bactrocera tryoni* (Queensland fruit-fly) is a member of the hAT family of transposable elements. Other members of this family include Hermes, from *M. domestica*, and hobo, from *D. melanogaster*. These elements have been successfully used as transformation vectors in *D. melanogaster* but most notably Hermes has been used to transform its host species species, the house fly, *M. domestica*.

Upon examination of the Qfly genome, using both PCR and Southern analysis, we have detected at least two classes of Homer element. 1.5kb regions of Homer1 and Homer2 were first isolated using degenerate primers designed to conserved regions within the large open reading frame of hAT elements. The Homer2 element was characterised in full using a composite of clones obtained by inverse PCR. Several overlapping clones show that Homer2 may be functional due to the presence of an long open reading frame which, when conceptually translated, appears to encode a hAT-like transposase. Homer2 has 12bp inverted terminal repeats and is ~3.8kb in length, although it appears there may be shorter copies of 3.2kb also present in the genome.

Work is now under way to investigate the potential of Homer2 for use as a gene vector in Qfly and other insect species by using transposition assays. Following on from this work it may be possible to design transformation vectors for a range of related tephritids, which are serious pests of the horticultural industry both domestically and overseas.

Polymorphic Markers for Ovine Cytokines Genes.

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Cytokines are a diverse group of proteins and polypeptides that, amongst other functions, are important in immune and inflammatory responses. Many cytokines have been postulated as candidates for mediating variation in the immune response to parasite infections. In order to examine potential disease linkage, polymorphic markers are required for the candidate genes. Previous work to identify microsatellites within ovine cytokine genes revealed only one diallelic microsatellite in intron 1 of the IL3 gene. Consequently, Single Strand Conformation Polymorphism (SSCP) techniques were employed to identify other useful markers. Oligonucleotide primers were designed to amplify various regions of the IL1A, IL3, IL4, IL5, IL6 and CSF2 genes via the PCR. The amplified products were subjected to a number of SSCP conditions to identify any polymorphism, and the PIC and position of the marker on the sheep linkage map were determined.

To date, SSCPs have been identified in the IL1A, IL3, IL4, IL5, and IL6 genes but not in the CSF2 gene. Linkage mapping has assigned IL1A to chromosome 3, IL6 to chromosome 4, and IL3 and IL5 to chromosome 5 in the sheep. In addition, linkage mapping revealed that a highly polymorphic marder, CSRD2138, is tightly linked to the IL3 and IL5 genes. As the SSCP markers for the IL3, IL4 and IL5 genes are not highly polymorphic, CSRD2138 appears to be the marker of choice for testing for associations between IL3, IL4, IL5 and CSF2 and resistance to parasites and other diseases in sheep.

P53

DNA SEQUENCE POLYMORPHISMS IN QUEENSLAND FRUIT FLY POPULATIONS

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The Queensland fruit fly, *Bactrocera tryoni*, is a major insect pest of commercial fruit crops in Australia. An understanding of the population biology of the species is critical to its control. A molecular analysis of the distribution of *B. tryoni* was carried out using restriction fragment length polymorphisms (RFLPs). Flies from seven locations (n = 294) were sorted into domains according to geographical area to ascertain if the population distribution was homogeneous or subdivided. Five polymerase chain reaction (PCR) targets for RFLP analysis were chosen to compare the level of polymorphism in the *white* gene with that in other genomic sequences and to analyse the distribution of polymorphic sites for flies from different geographic locations. A portion of exon 6 in the *white* gene has been previously shown to carry polymorphisms. Primer sets flanked three *white* gene regions and two randomly chosen genomic sequences. Direct sequencing was carried out in an attempt to confirm deduced restriction site maps.

The *white* gene and random genomic sequences showed similar levels of polymorphism with all five target regions containing at least one polymorphic site. The four different polymorphic sites found in the *white* gene were analysed as single loci and in a pair-wise fashion to test for population subdivision. Linkage disequilibrium was found between some pairs of loci and the amount of linkage disequilibrium was compared between different locations. No significant differences in the amount of disequilibrium between flies from different geographic locations were found. Hardy-Weinburg analyses for each sample from the seven locations revealed 4 samples not conforming to expectations. Various F-statistics analyses of RFLP allele frequencies did not reveal any population subdivision. It was concluded that the *B.tryoni* population is homogeneous, indicating a large amount of gene flow throughout the Australian distribution.

P54

The *white* gene of *Bactrocera tryoni* a marker for transformation in the *Bactrocera* genus

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The *white* gene was first isolated from *Drosophila melanogaster* and is thought to code for a transmembrane protein which is essential for tissue pigmentation. A 300bp PCR product containing putative *B. tryoni* exon 6 sequence was used to screen a *B. tryoni* genomic library and a lambda pGem clone ~ 15kb (w3°9.gem) was isolated. A 5.1kb *Xho*I subclone was sequenced and was found to contain exons 2 to 6 by sequence homology. Extensive nucleotide and amino acid identity was found between *B. tryoni and D. melanogaster*, *Lucilia cuprina*, *Ceratitus capitata* and *Anopheles gambiae*. Exon 1 could not be located in 7kb of sequence 5' to exon 2.

Using the technique of Rapid Amplification of cDNA Ends (RACE) with semi-nested primers, a *white* 5'cDNA fragment containing exons 1, 2 and part of 3 was generated, cloned and sequenced. Exon 1 is 604bp long with only 75bp of coding sequence. Several tests were then possible to locate exon 1 and the related promoter sequence in the genome.

Left/right primers were designed from exon 1 sequence to generate a 306bp fragment. PCR was successful on the 5'cDNA clone and *B. tryoni* gDNA but unsuccessful on w3°9.gem DNA. All attempts to PCR across intron 1 using a barrage of nested primers have failed. Subsequently we have found that intron 1 sequence just 3' to exon 1 has a complex repeat structure which may make long PCR difficult.

Inverted primer pairs were designed from the exon 1 sequence for inverse PCR. A genomic *Eco*R1 fragment of ~ 3kb was generated, subcloned and confirmed by sequence analysis. This clone has ~ 2.5kb of sequence 5' to exon 1 and 700bp of intron 1 sequence. This sequence will allow analysis of the promoter of the gene and should allow characterisation of intron 1. The size of intron 1 in the 3 species for which the genomic sequence has been isolated ranges from 3kb to only 4.5kb. This has led us to speculate that a lambda clone rearrangement may have occurred.

A *B. tryoni* mini *white* gene was generated with exons 1, 2 and 3 fused. From this clone, two constructs will be formed. One containing the native *B. tryoni* promoter, the other the *D. melanogaster* promoter to serve as a control in transformation experiments with eye colour recovery as the marker.

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P55

Gene delivery to insect embryos using electroporation and a slot cuvette

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Microinjection is the method used almost exclusively to deliver DNA constructs to insect embryos while electroporation is commonly used for DNA delivery to bacteria, cell cultures and certain plant tissues. This communication describes a method using an easily constructed slot cuvette and the electroporation technique for transfer of DNA to insect embryos for possible use in developing methods for germline transformation. This method eliminates time-consuming individual embryo manipulation and thus far has been found to be adaptable for use on several types of insect embryos. Using this method, we show successful transfer of plasmid DNA to embryos of the corn earworm moth, *Helicoverpa zea* and the house fly, *Musca domestica*. We also compare the relative efficiency of this method with that of embryo microinjection.

PCR amplification and direct DNA sequencing of DNA polymerase genes for inferring baculovirus phylogeny.

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Baculoviruses are a large family of viruses causing distinctive polyhedrosis and granulosis diseases affecting many different arthropods. Progress has already been made towards inferring phylogenetic relationships among these viruses using the DNA and polypeptide sequences of their occlusion proteins (known as polyhedrins and granulins), but these relatively small, highly conserved proteins yield only limited information on each taxon, and lack an outgroup. A different approach, using DNA polymerase gene sequences, is reported here. This large (≈ 3000 nt) gene is phylogenetically informative, and enables comparisons with other viral groups such entomopoxviruses. Sequence data on many baculovirus DNA polymerase genes (including six new taxa analysed in this study) have allowed us to design cross-reactive oligonucleotide primers suited to PCR-based amplification of the major part of the DNA polymerase gene of a wide range of new isolates. DNA is conveniently amplified directly from microlitre volume samples of infected larval cadavers. With appropriate cross-reactive primers we can rapidly obtain taxonomic data by determining genomic nucleotide sequences of new viruses directly from PCR products. From studies of DNA polymerase sequences we infer that the Lepidopteran baculoviruses diverged into two main lineages, and that the great extent of the sequence divergence between these two groups is consistent an origin in the mid-Tertiary period. Differences in trees based on polyhedrin as compared to DNA polymerase sequences suggests that the virus AcMNPV has exchanged its polyhedrin gene with a distantly related virus, emphasising the value of multiple genes for molecular phylogeny studies.

Identification of a linkage group with a major effect on resistance to *Bacillus thuringiensis* CryIAc endotoxin in the tobacco budworm *Heliothis virescens* (Lepidoptera: Noctuidae)

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A genetic analysis of resistance to *Bacillus thuringiensis* (Berliner) CryIAc toxin was conducted on the YHD2 strain of *Heliothis virescens* (F.). Resistance in this strain allows survival at high toxin doses that kill all susceptible individuals, and permits rapid growth at lower sublethal doses that inhibit growth of susceptibles. To quantify the number and relative effects of loci contributing to rapid growth on low doses of toxin, a genetic linkage analysis was conducted with markers on ten of the 31 chromosomes of *H. virescens* in a backcross design. Linkage Group 9 (marker locus *MPI*) contributed as much as 80% of the total resistance to CryIAc growth inhibition in YHD2. Recombination between the resistance locus or loci and the marker locus used to identify Linkage Group 9 occurred only when the informative hybrid parent was the father, which was expected because crossing-over does not occur in *Heliothis* females. Linkage Group 11 (marker locus *GDA*) made a smaller contribution to resistance that was only detectable when the effect of Linkage Group 9 was controlled for. Slight but significant differences between families still remained, above and beyond the effects of these two linkage groups, providing support for additional unlinked loci with a small effect on resistance. Measurements of the resistance levels conferred by a small number of genes with the largest effects may be useful in predicting the selection response of *H. virescens* in the field, following the release of transgenic cotton expressing *B. thuringiensis* toxins.

Behaviour of genetically engineered *Pseudomonas* biological control bacteria in small scale field release.

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The take-all fungal root rotting disease of wheat leads to a 10% loss of the annual Australian wheat crop, for which there are currently no effective control methods. *Pseudomonas* bacterial biological control protection against the take-all disease involves root colonization, exopolysacchardie production and specific anti-fungal metabolite (antibiotic) production by the bacteria, which have been shown to be the key components in take-all biological control protection (Nayudu *et al.* 1994). We have developed genetically engineered *Pseudomonas* bacterial strains which are more effective (in glasshouse trials) in take-all biological control. In Australia the release of any genetically engineered organism into the environment is only possible after satisfying the prerequisites of your local bio-safety Committee and the Genetic Monitoring Advisory Committee (GMAC) in Canberra. After consultation with GMAC we marked our *Pseudomonas* biological control strain AN5 with a luciferase marker (*lux*) which makes it glow in the dark. We showed this construct had no detrimental effect on a range of plant species in extended glasshouse trials. The process of application to GMAC for field release and the elaborate structure designed and built for release of this *lux* marked genetically engineered *Pseudomonas* bacteria in soil will be discussed. Studies relating to release of this *Pseudomonas lux* bacteria, monitoring it's spread in soil, competitive ability in mixed experiments with the parent strain and monitoring transfer of the transposon *lux* genes to other micro organisms will be presented. The safety and experimental aspects of field release of genetically engineered bacteria will be discussed.

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Characterisation of cryptic plasmids carrying *pco*-like copper resistance determinants in enteric isolates from pigs.

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Fecal swabs from pigs on farms of rural Victoria were plated on MacConkey agar and the enteric isolates purified. When plated on copper sulphate gradient agar plates, none of the new isolates demonstrated a copper resistant phenotype like the control *E.coli* strain carrying the plasmid pRJ1004 which encodes the previously characterised copper resistance determinant *pco*. They were phenotypically wild-type in their response to copper sulphate.

DNA hybridisation studies on the new isolates confirmed that they carried DNA with homolgy to *pco*.

Prolonged incubation of the new isolates at high density on nutrient agar containing a concentration of copper sulphate which is toxic to wild-type *E.coli* selected for copper resistant variants of the new isolates (10^{-8} to 10^{-9}) including *E.coli, Salmonella* and *Citrobacter* spp.

We believe a mutation in *pco* has altered the expression/regulation of the copper resistance phenotype. Genetic studies, PCR and sequencing are being performed to characterise these new isolates.

The miniaturized nuclear genome of a eukaryotic endosymbiont contains genes that overlap, genes that are cotranscribed, and the smallest known spliceosomal introns.

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Chlorarachniophyte algae contain a complex, multi-membraned chloroplast derived from the endosymbiosis of a eukaryotic alga. The vestigial nucleus of the endosymbiont, called the nucleomorph, contains only three small linear chromosomes with a haploid genome size of 380kb and is the smallest known eukaryotic genome. Nucleotide sequence data from a subtelomeric fragment of chromosome III were analyzed as a preliminary investigation of the coding capacity of this vestigial genome. Several housekeeping genes including U6 snRNA, ribosomal proteins S4 and S13, a core protein of the spliceosome (snRNP E), and a clpP-like protease were identified. Expression of these genes was confirmed by combinations of Northern blotting, *in situ* hybridization, immunocytochemistry, and cDNA analysis. The protein-encoding genes are typically eukaryotic in overall structure and their messenger RNAs are polyadenylated. A novel feature is the abundance of 18, 19- or 20-nucleotide introns; the smallest spliceosomal introns known. Two of the genes, U6 and S13, overlap whilst another two genes, snRNP E and clpP, are cotranscribed in a single mRNA. The overall gene organization is extraordinarily compact making the nucleomorph a unique model for eukaryotic genomics.

P61

cutE, the gene involved in divalent cations tolerance in *Escherichia coli*

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The copper-sensitive mutant of *Escherichia coli,* strain GME 135 *cutE*, was found to be additionally sensitive to zinc, cobalt, nickel and cadmium. A cloned 2.3 kb *EcoRI* fragment, carrying the wild-type gene, when transformed into *cutE* mutant cells complemented sensitivity to other divalent cations as well as copper sensitivity. The product of the *cutE* gene was identified using the T7 phage promoter expression system, as a polypeptide of about 50 kDa. This is consistent with the size predicted from the DNA sequence. This polypeptide was located in the bacterial inner membrane. Transcriptional fusions of *cutE* with the *lux* operon showed induction by copper, zinc, nickel, cobalt and cadmium and to a lesser degree by manganese and silver salts. Cells grown on elevated concentrations of these cations showed increased mRNA levels.

P62

Genetics in the Victorian Year 12 Biology Course

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The year 12 biology curriculum for Victorian schools contains a significant component of genetics. The content includes patterns of Mendelian inheritance; chromosomes, genes and their alleles; relationships between genotype and phenotype; DNA structure and function including the DNA code, its transcription and translation; genetic and environmental regulation of development; and aspects of genetic engineering including its application in a wide range of fields. Students are required to analyse natural selection and evolution in terms of the role that genetic events play in these two processes and examine social and ethical issues related to biotechnology and research associated with genetics and evolution. The unit also includes a practical component. Two of the three common assessment tasks (CATs) in year 12 biology deal with genetics. Each CAT forms one third of the assessment for the year. For CAT 2, each student must investigate an example of applied genetics and an issue arising from the selected practice. CAT 3 is an external examination of which the major part is genetics. So most students of year 12 biology spend more than fifty per cent of their time studying different aspects of genetics.

The poster will contain a more detailed statement of the genetics content in the unit of study. It will also compare that content with the genetics content in a selection of first year Victorian University biology courses. The poster will also contain sample text material to exemplify the level to which students study, a detailed statement of the assessment tasks, sample questions from the examination CAT and samples of student work.

CREATION OF A HIGHLY FECUND STRAIN OF HAIRLESS MICE

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Previous work has shown that the *hairless* mutation is caused by the integration of a retrovirus into the *hairless* locus on Chromosome 14. The mutation causes the hair cycle to stall at telogen, resulting in a hairless condition. All existing strains of *hairless* mice breed poorly and in some cases must be maintained using heterozygous females. Due to the importance of hairless mice in skin cancer research around the world, we set out to create a highly fecund strain of *hairless* mice, by backcrossing to the Inbred-Quackenbush Swiss (IQ) mice which have exceptional fecundity.

The breeding programme involved a cross between an SKH/HRA male and an IQ female with male progeny backcrossed to IQ females. From backcross 1 onwards, PCR genotyping was used to distinguish the hr/+ heterozygotes from homozygous normal mice. Primers are used which give a product of 411bp from the wild type allele, while other primers give a product of 409bp from the mutant allele. The susceptibility to skin cancer of these IQ/SKH is comparable to those of the original SKH mice. After ten generations of backcrossing, the new line will be essentially identical to the IQ line and inbred. An inter-se mating of the heterozygotes will be performed after ten generations, from which the hairless progeny will be used as a nucleus of congenic hairless IQ mice.

Mapping of DMP1 in sheep defines an inversion breakpoint with respect to human chromosome 4q.

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Comparison of the sheep map with maps of other species demonstrates that sheep chromosome 6 (OOV6) contains genes from a large portion of human chromosome 4 (HSA4) extending from 4p16 to 4q26. OOV6 has been shown to contain an inversion in which genes bounded by KIT (HSA4q12) and BMP3 (HSA4p14-q21) are inverted with respect to SPP1 (HSA4q11-q21). Genes distal to ANX5 on HSA4 map to OOV17.

The area around SPP1 is the focus of researchers mapping the human autosomal dominant disorder of dentin formation, dentinogenesis imperfecta type II (DGI1). A novel dentin-specific acidic phosphoprotein (DMP1) has recently been cloned and mapped to within 490kb of SPP1. A bovine cDNA for DMP1 was used to identify a 2 allele *Hin*dIII RFLP in sheep which was mapped in the AgResearch International Mapping Flock. DMP1 is located approximately 70cM from SPP1. These data confirm the large inversion on OOV6 and demonstrate that one breakpoint lies within the region of 500kb on the human genome bounded by SPP1 and DMP1. In mice, there is a breakpoint near SPP1 creating MMU5 and 3. Both DMP1 and SPP1 map to MMU5, indicating that this breakpoint is different to the breakpoint causing the inversion on OOV6. To date, DMP1 has not been mapped in any other species. Identification and characterisation of chromosomal breakpoints between species in this region may give us new insights into the evolution of mammalian genomes.

Use of Homologues of Human Batten's Disease Gene to Predict Protein Structure and Amino Acid Sites Important in Function

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Batten's disease is a human form of ceroid lipofuscinosis (CL), a lysosomal storage disorder that results in nerve degeneration. The yeast gene, YJL059w, is the homologue to the Batten's disease gene, CLN3. The highly significant probability for the alignment by BLAST is $3x10^{-70}$ and the protein products have 34% identity in amino acid residues and 47% similarity. The proteins are highly hydrophobic as might be expected for a protein interacting with the hydrophobic subunit 9 of mitochondrial ATP synthase which accumulates in the lysosomes in Batten's. The function of the protein is unknown. The hydrophobic regions are the most highly conserved, several with 80% identity. Chou-Fasman and GOR prediction of structure is mainly β-sheet with an α-helix in the middle and another towards the COOH end. The human protein has 2 large inserts at the NH2 end which are also predicted to form α-helix. It has been suggested that both the human and yeast proteins may be transmembrane (TM) proteins because they have several hydrophobic regions, however predictions of TM regions are usually reliable for water soluble proteins but falsely predict hydrophobic β-sheet to be TM. The PHDhtm on the Predict Protein Server at EMBL predicts many TM regions in this way. If the proteins are globular, PHDsec does predict fewer regions of β-sheet than CF or GOR.

We intend to disrupt the yeast gene to determine the phenotype of the null mutant in yeast. We are also cloning the CLN3 gene from dog and will include this in the alignment.

POLYMORPHISMS OF THE CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) GENE AND THEIR ASSOCIATION WITH LIPID PHENOTYPES IN GREEK AND ITALIAN MIGRANTS TO AUSTRALIA.

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In 1993 cardiovascular disease (CVD) was the major cause of death among Australians, accounting for 43.8% of all deaths. Coronary heart disease (CHD) was the major form of cardiovascular death accounting for 55.5% of such deaths and 24.5% of all deaths.

Many "modifiable" and "non-modifiable" risk factors have been identified and linked with CVD. The mechanisms by which modifiable risk factors, such as smoking, hypertension, obesity, diet, alcohol consumption, diabetes mellitus and a sedentary lifestyle, contribute to the development of CVD are generally well established and understood. The contribution of non-modifiable risk factors, such as age, race, sex, and family history, however are not as clear.

The aggregation of CVD in families has been well documented, and many studies have demonstrated the presence of a heritable component. Although many candidate genes for CVD have been identified, the exact role of many of these genes in the development of CVD is unclear.

This poster presents the results of an association study between lipid phenotypes and RFLPs in one of these candidate genes, the Cholesteryl Ester Transfer Protein (CETP) gene. The cholesteryl ester transfer protein is involved in reverse cholesterol transport and the eventual uptake of cholesteryl esters by the liver. This study, on two migrant populations, one Greek and one Italian, found a significant association between the *Taq*I B polymorphism and HDL-cholesterol levels in Greek males and females, and also between the *Taq*I A polymorphism and HDL-cholesterol levels in Greek females. Among Italians, with the sexes considered, either separately or combined, no such associations were detected.

Studies on the Menkes (MNK; ATP7A) P-type ATPase in copper-resistant CHO cells

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The Menkes (MNK; ATP7A) gene encodes a P-type ATPase which is involved in copper transport (1). Mutations in the MNK gene result in an X-linked disorder of copper transport in humans, Menkes disease. Patients with Menkes disease suffer from severe copper deficiency as a result of failure to absorb copper from the gut with copper accumulating in gut epithetial cells. Cultured fibroblasts from Menkes disease patients hyperaccumulate copper and are copper-sensitive (2).

Copper-resistant variants of cultured Chinese hamster ovary (CHO) cells were isolated by stepwise selection. These variants accumulate less copper than parental cells when incubated in copper-supplemented media. Kinetic studies using ^{64}Cu have demonstrated that the copper-resistant variants have enhanced copper efflux. As the copper-resistant variants have the opposite copper phenotype to cultured cells derived from Menkes disease patients, the organisation and expression of the MNK gene was investigated. It was found that the copper-resistant variants had increased levels of MNK mRNA and MNK protein (3). The MNK gene product was identified by immunodetection following SDS-PAGE, as a protein with an apparent molecular weight of 178 Kd. A correlation was observed between the level of copper-resistance, the extent of copper efflux and the levels of MNK mRNA and MNK protein, thus providing strong evidence that the MNK protein is involved in transmembrane copper efflux. This is consistent with the clinical findings in Menkes disease.

Southern blot and FISH analysis revealed that the molecular basis for overexpression of the MNK gene was amplification of this gene. MNK mRNA and MNK protein levels were not influenced by copper levels in the medium. However significant copper efflux was only observed when copper levels in the medium were high. This suggests that the MNK protein is activated by copper. The activity and regulation of the MNK copper pump has been investigated using everted vesicles isolated from the overexpressing cell lines. These studies have revealed that the MNK Cu pump depends on Cu^{+} (rather than Cu^{2+}), requires ATP, a protein gradient and critical sulfhydryl groups.

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Mutations in the 11s-Hydroxysteroid dehydrogenase Type II gene give rise to the syndrome of Apparent Mineralocorticoid excess.

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The enzyme 11s-hydroxysteroid dehydrogenase type II (11sHSD2) confers specificity on the non-selective renal mineralocorticoid receptor by inactivating glucocorticoids. The cloned human 11sHSD2 is a unidirectional enzyme, converting cortisol to the biologically inactive cortisone, and displays a low nanomolar Km for its substrate. Mutations in the HSD11B2 gene give rise to the syndrome of apparent mineralocorticoid excess (AME), a congenital condition characterized by sodium retention, low-renin hypertension, and often by growth retardation. The syndrome is inherited in an autosomally recessive manner and the predominance of some mutations in selected ethnic groups suggests founder effects in these populations. *In vitro* expression studies performed with mutants identified in AME patients revealed partially active and inactive 11sHSD2 enzymes, with some mutations resulting in unstable proteins. The enzyme has been localized immunohistochemically to the distal nephron and vascular smooth muscle cells, consistent with 11sHSD2 playing a role in the genesis of hypertension in AME. The localization of 11sHSD2 in mineralocorticoid target cells of sweat glands, salivary glands and colon suggests that a range of epithelia are affected in AME. It remains to be determined whether allelic variants of the HSD11B2 gene are associated with some forms of essential hypertension, and whether 11sHSD2 is involved in other conditions such as inflammatory bowel disease.

Absence Of Genetic Linkage Of Chromosome 5q31 With Bronchial Hyperreactivity And Atopy In The General Population

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We have demonstrated that the high affinity IgE receptor gene on chromosome 1lql3 is linked with bronchial hyperreactivity (BHR) but not atopy (van Herwerden *et al:* Lancet 1995;346:1262-1265). The chromosome 5q31 region has been suggested to be important in atopy and BHR (Postma *et al,* N Eng J Med 1995;333:894-900). We studied a polymorphic microsatellite marker in 5q31 (D5S399) in sibling pairs recruited from the general population who shared BHR and or atopy. DNA was isolated from peripheral blood leucocytes from 121 affected sibling pairs who had been characterised phenotypically with skin prick tests (atopy) and methacholine challenge tests (BHR). Genotyping for the D5S399 microsatellite marker was carried out by PCR using endlabelled primers with [^{32}P]-ATP. Our population displayed 13 different alleles, the most common being 117 (28%) and 127 (20%) basepairs in size. Based on this distribution we expected that sib-pairs chosen at random would share 1.24 alleles. In 103 sibling-pairs affected with atopy we observed 122 shared alleles, ie. an average of 1.18 shared allele per affected pair (P = 0.818). Concordance for BHR was found in 51 sibling-pairs in whom a total of 62 shared alleles were observed, giving an average of l.22 shared alleles per affected pair (P = 0.615). In an additional 14 pairs of siblings who did not share either atopy or BHR, we observed 18 shared alleles, an average of 1.29 alleles shared per pair. Therefore, there was no significant excess of shared alleles for D5S399 in sibling-pairs affected with atopy and or BHR. In our population sample which showed linkage between BHR and chromosome 1lql3, we could find no evidence of genetic linkage between D5S399 and either BHR or atopy.

A specific point mutation in an imperfect trinucleotide repeat region of the ARP gene in sporadic human renal cancer

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We have identified a human gene, called ARP, which encodes a previously unidentified arginine-rich protein that is 234 amino acids long. ARP maps to chromosome band 3p21, about 600 kb telomeric to the ACY1 locus. The entire gene is contained within 4 kb of genomic sequence; it has four exons130 to 471 bp in length and three introns 310 to 1000 bp in length. The ubiquitous transcript is 1 kb in size; the direction of transcription is toward the centromere. The gene is highly conserved at the DNA and RNA level, as shown by studies in human, hamster, rat, mouse, bovine, drosophila and budding yeast. We have detected a specific nucleotide substitution (ATG to AGG) in codon 50 or a deletion of the ATG at codon 50 in 10 of 21 sporadic renal cell carcinomas. These mutations remove a hydrophobic methionine from a hydrophilic region in which 15 of the 18 amino acids normally present are arginine residues. The mutations are not simply due to microsatellite instability in this imperfect trinucleotide repeat region, and the region is not polymorphic in the population. The wild-type allele is also present in the tumor cells, so the mutation in codon 50 may act as a dominant gain of function mutation, like the codon 12 or 61 mutations in the *ras* oncogene seen in many cancers.

P71

MHC Diversity and Disease Risk

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Low diversity at the major histocompatibility complex (MHC) has been suggested as a significant factor for disease risk to a population. MHC molecules are the central component of the vertebrate immune system and present self- and foreign-peptides to T-cells. Many species contain very high levels of MHC diversity, presumably the result of some type of balancing selection in response to parasite diversity. The argument is that a new and virulent parasite will be detected by some MHC alleles and not others, and as allele frequency is inversely proportional to diversity, then so will be the frequency of disease.

However, there is no empirical evidence to support the argument that there will be a strong correlation between MHC diversity and disease risk, and a substantial body of evidence to indicate that correlations will typically be weak and of limited importance to the conservation and management of vertebrate populations. Briefly, (i) the specificity of peptide presentation by MHC molecules is broad, (ii) correlations between MHC alleles and infectious disease are typically not evident, and at most are weak, (iii) the trans-species nature of MHC evolution is incompatible with selection by highly virulent, rapidly evolving, and often species-specific parasites, and (iv) the strength of selection on MHC genes is low.

The point is that historical processes of small effect over evolutionary time can result in a high level of genetic diversity. This diversity may have little relevance for future responses over ecological time.

P72

A Putative Human Ubiquitin Specific-Protease: HUBP

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Ubiquitin specific-proteases (Ubps) are thiol proteases which act on both ubiquitin-precursor molecules and ubiquitin-protein conjugates as part of the ubiquitin-dependent proteolytic pathway. The cDNA clones of a putative human Ubp was identified by screening DNA databases for similarity to two conserved sequence domains (containing conserved cysteine and histidine residues) characteristic of yeast Ubps.

Initially, Northern hybridisation analyses was conducted to confirm the human origin of the partial cDNA clone, HSAAADQEI, and identify the size of the full length clone. This however, produced unusual bands. Sequence analysis conducted concurrently not only confirmed the partial nature of HSAAADQEI but also demonstrated that this clone was actually two separate clones fused; HUBP (the ubiquitin-specific-protease clone) and HumTrip1 (from human thyroid receptor interactor mRNA). This dual nature of the HSAAADQEI clone explained the unexpected bands resulting from the Northern analysis.

The approx. 770bp HUBP clone (containing the complete cysteine-containing conserved Ubp domain common to most Ubps) was isolated from HSAAADQEI and used for subsequent experiments. Hybridisation analyses using HUBP as the probe confirmed its human origin and size, and indicated that multiple genes might exist in humans. Screening of a human testis cDNA library for the full length clone detected numerous positive clones. Sequencing and analysis of these clones is currently underway and already the sequence size of HUBP has been at least doubled. In addition, the histidine-containing conserved Ubp domain common to all functional Ubps has been identified as part of the new HUBP sequence. Most interesting however, is the strong identity shown between the HUBP sequence and the sequence of the human proto-oncogene TRE-2. This may have important implications for analysing the functions of the HUBP protein. Once full length copies of HUBP gene has been obtained, it is planned to characterise HUBP protein products and investigate its functional roles both *in vitro* and eventually *in vivo*.

Absence Of Mutations In The Coding Regions Of The High Affinity IgE Receptor Gene In Asthma

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We have reported linkage between the high affinity IgE receptor subunit (Fcε RIß) gene and asthma (van Herwerden *et al:* Lancet 1995;346:1262-1265). This linkage was explained by non-specific bronchial hyperreactivity (BHR) rather than atopy. It has been suggested that mutations in the Fcε RIß gene may alter the function of the receptor and predispose to asthma (Shirakawa *et al:* Nature Genet 1994;7:125-129). The aim of this study was to determine whether mutations in the exons of the Fcε RIß gene could explain the observed linkage with BHR. Relevant parts of the Fcε RIß gene were amplified using PCR with primers derived from the published gene sequence. Exons 1, 2 & 3 were amplified together on a 2.2 kb fragment. Exons 4, 5 and 6 were amplified on a 1.5 kb fragment and exon 7 was amplified separately. PCR products were purified and subjected to direct manual sequencing using end-labelled primers. From our original group of affected sibling pairs we sequenced all exons in 34 subjects with BHR alone, 2 subjects with BHR and atopy and 4 subjects with atopy alone. In none of the subjects did we detect any deviation from the published gene sequence in any of the exons. A polymorphism in intron III at position 4390 was detected but is of uncertain significance. Mutations in the promoter region of the Fcε RIß gene or in adjacent genes may explain the linkage between chromosome 1lql3 and BHR.

Expression of Mutated and Wild Type Dystrophin After Myoblast Transfer Therapy in the *mdx* Mouse

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Duchenne Muscular Dystrophy (DMD) is a debilitating disease which affects 1 in every 3500 male births. The underlying defect to the dystrophin gene at Xp21 results in the absence of the protein. The lack of this protein results in progressive degeneration of muscle resulting in death within the second decade of life. The *mdx* mouse model of DMD carries a point mutation within the dystrophin gene which introduces a premature stop codon again resulting in the absence of the protein. Much attention has focused on correcting the genetic defect in DMD through both cell and gene therapy. Transfer of normal myoblasts into *mdx* muscle has shown some early promise in increasing the level of dystrophin within diseased muscle. One potential limitation is the suspected loss of myoblast activity soon after transplantation. In order to overcome this and enhance the efficacy of myoblast transfer we have utilised the cytokine Leukaemia Inhibitory Factor (LIF). LIF has been shown to have a number of beneficial effects on skeletal muscle, including activation of myoblast proliferation *in vitro* and a direct beneficial effect on skeletal muscle regeneration *in vivo*. One can measure the efficacy of myoblast transfer by comparing ratios of wild type verses mutated dystrophin mRNA produced. The correction of dystrophin deficiency using myoblast transfer therapy in combination with LIF remains one of the more promising therapeutic alternatives. In addition to this an intricate understanding of the molecular mechanisms underlying the positive effect of LIF on myoblasts is essential, to this end the activation of immediate early growth response gene expression has also been studied.

IDENTICATION OF PC8 THE SEVENTH MEMBER OF THE CONVERTASE FAMILY

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A novel subtilisin-like protein, PC8, was identified by polymerase chain reaction using degenerate primers to conserved amino acid residues in the catalytic region of members of the prohormone convertase family. PC8 was predicted to be 785 residues and was structurally related to the mammalian convertases furin, PACE4, PC1 and PC2, sharing greater than 50% amino acid identity over the catalytic region with these family members. PC8 possessed the catalytically important Asp, His, Asn and Ser amino acids and the homo B domain of this family of enzymes. In addition, PC8 possessed a C-terminal hydrophobic sequence indicative of a transmembrane domain. Structurally, PC8 is more related to furin and PACE4 than PC1 or PC2. Furthermore, like furin and PACE4, PC8 mRNA of 4.5 kb was found to be ubiquitously expressed; this is in contrast with PC1 and PC2 which have a restricted distribution. A less abundant transcript of 3.5 kb was also detected in most tissues. Unlike furin and PACE4, both of which map to chromosome 15, PC8 maps to chromosome 11q23-11q24 suggesting that this gene may have resulted from an ancient gene duplication event from either furin or PACE4, or conversely that these genes arose from PC8.

Detection of antibodies reactive to the early endosomal marker (EEA1) in disparate autoimmune sera

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Autoimmune sera have been used as diagnostic markers to disease states and as probes for the molecular cloning of the reactive autoantigen (Tan, 1989; Yeo & Toh, 1994). We have used this approach in the molecular cloning and characterisation of a novel early endosomal autoantigen, EEA1 (Mu *et al.*, 1995). EEA1 is a 162 kDa coiled-coil protein localised to the cytoplasmic face of early endosomes and the cytosol. The C-terminal region of EEA1 contains a double zinc finger binding motif (FYVE finger) which is apparently required for the molecules specific localisation to early endosomes (Stenmark *et al.*, 1996).

Autoimmune serum reactive to EEA1 was initially observed in a patient presenting with sub-acute cutaneous lupus erythematosus (LE). We have investigated the occurrence of EEA1 reactive autoantibodies in a pool of over 30 sera referred for diagnostic detection of anti-nuclear antibodies (ANA). All sera investigated displayed cytoplasmic speckling when used in indirect immunofluorescence with commercially prepared HEp2 test slides. These sera were further characterised by their reactivity to HeLa cells and an EEA1 fusion protein. Co-localisation of sera reactive molecules with the transferrin receptor—a marker of early endosomes—was also investigated. We have identified four sera from clinically distinct patients containing autoantibodies reactive to EEA1.

Mu, FT. *et.al.J. Biol .Chem.* **270,**13503-13511.
Stenmark H. *et al.* (1996) *J. Biol. Chem.* Submitted
Tan, EM.(1989) *Advances in Immunology* **44**,93-151.
Yeo, J-P. and Toh, B-H., (1994) *Autoimmunity* **18**, 291-300.

Quantitating the number of *mdr-1* molecules per cell using competitive PCR

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Competitive RT-PCR involves the co amplification of a target gene and internal standard (competitor) resulting in competition for PCR primers and amplification. The amplification efficiency remains constant for the target and competitor molecules during cycling, because they share the same primer sequence. Hence absolute quantitation of the products can be achieved by comparing the amount of target gene, relative to the standard and the data need not be reliant to the plateau phase of amplification. Heterologous competitors are constructed to differ from the target sequence only in size, demonstrated by gel electrophoresis. Using low stringency PCR to promote spurious product amplification, we constructed heterologous competitors for human multidrug resistance (*mdr-1*) gene and as a internal reference the ubiquitously expressed human histone variant 3.3 (H3.3) gene. We have verified that the competitor molecules are of consistent size and the amplification efficiencies of the target (t) and competitor (c) molecules do not alter at different stages of PCR. This has been demonstrated for individual competitors and when competitors are co amplified with the target genes. The ratio (t/c) remains constant at every stage of amplification and does not alter when the reaction is well into plateau phase. Amplification efficiencies are independent of template concentrations and do not shift under changing DNA levels. We have used competitive PCR to quantitate mdr-1 gene levels in drug sensitive (*mdr-1*-) CEM-CCRF cell line, drug resistant (*mdr-1*+) CEM-A7+ cell line and in a number of patients with B-cell chronic lymphocytic leukaemia. The greatest advantage of competitive RT-PCR is its sensitivity and hence the ability for fine resolution from finite biological material.

Is the reported sequence of RMSA-1 a pseudo gene?

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Previously we reported the availability of an autoimmune serum from a patient with discoid lupus erythematosus which is reactive to the chromatin of dividing cells, but non-reactive to the chromatin of cells in interphase (Yeo *et al.*, 1994). This serum immunoblots a protein of apparent molecular weight 49 kDa in dividing cells. The 49 kDa protein is thought to be required for the assembly/stabilisation of the microtubules which form the mitotic spindle in dividing cells. The autoimmune serum was used to screen a human hepatoma Hep-G2 cDNA expression library. We have reported the sequence of a clone designated RMSA-1 (after regulator of mitotic spindle assembly-1) which is reactive to the autoimmune serum (Yeo *et al.*, 1994). However, this clone contains two almost complete Alu repeats arranged in tandem, contributing to over 40% of the reported ORF sequence (open reading frame; Margalit *et al.*, 1994; Tugendreich *et al.*, 1994). Although Alu sequences have been previously reported in coding regions of mRNA (Makalowski, 1994), the substantial contribution the Alu sequences make to the reported ORF of RMSA-1 has raised the question of whether or not the reported RMSA-1 cDNA is in fact a pseudo gene (Tugendreich *et al.*, 1994).

Here we report on the relationship of ESTs (expressed sequence tag) yi80b01 (http://genome.wust1.edu/est/esthmpg.html), humgso01269 (NCB1 sequence ID: 501392) and three additional cDNA clones we have identified while screening a HeLa cDNA library with a DNA probe corresponding to the 5' end of the reported RMSA-1 ORF to the previously reported RMSA-1 cDNA clone.

Makalowski, W. (1994) *TIG* **10**, 188-193.

Margalit, H. *et al.* (1994) *Cell (letter to editor)* **78**, 173-174.

Tugendreich, S. *et al.* (1994) *Nature (Scientific correspondance)* **370**, 106.

Yeo, J-P. *et al.* (1994) *Nature* **367**, 288-291.

Characterisation of rat testis antigens reactive to serum from a patient with discoid lupus erythematosus (DLE)

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Autoantibodies are useful diagnostic markers for autoimmune disease and as probes for the molecular cloning and characterisation of the pertinent antigens. It is often the case that these autoimmune sera recognise evolutionary conserved functional domains of the autoantigens (Tan, 1989; Yeo & Toh, 1994).

Serum collected from patient (ES) with DLE is reactive by immunofluorescence to the condensed chromatin of dividing cells, but not to the chromatin of cells in interphase. By immunoblotting ES serum appears to react with a single antigen of 49 kDa (p49) in protein extracts of mitotically active cultured cells (Yeo *et al.*, 1994a). Similarly, by immunofluorescence ES serum is reactive to the chromatin of meiotically dividing cells (in prophase I-anaphase I and prophase II-anaphase II), however, in the case of invertebrate cells, the serum appears to react with a single antigen of 31 kDa (Yeo *et al.*, 1994b).

We have begun to investigate antigens reactive to ES serum in the rat testis. This organ is not only highly mitotic, but also highly meiotic. As expected, by immunofluorescence ES serum is reactive to the chromatin of the mitotically active spermatogonia and the meiotically dividing spermatocytes found in the tubules of the testis. We have characterised the ES-reactive antigens by their molecular weight, and distribution in whole testis, specific cell types, and subcellular fractions by immunoblotting. The rat testis ES reactive protein(s) has a molecular weight of 49 kDa, is predominantly localised in the cytosol, and surprisingly, was found to be particularly abundant in mature spermatozoa tail. In addition, we have been able to demonstrate that the ES serum reactive protein can be pelleted with microtubules stabilised with taxol, and released in the presence of either ATP or GTP. We are currently screening a testis λgt 11 expression library with ES serum to facilitate the molecular cloning of the rat p49 cDNA.

Tan, EM. (1989) *Advances in Immunology* **44**, 93-151.
Yeo, J-P. & Toh B-H. (1994) *Autoimmunity* **18**, 291-300.
Yeo, J-P., Alderuccio, F. and Toh, B-H. (1994a) *Nature* **367**, 228-291.
Yeo, J-P., Forer, A. and Toh, B-H. (1994b) *J Cell Sci.* **107**, 1845-1851.

P80

Preliminary identification and characterisation of nervous system autoantigens reactive to a serum collected from a patient with discoid lupus erythematosus

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Autoimmune sera have proven to be useful as diagnostic markers for certain disease states and as probes for the characterisation and molecular cloning of the pertinent autoantigens (Tan,1989; Yeo & Toh, 1994).

Using a serum collected from a patient with discoid lupus erythematosus, we have previously identified an autoantigen, of apparent molecular weight 49kDa, present on the chromatin of dividing cells, but not detected on the chromatin of non-dividing cells (Yeo, *et al.*, 1994). We have hypothesised that this autoantigen, RMSA-1 (after Regulator of Mitotic Spindle Assembly-1), maybe involved in the assembly/stabilisation of microtubules which from the mitotic and meiotic spindle of dividing cells.

We have investigated the distribution of the RMSA-1 autoantigen by immunoblotting and immunofluorescence in tissues collected from a model animal—the mouse (Gilitis,1995). As expected RMSA-1 was most abundant in tissues with high mitotic/meiotic indices. Surprisingly, however, the brain was found to be reactive to the autoimmune sera by immunoblotting, but not by immunofluorescence. In the mouse brain proteins of apparent molecular weights: 80, 70, 49 (RMSA-1?) and 33 kDa were all found to be reactive to the autoimmune serum. The brain is an organ which is generally considered to be non-mitotic. We have investigated the relationship of the 49 kDa brain autoantigen to the 49 kDa RMSA-1 autoantigen. In addition, we have began to characterise the other autoantigens present in the nervous system which are reactive to the autoimmune serum.

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Tan, EM. (1989) *Advanc. Immunol.* 44, 93-151.

Yeo, J-P. & Toh, B-H (1994) *Autoimmunity* **18**, 291-300.

Yeo, J-P, *et al.*, (1994) *Nature* **367**, 288-291.

MITOCHONDRIAL DNA DELETION DETECTION AND QUANTIFICATION IN CARDIOMYOPATHIC HEARTS.

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Mitochondria contain numerous copies of their own 16.5kb circular genomes with copy number varying from 5-1000 depending on cell type. Deletions of the mitochondrial DNA have been implicated in mitochondrial dysfunction leading to the pathogenesis of ageing and certain mitochondrially mediated diseases. Mitochondrial dysfunction has also been implicated as a factor involved in cardiomyopathy, although its role is unclear. Detection and quantification of point mutations and deletions can be complicated due to intra and inter-mitochondrial heteroplasmy. We analysed cardiomyopathic heart tissue by PCR to detect mitochondrial DNA deletions present in low amounts and southern blot analysis for the detection of deletions present at high levels. The PCR results revealed the presence of a commonly found 5kb deletion in most samples with some individuals also having additional larger deletions of the mitochondrial genome. Quantification of these deletions by kinetic PCR revealed that deletion levels varied between individuals and in most cases were not detectable by southern blot analysis. Mitochondrial DNA deletions may contribute to a decline in respiratory activity as observed in the individuals in this study.

POPULATION STUDIES AND FORENSIC APPLICATION OF THE CTT MULTIPLEX STR SYSTEM.

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Forensic scientists are moving away from using blood, biochemical, RFLP, and VNTR-PCR markers to using STR-PCR markers for generating profiles of individuals. Short Tandem Repeats (STRs) have several advantages including; ability to compare typings from a range of biological materials, able to type minute amounts (1-5 ng) and low quality DNA, greater resistance to environmental factors, genetics and typings are distinct, greater discrimination power (DP), and provide the opportunity to be grouped into multiplexes. The use of multiplexes, as opposed to series of monoplexes, can provide a high DP in a more time efficient manner and will also further reduce the amount of template DNA required.

A multiplex system that has recently become available, which includes three loci; CSF1PO (5q33.5-34), TPOX (2p23-2pter) and THO1 (11p15.5), is currently being tested for use by VFSC and has a matching probability of approximately 1 in 500 to 1 in 1500 depending on the population.

Before a new genetic marker can be used in forensic casework appropriate population studies need to be conducted. These include: determining the allele and genotype frequencies, H-W equilibrium, comparisons with other populations, and independence of the new locus from other loci being used to establish whether the multiplication rule can be applied.

A survey of published population databases reveals that there are limited allele frequency differences among diverse ethnic populations for the loci CSF1PO and TPOX. For THO1 there is limited variation among numerous Caucasian populations, but significant allele frequency differences between each of the following major ethnic groups; Caucasian, Asian, American Blacks, Hispanics and Greenland Eskimos.

Appropriate Victorian population databases are in the process of being generated for analysis.

POPULATION STUDIES OF A SEVEN LOCI MULTIPLEX PCR SYSTEM.

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A new multiplex PCR system, developed by the Forensic Science Service in the United Kingdom, allows for coamplification and typing of six STR loci; HUMVWA (chr 12p), HUMTHO1 (chr 11p), D6S502 (chr 6), HUMFGA (chr 4q), D21S11 (chr 21), D18S51 (chr 18q) and the sex determining marker Ameloginin. This system has a Match Probability value (PM) of approximately 1.2×10^{-8} - 1.0×10^{-9}.

Population studies are essential before any new locus can be introduced into forensic casework. Several population databases are available for loci HUMTHO1 and HUMVWA. They reveal limited variation among numerous Caucasian populations, but significant allele frequency differences among major ethnic groups. Only a few databases are available for D6S502, D18S51, D21S11 and HUMFGA. The limited data indicate some significant allele frequency differences between distinct ethnic groups.

We are currently in the process of generating appropriate Victorian population databases for analysis of; allele frequencies, genotype frequencies, Hardy Weinberg equilibrium, differences among populations and independence of these loci from each other and other loci already used for routine forensic casework.

LOH AT 9p21 CENTROMERIC TO CDKN2a: ANOTHER TSG?

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Frequent observations of deletions from chromosome 9p in many tumour types strongly suggests the presence of a tumour suppressor gene (TSG) at this location. We have studied non-small cell lung cancer (NSCLC) and found the most frequent cytogenetic abnormality in NSCLC was a deletion of chromosome 9p. By further molecular studies of NSCLC and MM cell lines, we have since more precisely defined the minimum common region of homozygous deletion to 9p21.

A candidate TSG CDKN2a has been identified at 9p21. CDKN2a encodes the $p16^{INK4}$ protein which acts as an inhibitor to the cell cycle complex cyclin D/cdk-4. CDKN2a was initially found to be deleted in many different types of tumour cell lines. However, a high incidence of homozygously deleted or mutated CDKN2a in fresh tumour tissue, as opposed to cell lines, has so far only been reported in melanoma, esophageal carcinoma and pancreatic carcinoma.

In order to investigate the possibility of a second TSG locus at chromosome 9p21 we have studied 32 primary lung tumours for loss of heterozygosity (LOH) in this region. We have used the technique of microdissection to acquire relatively pure samples of tumour and normal lung cells and performed PCR directly on lyzed cells. Primers to 12 microsatellite markers and gene loci spanning the region 9p13 to 9p23 have been used to screen for LOH. Our results indicate a common region of loss more centromeric to the CDKN2a locus. Loss of one or more alleles was found in 18-47% of informative cases at markers within 9p21 but more centromeric to CDKN2a, while LOH at this gene was found to be only 7.5%. We propose that there is a second TSG proximal to the CDKN2a locus which may play a more important role in the tumourigenesis of NSCLC.

PPD11 BANDING: SIMPLY BRILLIANT CHROMOSOMAL IDENTIFICATION

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DAPI staining is usually employed to identify chromosomes for fluorescence *in situ* hybridization (FISH) and primed *in situ* synthesis (PRINS) experiments. Unfortunately DAPI bands are rather vague and require UV excitation. The most common fluor used to label DNA, FITC, must be viewed under blue excitation using propidium iodide (PI) counter-staining. An alternative to viewing bands and signal separately is provided by PPD11 banding (Lemieux et al., 1992), which only requires 10 minutes more time than solid staining with PI. PPD11 bands are so clear that the method is applicable to routine karyotypic analysis, as will be demonstrated for marsupial and eutherian chromosomes.

For PPD11 banding, the chromosomes must be labelled with 5-bromodeoxyuridine (5-BrdU). Pulse-labelling in early S-phase produces G-banding (admixed with about 10-30% R-banding) and involves rinsing the cells. Alternatively, pure R-banding may be easily produced by continuously labelling with low levels of 5-BrdU without rinsing the cells.

PPD11 banding of 5-BrdU labelled chromosomes simply involves staining slides with approximately 5µg/ml propidium iodide in PBS for 5 minutes and rinsing 3 times with PBS. A coverslip is then mounted with 1.0 mg/ml para-phenylenediamine in 90% glycerol/10% PBS at pH 11.0. The bands produced are very bright red under green excitation and suitably subdued for FISH or PRINS under blue and UV excitations. PPD11 banding will work with very old or fresh slides.

Examples demonstrating the use of the PPD11 banding in FISH will include localization of transgenes in sheep, satellite repetitive DNA sequences in cattle and homologues of *Drosophila* genes in humans.

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MALE-SPECIFIC EXPRESSION OF *cSOX9* IN CHICKEN SEX DETERMINATION

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SOX9 has been implicated in male sex determination since the discovery that *SOX-9* mutations result in campomelic dysplasia, a bone dysmorphology and XY sex reversal syndrome. We have investigated expression of *cSOX9* during chicken gonadogenesis to establish whether *SOX9* is likely to have a conserved role in vertebrate sex determination.

Using whole-mount in situ hybridization, *cSOX9* expression was detected in male (ZZ), but not female (ZW), chicken embryos over the sex-determining period. *cSOX9* expression was also observed in both sexes in the skeletal system and in the collecting ducts of the metanephric kidney. Southern analysis was performed to determine whether *cSOX9* is sex-linked. This experiment suggested that *cSOX9* is not located on either the Z or W chromosome. However, the timing and localization of *cSOX9* expression in the developing genital ridge suggests that *SOX9* has a conserved function in vertebrate gonadogenesis.

Localisation of the sex reversing *SOX9* gene to the 2q region of the Tammar Wallaby *(Macropus eugenii).*

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Mutations in *SOX9* have been found to cause a skeletal dysmorphology syndrome known as Campomelic Dysplasia as well as male to female sex reversal in humans, suggesting that it is another gene in the sex determining pathway. *SOX9* (SRY-like HMG box containing) gene is found to map in humans at 17q24. Its HMG domain i s highly conserved between species and shares 71% amino acid similarity with that of *SRY,* the testis determining gene.

It has been suggested that *SOX9* may originally have been a sex determining gene on the ancestral sex chromosome and may still be located on the X-chromosome in metatherians. In order to investigate this possibility, Tammar Wallaby SOX9-homologous clones were isolated by screening a Tammar genomic library and mapped by fluorescence *in situ* hybridisation to Tammar metaphase spreads. One of the clones was partially sequenced and was found to encode an amino acid sequence that is 100% identical to the corresponding region of human S0X9. This *SOX9* clone was mapped to the q region of Tammar chromosome 2 by fluorescence *in situ* hybridisation, next to other HSA 17 genes.

The autosomal location of metatherian S0X9 makes it unlikely that S0X9 was part of the original dose-dependent sex determination system, although it does not rule out a direct role in mammalian sex determination.

P88

Mapping of human Xq13 genes in marsupials: Implications for X chromosome inactivation.

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X chromosome inactivation involves stable and somatically heritable repression of thousands of physically linked genes on a single chromosome. It is an important example of large scale transcriptional control, the molecular basis of which is not yet understood. In eutherian mammals this involves the random selection of one X chromosome to remain inactive and the initiation and spreading in *cis* of inactivation on any additional X chromosomes. There is evidence from translocations that XCI is initiated at a unique controlling locus at Xq13 from which inactivation spreads, and a gene *XIST* has been cloned from this region and shown by gene targeting to be essential for the initiation of X chromosome inactivation.

Marsupial (metatherian) X chromosome inactivation is similar to that observed in eutherians but is distinguished by the non random selection of paternally derived X chromosomes for inactivation, incompleteness, tissue specificity and the apparent absence of CpG island methylation. In order to clone a marsupial homologue of the *XIST* gene and to determine if the molecular basis of X inactivation is the same in both eutherian and metatherian mammals genes from within the region demonstrated to contain the eutherian X inactivation centre have been mapped in Tammar Wallaby. This work has determined that genes from this region are conserved on the marsupial X chromosome and the conservation of this region, and its implications for various models of X inactivation will be discussed.

Human DYZ1 Y-specific sequences are detected by PCR on the marsupial Y chromosome

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The long arm of the human Y chromosome contains a heterochromatic region composed largely of the DYZ1 3.4kb Y-specific repeated family, and this tandem array of pentanucleotides varies in length from 800 to 5000 copies within the normal male population. PCR amplification studies have shown that the base sequence corresponding to the human DYZ1 repeat is present in cattle. pigs, rats, and mice. We have used PCR analysis to detect human DYZ1 related sequences in marsupials and monotremes. Male-specific fragments were observed against a background of shared fragments (presumably representing related satellite DNA) in the marsupials *Sminthopsis macroura* stripe-faced dunnart) and *Macropus eugenii* (tammar wallaby), but not in the monotremes *Tachyglossus aculeatus* (echidna) and *Ornithorhyncus anatinus* platypus). Hybridization studies using cloned male-specific PCR products from both *S. macroura* and *M. eugenii* reveal male-specific banding patterns in the marsupial source and in at least one other marsupial species, confirming that this sequence is conserved on the Y chromosome in marsupials. Hybridization intensities suggest that the DYZ1 repeat sequence is not highly repeated on the marsupial Y chromosome, which is characterized by a paucity of heterochromatin. We suggest that DYZ1-related sequences have a conserved male-specific function in therian mammals, and that while amplification of these sequences has occurred in several eutherian species, it is absent or minimal in marsupials.

Enrichment of a marsupial testis cDNA library for male-specific sequences by subtractive hybridization

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Subtractive hybridization is a technique used to isolate target sequences that differ in abundance/presence between two similiar nucleic acid populations. The technique exploits the differential annealing rates that occur under hybridization conditions in which driver DNA (depleted in target sequences) is in excess over tester DNA (enriched in target sequences).

The dasyurid marsupial *Sminthopsis macroura* has a tiny Y chromosome which appears to contain at least some genes in common with the human and mouse Y chromosome, and thus presents itself as an ideal model species for the study of Y-specific expressed sequences. In order to isolate male-specific sequences from this marsupial, subtractive hybridization was carried out using vector primer PCR-amplified *S. macroura* testis cDNA sequences as the tester nucleic acid, and direct incorporation biotinylated DOP (degenerate oligonucleotide primer) PCR-amplified genomic DNA from female *S. macroura* as the driver nucleic acid. Double-stranded female-female/male-female and single-stranded female DNA were removed by biotin-avidin magnetic bead separation, and the remaining testis cDNA sequences were amplified in successive rounds of PCR using internal vector primers. PCR analysis of the enriched *S. macroura* cDNA library has shown it to be positive for Y-specific SRY sequences, but negative for autosomal ZFXY sequences. The enriched cDNA library was ^{32}P-labelled by direct incorporation PCR, and hybridization of this population of sequences to male and female *S. macroura* genomic DNA southern blots reveals an at least two-fold enrichment of the library for male-specific sequences.

Are Candidate Human Spermatogenesis Genes Conserved on the Y in Marsupials?

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In man and mouse, the Y chromosome contains spermatogenesis genes that are independent of the sex-determining function of *SRY*. In humans, deletions of part or all of the long arm of the human Y chromosome are associated with azoospermia. Deletion analysis has mapped the putative *AZF* (azoospermia factor) gene at Yq 11.23. Two putative candidate *AZF* genes have been identified which map to this region, the *RBM* (RNA binding motif) gene family and *DAZ* (deleted in azoospermia). Both these Y-specific candidates are expressed only in adult testis tissue, and encode different RNA-binding proteins. As the function of these genes in human spermatogenesis is unknown, conservation of sequence, location on the Y, and testis-specific expression in more distantly related mammals would be powerful evidence for a conserved function in mammalian spermatogenesis.

Southern analysis in marsupials using human *RBM* and *DAZ* cDNA clones as probes at low stringency shows that *RBM* sequences are male-specific in marsupials whereas *DAZ* sequences appear to be autosomal. Sequence analysis and RT-PCR has confirmed the identity and testis-specific expression of *RBM* sequences, and fluorescent in situ hybridisation has confirmed their location on the Y. Thus the *RBM* gene has been conserved on the mammalian Y chromosome for at least 150 MYr, and may prove to be a more promising candidate for a crucial role in spermatogenesis than the *DAZ* gene, whose role in the spermatogenic pathway may have been more recently acquired.

Sex determination and differentiation in intersexual marsupials.

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In marsupials, sexually dimorphic structures, such as the mammaries and scrotum, begin to differentiate before the gonads are morphologically distinguishable on the basis of sex. Development of the scrotum in eutherians, unlike its counterpart in marsupials, relies on secretion of androgens from the testes. Gonadal sex in all mammals is determined by the Y chromosome. Both marsupial and eutherian mammals have an XX/XY female/male sex chromosome dimorphism. We describe an intersexual agile wallaby (*Macropus agilis*) whose sex chromosome constitution is XXY in lymphocytes and cultured fibroblasts. This animal has a penis, a pouch and four teats. We also describe an intersexual eastern grey kangaroo (*Macropus giganteus*) with a small empty scrotum and no penis, and an abnormal red kangaroo (*Macropus rufus*) with no penis, pouch or teats. Both the grey and red kangaroos had XX sex chromosome complements. The SRY gene was investigated in these animals. SRY has been shown to be testis determining in mice and humans. We conclude that the agile wallaby and the grey kangaroo described in this paper provide further evidence that scrotal development in marsupials is Y chromosome independent. More detailed molecular analysis is required before the cause of abnormalities in the XX individuals presented here can be ascertained. This analysis awaits identification of the genes involved in pouch, mammary or scrotal development in marsupials.

Isolation and Characterisation of the sex reversing *DAX*-1 gene in marsupials: Implications for the evolution of sex determination.

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A number of cases of XY female sex reversal were found in association with a duplication of the short arm of their X chromosome. A gene within the duplicated region was proposed to override *SRY* and cause female development when present in two copies. A candidate gene *DAX*-1 was cloned from the duplicated X-linked region and was found to be very conserved across a number of animal groups.

The conservation of *DAX*-1 and its proposed dosage dependant sex determining effect suggested that this gene could represent an ancestral X borne sex determining gene that worked, not by a dominant effect (as does modern *SRY*) but by gene dosage. Dosage sensitive sex determination (DSS) has been proposed to be the method of sex determination in the first mammals, before the evolution of *SRY* and complete X inactivation. Comparative mapping of mammalian X chromosomes has shown that the marsupial X represents the original mammalian X shared by all mammals. If *DAX*-1 were the ancestral DSS mechanism it should lie on the X chromosome in marsupials as well as eutherians.

We have cloned the *DAX*-1 homologue in the tammar wallaby, and isolated a large λ clone. This clone was then mapped to tammar wallaby chromosome spreads by FISH. The *DAX*-1 gene was not on the X or Y, but clearly localised to chromosome 5p, next to *ZFX/Y* and *DMD* (its neighbours on the human X chromosome). The autosomal location of *DAX*-1 in marsupials would imply that it was autosomal in the common ancestor to all mammals and not X linked. The autosomal location of the *DAX*-1 gene in the common ancestor suggests it was not the ancestral DSS mechanism.

P94

Multiple Copies of *SRY* in a Dasyurid Marsupial

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Male sexual development in humans and other mammals is triggered by the determination of testis, which is controlled by a testis determining factor (TDF) on the Y chromsome, *SRY*[1]. Present as a single exon in all eutherians and most marsupials, *SRY* is assumed to share a sex determining role although it is poorly conserved between species[2]. *SRY* expression in marsupials has been shown to follow the same expression pattern as in humans and presumably shares the same control signals[3]. We have found that the *SRY* gene is present in three distinct copies, characterized by distinct sequence, in the dasyurid marsupial, *Sminthopsis crassicaudata*, only one of which is transcibed in adult testis. Since the closely related species *Sminthopsis macroura* has only a single copy of this gene, these multiple copies must have appeared very recently. This unique system provides the opportunity to identify the promoter region of *SRY* and its binding activity through comparisons of regions upstream of the transcription start site between transcribed and silent copies.

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Transposable Element Evolution in Rock Wallabies (*Petrogale*)

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Rock wallabies (*Petrogale*), represented by 20 distinct chromosome races comprised of 15 species (and 6 subspecies)[1], have undergone a recent radiation, probably due to rapid chromosomal rearrangements. Chromosomal changes have been shown to occur most readily in sympatric populations and those in multiple zones of parapatry, indicating a correlation between these rearrangements and reproductive isolation[1]. Such chromosomal rearrangements and their contribution to postmating isolation have been linked to transposable elements in other organisms studied in isolation, such as *Drosophila* [2,3], yet any such correlation has yet to be identified in natural populations. Here we examine the frequency and distribution of several endogenous mammalian retroviruses found in *Petrogale* for any correlation between chromosomal rearrangements, rapid speciation and reproductive isolation.

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P96

ANALYSIS OF MATRILINES IN EUROS USING MITOCHONDRIAL DNA

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The euro *(Macropus rohMsfus erubescens)* is a species of macropods well-adapted to the semi-arid and arid regions of Australia. Euros are sedentary animals with small, stable home ranges. Recent studies of the euro population at Fowlers Gap Research Station found evidence of female philopatry. These studies suggested that the occurrence of female philopatry, and the sedentary nature of euros might lead to the formation of geographically localised matrilines.

The mitochondrial d-loop was used as genetic marker for identifying matrilines. Polymorphic restriction enzymes were used to characterise mtDNA genotypes in members of the euro population at Fowlers Gap. This mtDNA marker system was checked with known mother-offspring pairs. The amount of genetic variation within the euro population was estimated, and compared with values observed for other species.

The main aim of this study was to investigate the possibility that euros show geographically localised matrilines. Using the Mantel test, geographic distance between centres of female home ranges, and percent home range overlap between females, were tested separately for correlation with genotypes of the animals. The Nearest Neighbour test was carried out to determine whether animals of the same genotype are more likely to be each other's nearest neighbour than random expectations. All analyses showed that there is no significant correlation between the spatial distribution of the animals and their genotypes.

Genetic data at present does not support the hypothesis that geographically localised matrilines exist in the euro population at Fowlers Gap. Possible reasons for not detecting these matrilines are discussed.

Updating the sheep genetic linkage map.

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We have been taking two approaches to increase the density of markers on the ovine linkage map. Firstly, new sheep microsatellite markers have been developed by screening ovine genomic and cDNA libraries with a radio-labelled (CA)n probe. Positive clones have been subcloned, sequenced and oligonucleotide primers designed for the regions flanking the microsatellites. Secondly, a number of published bovine microsatellite primers have been obtained and PCR conditions adjusted so that the primers amplify the homologous ovine microsatellite. Ovine chromosomal map positions and PICs have been determined for both types of markers.

Mapping homologous loci for the bovine and ovine genomes also enables us to make comparisons between their genetic maps and thus examine what chromosomal similarities and rearrangements exist between the two species.

The increasing number of markers being placed on the sheep and other genetic linkage maps, makes it necessary to develop facilities to disseminate this information in a timely fashion. The ease of access to the World Wide Web makes it an excellent medium for presentation of this information. We have set up a web page detailing the markers that have been mapped by the Centre for Animal Biotechnology and other sheep gene mapping groups (http://rubens.its.unimelb.edu.au/~jillm/jill.htm). Marker details include chromosomal positions, primer sequences, polymorphism information, and PCR conditions. Additionally, the site details chromosomal positions of homologous loci that have been mapped in a variety of other species.

Isolation and preliminary characterisation of the Leukaemia Inhibitory Factor (*LIF*) gene in the marsupial *Sminthopsis crassicaudata*

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Leukaemia Inhibitory Factor (LIF) is a polyfunctional cytokine that has diverse effects *in vitro* on the growth and differentiation of various cell types, ranging from the induction of monocytic differentiation in mouse leukaemic cell lines, to the suppression of differentiation of totipotent embryonic stem cells. Studies of *LIF* gene expression during the early stages of mouse embryogenesis indicate that *LIF* is synthesised in extra-embryonic tissue, acts on embryonic tissue, and possibly plays a role in implantation. The feto-maternal relationships in eutherians and marsupials are very different and some species of marsupial exhibit embryonic diapause. Clearly, a study of *LIF* gene expression during development in marsupials will be of great interest.

A sequence encoding *LIF* (referred to as *sLIF*) has been isolated from genomic DNA library of *S. crassicaudata* by screening with mouse and human *LIF* probes. The DNA sequence of this clone has been determined and compared with that of several eutherian species including human and mouse. The putative coding regions show high homology (>75%) while the non-coding regions are very different in sequence. *sLIF* cDNA has been derived and cloned into $p^{Bluescript}$ and p^{GEX2T} vectors. Studies on the expression of *sLIF* have so far used the techniques of Northern analysis and RT-PCR. These studies are being extended to include *in situ* hybridisation to uterus/embryo tissue sections. Preliminary results based on RT-PCR indicate that *sLIF* transcripts are present during the early stages of development in *S. crassicaudata*.

THE MOLECULAR BIOLOGY AND EVOLUTION OF ß-GLOBIN GENES IN MONOTREMES

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The globin gene families have been extensively studied in mammalian and non-mammalian species and have provided an ideal model system for investigating molecular evolution. Amongst mammals, work on globin genes has focused on eutherians and to a lesser extent marsupials; very little is known about the molecular biology of monotreme globins. This is a significant deficiency, given the ancient divergence of the monotreme lineage. We are characterising the ß-globin gene families in the three extant species of monotreme, the Australian echidna (*Tachyglossus aculeatus*), the Niugini echidna (*Zaglossus bruijni*) and the platypus (*Ornithorhynchus anatinus*). Using standard cloning and PCR methods we have isolated and characterised putative ß-globin genes from all three species. Nucleotide sequence analyses of promoter and coding regions reveal that one of Australian echidna genes (Hbß-E^1), and two of the platypus genes (Hbß-P^1 and Hbß-P^2) are orthologous to the adult-expressed ß-globins of eutherians and marsupials. The sequence of Hbß-E^1 and Hbß-P^1 translate into the published amino acid sequence for the major ß-globin found in the adult Australian echidna and platypus respectively. Our results suggest that monotremes have more than one ß-globin gene and that one of these genes has been conserved to an unexpected extent in the Australian echidna and platypus.

T-CELL CYTOKINES IN THE MARSUPIAL, *MACROPUS EUGENII*

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Cytokines play a key role in regulating the immune response of birds, fish, amphibians, reptiles and eutherian mammals. Their role in the marsupial immune system remains largely unexplored and it has been generally assumed that their form and function in these animals parallels what has been documented in eutherians. This assumption is to a significant extent supported by what is known about the metatherian immune system. Histological studies, for instance, show clear similarities between structure and development of both the metatherian and eutherian lymphoid tissues. At a molecular level, marsupials have been shown to produce immunoglobulins A and G; have both MHC Class 1 and 2 equivalent loci and share some common T and B cell specific (CD) markers with eutherian mammals.

The cytokines, interleukin 2, 4 and 6, have been studied in the macropod *Macropus eugenii* using PCR consensus primers designed from known eutherian mRNA sequences. All cytokines were expressed in adult spleen and mitogen stimulated lymphocytes but only interleukin 2 was detected in unstimulated lymphocytes.In order to determine the full coding sequence of each cytokine .a cDNA library was constructed from adult tammar spleen using the lambda zap cloning system. The library was screened for putative clones in 2 ways. 1.using a mouse probe generated through random priming of a PCR fragment produced using consensus primers and mouse spleen cDNA and 2.using a tammer probe generated from sequenced PCR products.The degree of homology with the known eutherian sequences for each cytokine was found to be between 40 and 50 percent.

Taxonomy of Quolls (Dasyuridae:Marsupialia) at the Species, Subspecies, and Population Level.

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Over 200 individual quolls representing the four Australian Dasyurus species and up to 15 different extant and historic populations are being examined for genetic variation at the species, subspecies, and population levels using microsatellite and mitochondrial markers.

Genetic libraries have been manufactured and screened for (CA)n microsatellites and primers have been designed for 12 loci to date. Four of these loci have been analysed for variation.

Preliminary results of microsatellite analysis indicate that all populations that have suffered declines in numbers and range (tiger quolls, eastern quolls, and western quolls) show low levels of allelic diversity except for western quolls. Western quolls still retain very high levels of genetic variation despite the dramatic decline in this species. Northern quolls have not suffered from declines until recently and have high levels of allelic diversity as is expected.

Tiger quolls from the five sample populations analysed to date (NSW: Barrington House, Copeland, Chichester State Forest; TAS: Central Tasmania; QLD: Mt. Windsor Tableland) appear to be fairly similar in genetic distance analyses, although as expected in widely geographically separated populations there is a range of variation. A similar situation exsists within the eastern quoll populations (TAS: Vale of Belvoir, Central Tasmania; NSW: Historic). Historic populations of eastern quolls from the mainland appear to be quite distinct from populations in Tasmania, however the two Tasmanian populations cluster quite closely in multidimensional scaling analysis.

In neighbour joining trees, tiger and eastern quolls appear to be sister groups with western quolls as an intermediate group, and northern quolls being the most differentiated from all other groups.

P102

Phylogenetic relationships within the genus *Potorous* (Marsupialia: Potoroidae) using allozyme electrophoresis and sequence analysis of the cytochrome B gene

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Genetic analyses were used to examine relationships within the genus *Potorous.* There have been many changes in the taxonomy of this genus. The recent rediscovery of Gilbert's Potoroo, in south-western Western Australia, has allowed us to re-examine the relationship of this species to other extant Potoroos. No suitable material was available for *P. platyops.* Protein electrophoresis and mtDNA sequencing of the cytochrome B gene were carried out. There was considerable concordance between the two data sets. Gilbert's Potoroo was slightly more closely related to *P. longipes* than to *P.tridactylus.* There was 4.5% sequence divergence between the two *P. tridactylus* subspecies and less than 1% divergence between individuals of the same species based on the cytB data. Dates for the divergence of Macropodids and Potoroids were also estimated. Overall, the fixed differences in the allozymes and the large degree of sequence divergence between the three taxa clearly warrants a revision of earlier descriptions and the recognition of the separate identity of Gilbert's Potoroo.

Comparative Phylogeography of Aquatic Fauna in the Tully and Herbert Rivers - Evidence for an Altered Drainage Pattern.

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CRC -Tropical Rainforest Ecology and Management

It has been suggested that river capture (i.e. drainage rearrangement when one river experiencing greater headwater erosion 'captures' part of an adjacent river) has played a major role in the formation of the current drainage patterns observed on the Atherton Tableland in north-eastern Queensland. In fact several rivers flowing to the east coast display classic river capture characteristics (e.g. elbow of capture, barbed drainage pattern). The Tully River is one particular system that exhibits these traits as it initially flows NNW before dropping over the escarpment and flowing ESE to the coast. Several hypotheses regarding the drainage evolution of the Tully River and the adjacent Herbert River headwater streams have been postulated, however the actual nature of drainage rearrangement and therefore approximate timing of the event remain unclear.

Phylogeographic techniques have been widely used to evaluate the relative roles that gene flow, population bottlenecks, and historical physical or ecological barriers have played in determining current spatial patterns. The present study involves the comparative phylogeography of 3 totally aquatic taxa (2 species of fish, *Mogurnda adspersa* and *Melanotaenia. sp.*; 1 species of shrimp, *Caridina zebra*) using allozyme, mtDNA and microsatellite data in order to determine the likely time when these populations separated. This presentation will include allozyme data for *C. zebra* and allozyme and mtDNA data for *M. adspersa.*

Evolutionary Relationships Within the *Litoria pearsoniana* Complex

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The *Litoria pearsoniana* species complex comprises rainforest restricted frogs found along the East coast of Australia from central Queensland to southern Victoria. Fragmentation and contraction of rainforests during the Pleistocene may have resulted in restriction of gene flow between populations of these frogs. The degree of genetic divergence between rainforest isolates may reflect the severity of the dry barrier between such isolates. Recent allozyme data suggests the complex consists of four species. However, nucleotide sequences from the mitochondrial COI gene are not concordant with these groupings. The mitochondrial data shows genetic divergence between populations in south-east Queensland, but no divergence between populations in central New South Wales. Comparisons within a rainforest block reveals no evidence of genetic divergence of *L. pearsoniana* within the Conondale Ranges. The level of genetic diversity within the Conondale Ranges is less than in populations at Mt Glorious and Lamington Plateau, possibly reflecting historically lower effective population size in the Conondale Ranges.

P105

Mitochondrial DNA for use in species identification and phylogenetic analysis of the Heliothinae (Lepidoptera: Noctuidae)

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The Heliothinae are a cosmopolitan subfamily of noctuid moths, consisting of about 400 species. Australia supports a wide range of species including the major pests, *Helicoverpa armigera* and *Helicoverpa punctigera*, and 76% of the identified species comprising the genus *Heliocheilus*. An accurate demarcation of species limits and an understanding of the phylogenetic relationships among species are essential in the search for natural enemies and other means to suppress specific pest species. Molecular phylogenetic studies of the Heliothinae to date have used nuclear gene loci to assess relationships. These have, in general, supported morphometric analyses but uncertainties still prevail. Previous study has shown that *H. armigera* and *H. punctigera* are easily distinguished using sequence information from the variable A+T-rich region of the mitochondrial DNA. The present study assesses mitochondrial sequence information for a further twenty one Australian species and from two other major pests, *Heliothis virescens* and *Helicoverpa zea*. All show distinct mitochondrial sequences. The mitochondrial 12sRNA gene, amplified in tandem with the variable A+T-rich region, has been used here to clarify and improve the taxonomy and phylogeny of the group.

P106

Phylogenetic Position of Hoop Pine (*Araucaria cunninghamii*)

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A variety of Gymnosperms (14 species from 6 families and one non conifer) were collected and the small ribosomal subunit (18S rRNA) was sequenced by direct sequencing. Fourteen species were included in the study which included widely separated provenances of *Araucaria cunninghamii* to determine the phylogenetic relationship of these gymnosperms. Two of the four conifers families found in Australia which were included in the study (Araucariaceae and Pocarpaceae) separated as expected with further separation of genera and species within these families. Homologies between the Gymnosperms varied from 89% to 98%. Four geographically distinct groups, determined by extensive RAPD data, within *A. cunninghamii* were separated phylogenetically. This study suggests that *A. Cunninghamii* was the most recent *Araucaria* species to evolve, with Papua New Guinea provenances undergoing the fastest evolutionary changes. This type of analysis has provided molecular evidence to support the taxonomy of the these Coniferales. It has shown, contrary to an established understanding in Angiosperms, that sequence information of the 18S rRNA genes provides a useful means of studying phylogeny in the Gymnosperms at the species level.

The phylogeny of socially parasitic bees

Roger Lowe and Ross Crozier

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Many aculeate Hymenoptera are obligate social parasites on other species. Invariably, the host and parasite are closely related - a phenomenon known as Emery's rule. How strictly the relationship conforms with Emery's rule has been tested, using molecular techniques, in a number of groups. The use of molecular data avoids the problem that the parasitic condition might cause parallel morphological changes in independent parasite lineages. Bees of the Australian native allodapine genus *Inquilina* parasitise members of the closely related genus *Exoneura*. The phylogeny of this group was tested using DNA sequence data from a region of the mitochondrial cytochrome *b* gene. Analysis of the data gave consistent results under the application of various methods of phylogeny reconstruction. The results strongly supported a single origin of the parasitic condition in the genus *Inquilina*, indicating that these parsites show only loose conformity with Emery's rule. Various explanations of the evolution of parasitism in these bees are discussed.

Phylogenetic relationships of non-LTR retrotransposons in the genus *Chironomus* (Diptera)

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Jon Martin, *Genetics Department, University of Melbourne*

Non-LTR retrotransposons (NLRs) are widely distributed in nature and, therefore, it is not surprising that they have been found in *Chironomus*. The NLR, NLRCth1, was first reported in the European species *C. thummi* and more recently in *C. tentans*, a member of the subgenus *Camptochironomus*. Such elements offer particular advantages in phylogenetic analyses because relationships can be assessed on the basis of whether NLRs are present or absent, as well as comparison of the actual DNA sequences of the element. For this reason an investigation of the distribution and sequence of elements related to NLRCth1 was undertaken in a sample of about 30 species of the subgenera *Chironomus* and *Camptochironomus* from Siberia, North America and Australia and the related genus *Kiefferulus* from Australia. Preliminary results support some relationships previously suggested by cytological comparisons, but suggest the need for revision of the relationship between the species of northern and southern Australia. Some results also suggest the possibility of horizontal transfer.

FISHing the cattle tick genome

Andrea Crampton and Steve Barker

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Boophilus microplus (the Australian cattle tick) is internationally a major pest of cattle. Introduced to Australia from Java in 1872, *B. microplus* has become a multi-million dollar concern for the Australian meat and livestock industry. The costs incurred include production losses from cattle infested with ticks and tick-borne diseases as well as costs associated with developing and implementing control strategies. The economic importance of this arthropod parasite along with the ease with which it can be cultured in the laboratory environment makes *B. microplus* an ideal arachnid for genomic research.

B. microplus has a XX, XO sex determination system, the male is heterogametic. The diploid chromosome number is 22 for females and 21 for males. The sex chromosomes are over 4 times the size of the autosomes. When viewed at metaphase most of the autosomes appear to be about the same size, making identification of specific chromosomes difficult.

This project involves fluorescent *in situ* hybridisation (FISH) to physically map the genome of *B. microplus*. Over 100 expressed sequenced tags (ESTs) have been generated from a *B. microplus* cDNA library. Twenty two percent of the ESTs represent known genes, as identified via homology searches of genebank and EMBL. The genes identified include those for ribosomal proteins, elongation factors, histidase, the sodium channel and homeobox related protiens. Several of the ESTs are significantly homologous to ESTs generated from other mapping projects eg. *Sus scrofa, Drosophilia melanogaster*, *Caenorhabditis elegans* and *Homo sapien* mapping projects.

P110

Evolution of the $(AC)_n$ microsatellite at the IGF-I locus in Artiodactyls.

Mohammad Reza Shariflou and Chris Moran

Department of Animal Science, University of Sydney NSW 2006

The position and flanking sequence of microsatellite repeats are frequently conserved across quite distant mammalian lineages. An $(AC)_n$ microsatellite occurs in the 5' non-coding region of the insulin-like growth factor I (IGF-I) in humans, mouse and rat and the flanks are sufficiently conserved that PCR primers have been designed to amplify the polymorphic repeat in pigs and cattle (Kirkpatrick, 1992). We have used these primers to amplify and sequence the microsatellite region from cattle, deer and camel for comparison with the published sequences for sheep, goats and pigs. Our initial objective was to determine the time of origin of an AATA interrupt within the AC repeat motif. The interrupt was known to be present in sheep and goats, but absent from pigs, which diverged about 55 million years ago from the lineage leading to Bovids. Our sequence analysis revealed that the interrupt is conserved in deer, but is absent from the camel, thus refining the estimated time of origin of the interrupt to 19-35 million years ago. The camel was also found to have a 12 nucleotide deletion commencing one base before the start of the AC repeat. The deleted region presumably comprised a true palindrome (reversed repeat) which is conserved in the other Artiodactyl species. The unusual pairing possibilities of this reversed repeat may explain why it has been deleted in the camel.

Reference

Kirkpatrick, B.W. (1992) Identification of a conserved microsatellite site in the porcine and bovine insulin-like growth factor-I gene 5' flank. *Animal Genetics* **23:** 543-548.

Current status of the marsupial and monotreme genetic maps

Pino Maccarone and Jennifer A. Marshall Graves.

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Marsupials and monotremes represent two of the three extant groups of mammals. Marsupials diverged from eutherians (RplacentalsS), at least 150 MYrBP, and monotremes even earlier, so comparisons between the three groups provide information about very early events in mammalian genome evolution.

We are constructing outline physical maps of the genome of a marsupial and montreme, in order to compare them with the human gene map, and thereby charting the various changes that have occurred in the genomes of each lineage. We have focused our efforts on the Tammar Wallaby (*Macropus eugenii*) and the Platypus (*Ornithorhyncus anatinus*).

Two methods have been employed to construct these maps:

i/ radioactive *in situ* hybridization, whereby eutherian cDNA probes are mapped directly to the Tammar and Platypus chromosomes;

ii/ FISH. We have constructed large-insert phage libraries of Tammar and Platypus genomic DNA, screened them for homologous clones and used these directly for FISH.

Summary maps of the Tammar and Platypus genomes are presented. It is already evident that some gene arrangements have been conserved since prior to the radiation of the three mammalian lineages early in mammal evolution.

Physical and Linkage Mapping of Microsatellites in the Porcine Genome

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The major goal of gene mapping projects is to physically and linkage map a set of closely spaced markers on each chromosome. These will provide a foundation for further genetic analysis of economically important traits such as growth, meat quality and disease resistance. Microsatellites comprise a class of highly polymorphic loci that exhibit variation in the number of tandem repeats. They are now playing an important role in the development of linkage maps in almost all species. Microsatellites isolated from a cosmid library can be used not only for linkage analysis, but the cosmid also can be used *in situ* hybridisation for physical mapping.

A porcine cosmid library was screened with a labelled $(GT)_{10}$ probe. Sequence from one flank of the repeat in positive cosmids was obtained using one of a subset of twelve $(AC)_n$ specific primers (Yuille et al. 1991). A primer from this flanking sequence was used to sequence through the microsatellite and into the other flank from which a second primer was designed. Microsatellites have been genotyped on the European PiGMaP reference family to linkage map the loci. Cosmid DNA has been mapped by fluorescent *in situ* hybridisation. Eleven microsatellites have been linkage mapped to chromosome 1, 3, 7, 15, and 18 with seven of them physically mapped to the corresponding chromosome.

P113

HETERODUPLEX POLYMORPHISM (HPA) AND SSCP ANALYSES USING ENTANGLED SOLUTION CAPILLARY ELECTROPHORESIS (ESCE) FOR RAPID IDENTIFICATION OF INTERSTERILITY GROUP IN *HETEROBASIDION ANNOSUM*

Jing Cheng (1), Takao Kasuga (2) and Keith Mitchelson (3)

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(2) Samuel Roberts Noble Foundation, Plant Biology Division, Ardmore, Oklahoma, USA.
(3) ForBio Research Pty. Ltd., 50 Meiers Road, Indooroopilly, Qld 4068, Australia.

Heterobasidion annosum is a root and butt rot fungus prevalent in conifer forests in the northern hemisphere. Three intersterility groups (ISG-P (pine), ISG-S (spruce) and ISG-F (fir)) have been identified in Europe using classical mating tests, which correlate with host preference. The ISG-P group however has a broader specificity than ISG-S and ISG-F and may be found commonly on young spruce as well as exotic conifers and on broad-leaf species. The groups are difficult to distinguish morphologically and the detection of DNA polymorphism can provide analysis of fungal genotype, population structure and distribution. Capillary electrophoresis is a new electrophoretic format offering high speed, high resolution, high reproducibility and low cost as well automatic data acquisition and data analysis. We have developed an entangled liquid sieving medium which provides good resolution of DNA fragments in the range 25 bp to 1.2 kb. Sequence polymorphism in the internal transcribed spacer region 2 of the ribosomal repeat (ITS2) can be used to distinguish the intersterility groups of *H. annosum*, however the variant nucleotides are not amenable to restriction analysis. The intersterility groups could be rapidly distinguished by either heteroduplex polymorphism assay, single strand conformation assay (SSCP) or by amplification-refractory mutation system (ARMS) assays adapted for ESCE analysis. Additionally, isolate specific sequence polymorphism could be visualized in abundant repeated DNA motifs following restriction of high quality genomic DNA from fungal mycelia, or following restriction of the PCR-amplified fragments from the intergenic ribosomal spacer region 1 (IGS1).

P114

POLYMORPHISM IN THE RIBOSOMAL ITS DISTINGUISHES SEXUAL FORMS OF THE PINE BLISTER RUSTS *CRONARTIUM FLACCIDUM* AND *PERIDERMIUM PINI*

Salvatore Moricca (1), Takao Kasuga (2), Keith Mitchelson (3), Alessandro Ragazzi (1) and Stephanos Diamandis (4)

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(2) Samuel Roberts Noble Foundation, Plant Biology Division, Ardmore, Oklahoma, USA.
(3) ForBio Research Pty. Ltd., 50 Meiers Road, Indooroopilly, Qld 4068, Australia.
(4) Forest Research Institute, University of Thessalonica, Thessalonica, Greece.

The pine blister rusts *Cronartium flaccidum* and *Peridermium pini* occur on two needle pines in Europe and represent heteroecious and monoecious forms respectively, which are virtually indistinguishable from examination of spore morphology or hyphal form in culture. In this study (1), the internal transcribed spacer region (ITS) of the ribosomal gene repeat has been used to identify sequence polymorphism between single-tree, mixed aeciospore isolates of *Peridermium pini* from Britain (2 samples) and Greece (1 sample) and in *Cronartium flaccidum* from Italy (6 samples). Sequence polymorphism was detected in all samples, suggesting that genetic heterogeneity exists in asci found on a single tree. Polymorphism occurred at several sites in *Peridermium pini* ITS1 and ITS2 regions, with some different variants detected in samples from Britain and Greece. Sequence polymorphism was also detected at the same sequence loci in *Cronartium flaccidum* from Italy, as well as at additional sequence loci. Overall, a large number of sequence variants were found to be common to each of the six *Cronartium flaccidum* samples from geographically different regions of Italy. The ITS region of single-tree, mixed aeciospore samples of *Cronartium flaccidum* display more polymorphism than samples of *Peridermium pini*. SSCP analysis of the PCR products from *Cronartium flaccidum* showed additional bands to *Peridermium pini* products on non-denaturing PAGE gels, consistent with the DNA sequence analysis. Although the SSCP test was rapid, the sequence polymorphism in the ITS could not be readily used to distinguish populations of *Cronartium flaccidum,* however it might be used to distinguish between *P. pini* and *C. flaccidum.*

(1) Moricca, S., T. Kasuga, KR. Mitchelson, A. Ragazzi and S. Diamandis, (1996). Sequence polymorphism in the ribosomal internal transcribed spacer of the pine blister rusts *Peridermium pini* and *Cronartium flaccidum*. Current Genetics 29, 388-394.

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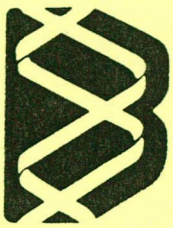

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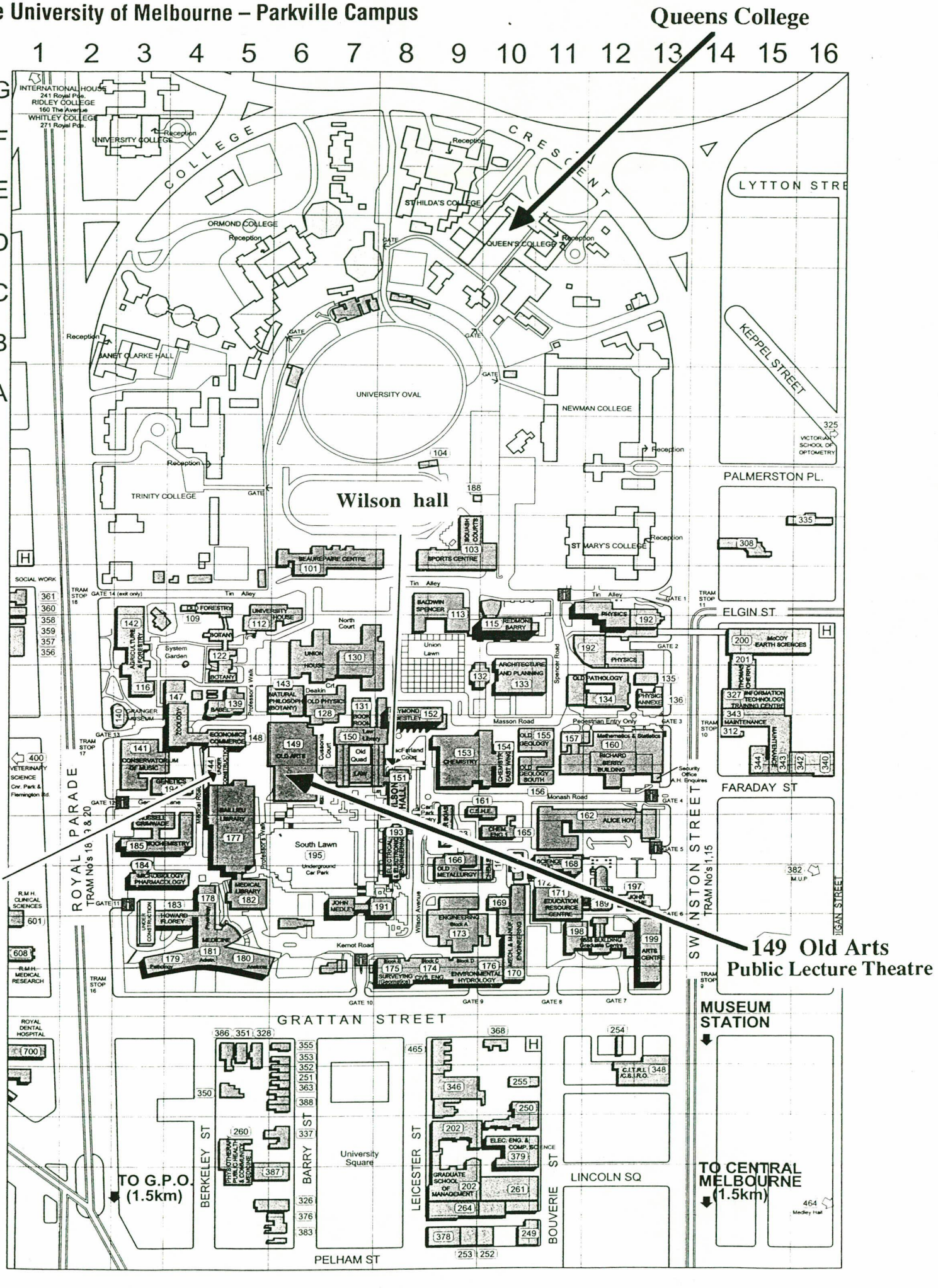
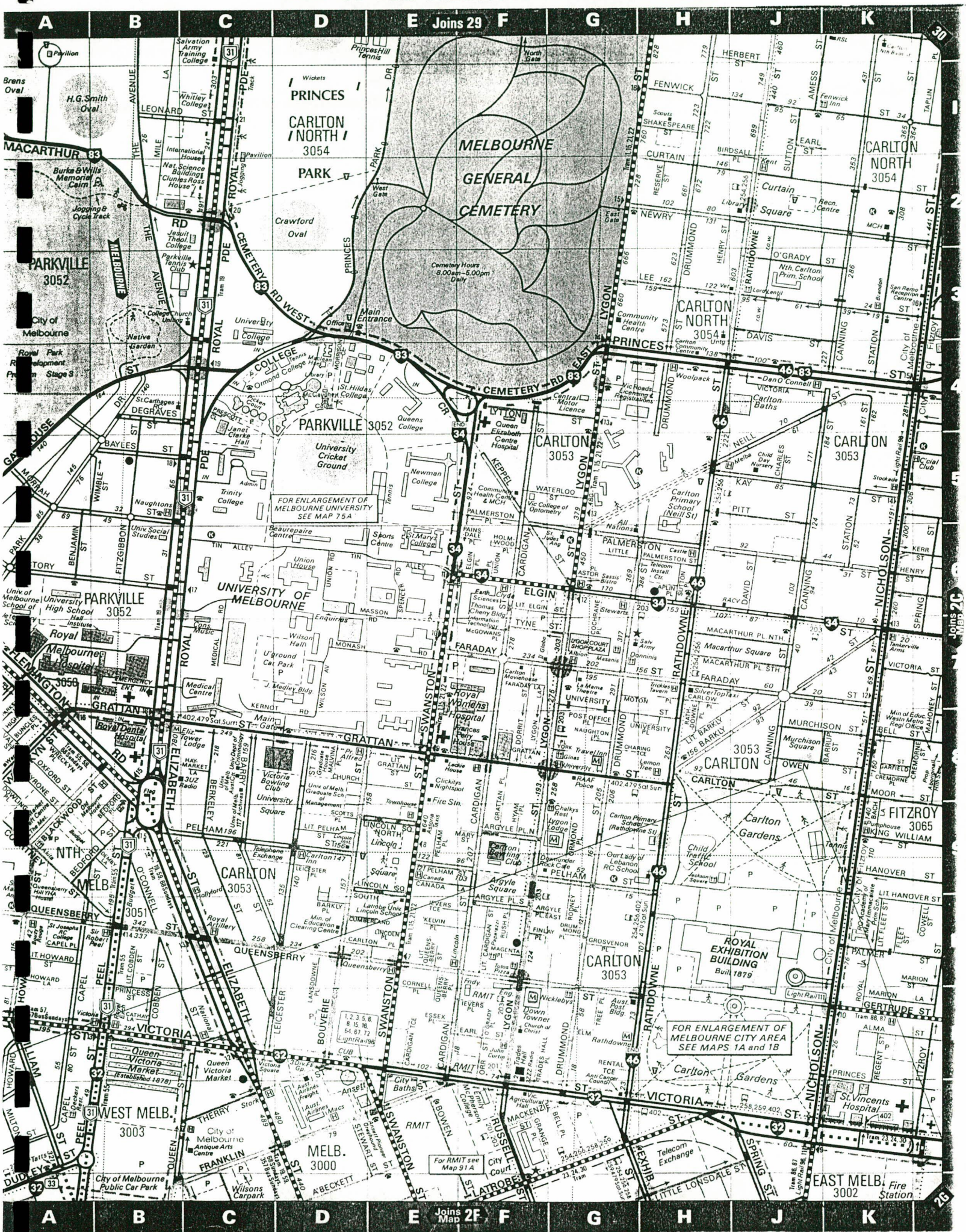


MAP OF THE PARKVILLE/CARLTON AREA



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8/7/96 Lunch $3-10
Coffee $1-80

Dinner $25-00

9/7/96 Dinner $22-00

10/7/96 Lunch $3-10

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