CHRIS MORAN

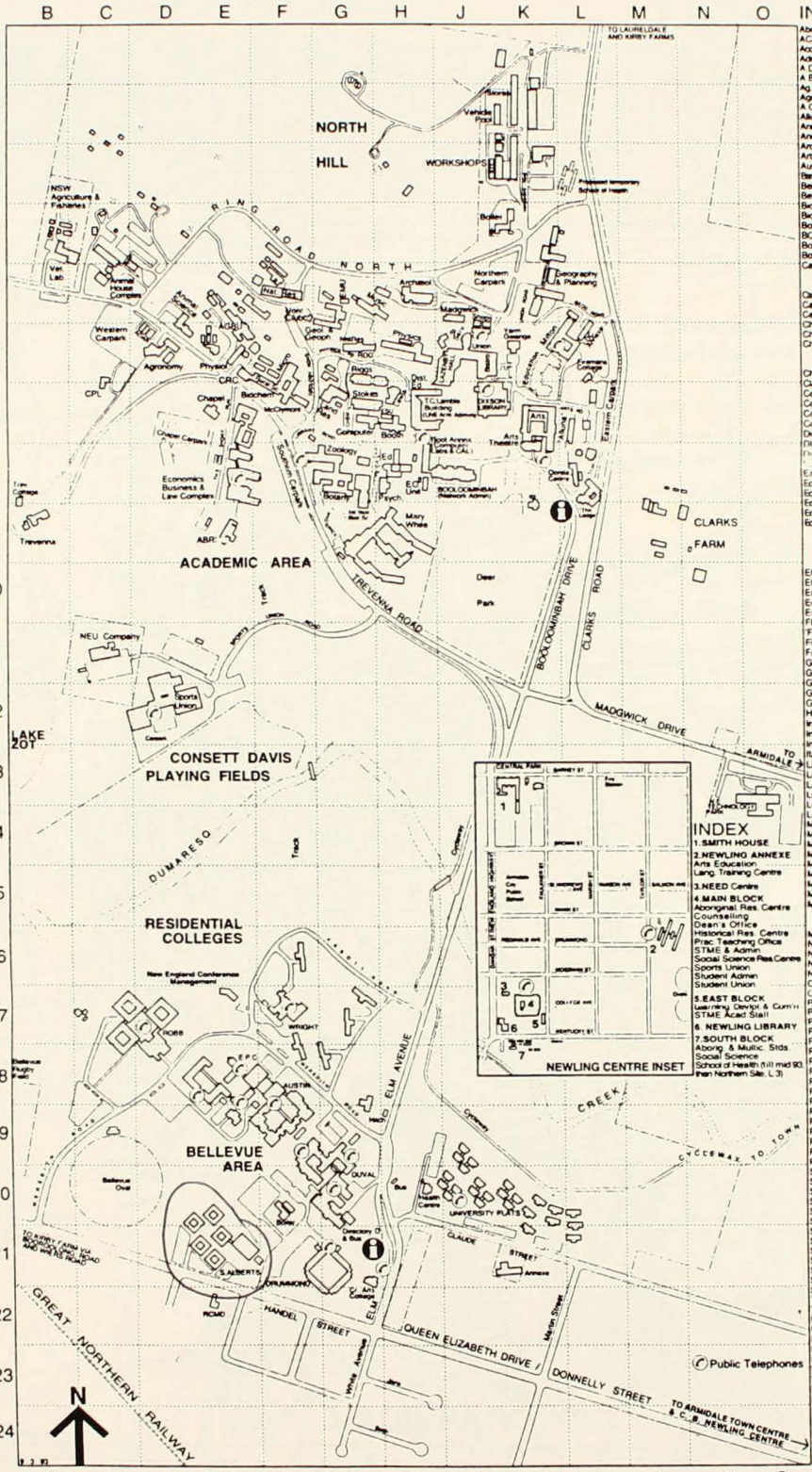
GENETICS SOCIETY OF AUSTRALIA
41ST ANNUAL MEETING

PROGRAM AND ABSTRACTS

UNIVERSITY OF NEW ENGLAND
ARMIDALE, N.S.W.

JULY 3-6, 1994

Tell Mick to
get new set of
instructions from Bresatec.
- new buffer system
- high salt.



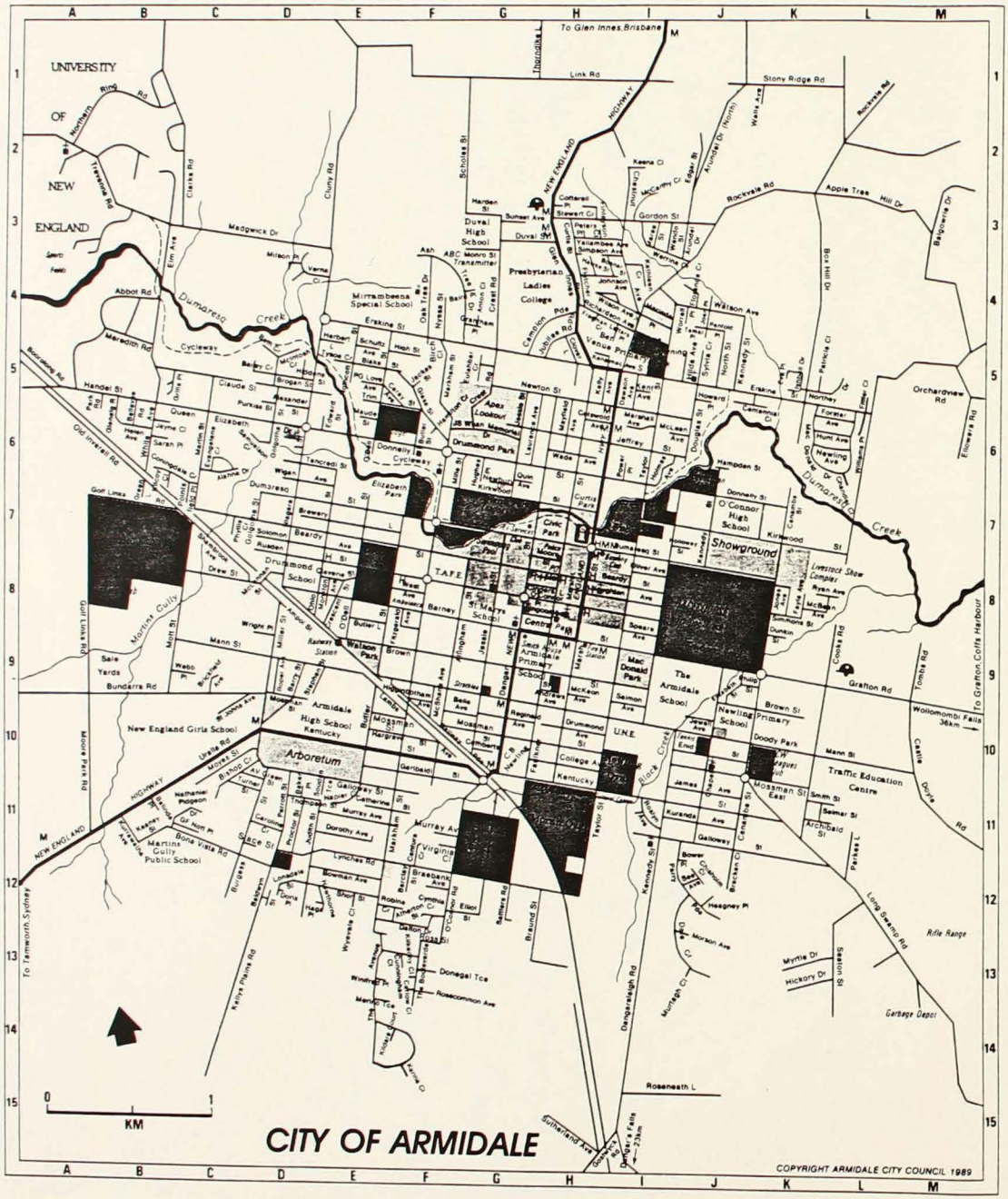
INDEX

INDEX

UNE–ARMIDALE NORTHERN SITE DIRECTORY '93

C. B. NEWLING CENTRE IN INSET
100 METRE GRID
2 FEB '93

ARMIDALE CITY MAP



EDWARDS COACHES

72 3116

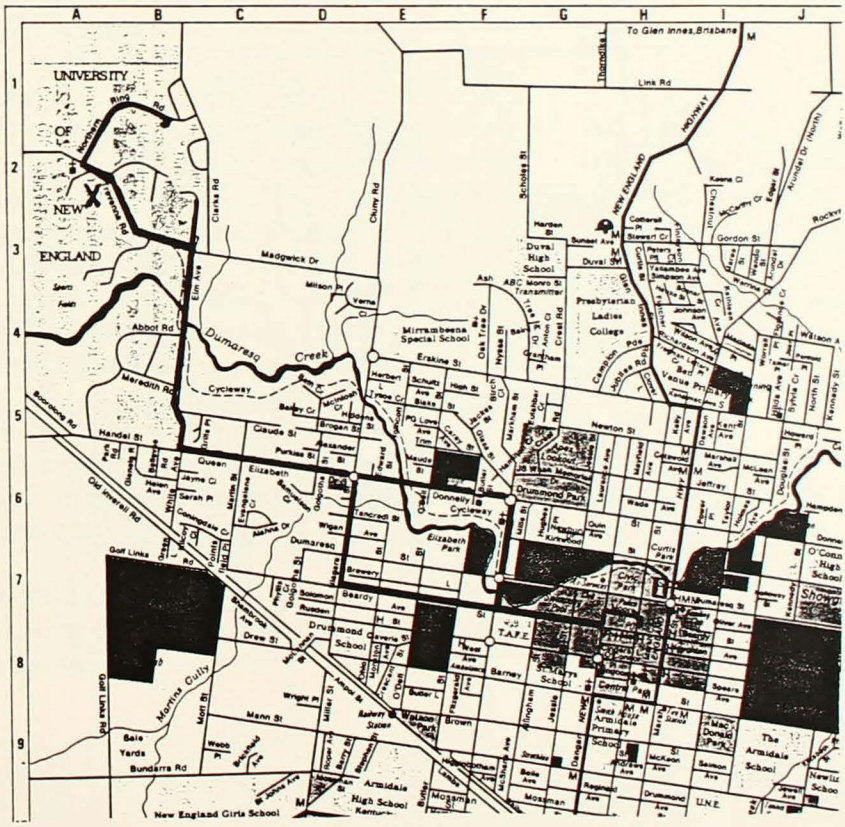
CITY SERVICE TIMETABLE

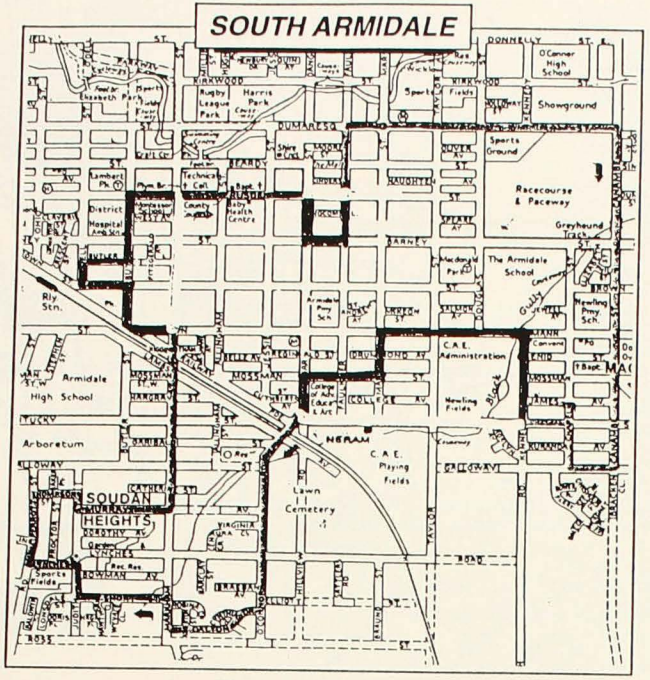
UNE SERVICE

Monday - Friday

Departs: Courthouse	Route	Departs: Nrth Carpark
8.35 am	1	8.50 am
9.15 am	1	9.30 am
9.45 am	2	10.00 am
10.15 pm	1	10.30 pm
10.45 pm	2	11.00 pm
11.15 pm	1	11.30 pm
11.45 pm	2	12.00 md
12.15 pm	1	12.30 pm
12.45 pm	2	1.00 pm
1.15 pm	1	1.30 pm
1.45 pm	2	2.00 pm
2.15 pm	1	2.30 pm
2.45 pm	2	3.05 pm
-		-
3.45 pm	1	4.05 pm
-	2	4.30 pm
4.45 pm	2	5.05 pm
5.15 pm	1	5.30 pm
5.45 pm	1	6.05 pm

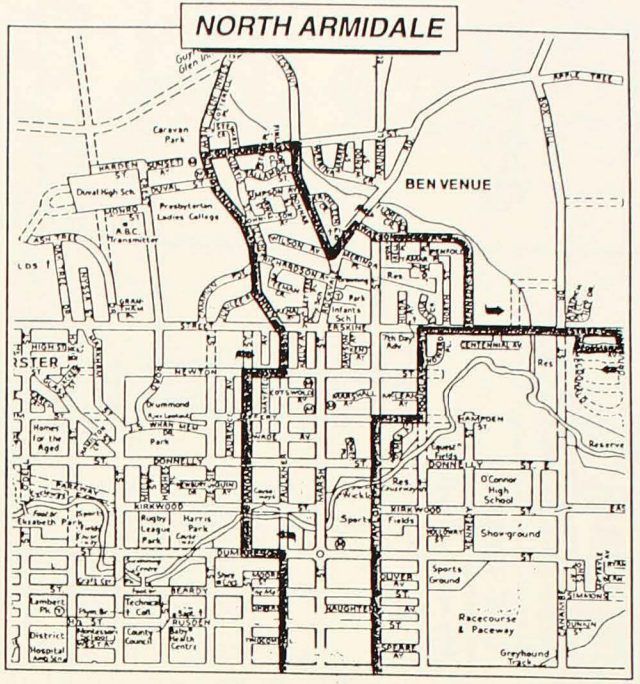
Route No.1: Beardy St, Niagara St, Queen Elizabeth Drive to UNE. Return to Courthouse via Newling Campus
Route No.2: Beardy St, Markham St, Donnelly St, Queen Elizabeth Drive, UNE top. Return to Courthouse via Newling Campus





Monday - Friday

Departs Courthouse: 9:45am, 10:45 am, 11:45 am
12:45pm, 1:45pm, 2:45pm, 4:45pm, 5:45pm



Monday - Friday

Departs: Courthouse	Departs: Cnr Gordon & Chestnut to Courthouse
9.20 am	
10.20 am	10.25 am
11.20 am	11.25 am
12.20 pm	12.25 pm
1.20 pm	1.25 pm
2.20 pm	2.25 pm
4.20 pm	4.25 pm
5.20 pm	5.25 pm

GENETICS SOCIETYOF AUSTRALIA

41ST ANNUAL MEETING

TRANSPORTATION TO UNE

The Faculty of Economics, Business and Law is a 20 minute walk from Drummond College. Shuttle buses will be available to take people from Drummond College to the conference. The buses will depart from Drummond College at 8:15 a.m. Buses will also be provided to return peoplet to the college for lunch and at the end of the day. For those staying off campus, parking is available at the Southern Carpark next to the Faculty of Economics, Business and Law. A timetable of bus service in Armidale is included in this booklet. Armidale Taxis can be reached on 71-1455.

MIXER

A mixer will be held in the Drummond Seminar Room from 7:00-10:00 p.m. on July 3rd. Drinks and finger foods will be provided.

MEALS

All meals are included in the accommodation at Drummond College. Breakfast will be served from 7:00-8:30 a.m. and dinner will be served between 6:00-6:30 p.m. (You don't have to finish eating by 6:30 p.m. but you should arrive before 6:30 p.m.) Non-residents who did not purchase meal tickets for lunch at Drummond College can eat at the Union. The Annual Conference Dinner will be held in Drummond College on Tuesday July 5th. Wine and non-alcoholic drinks will be provided at the dinner. There will be a band for after dinner entertainment.

EXCURSION

A joint excursion with the Australasian Gene Mappers will be held on the afternoon of July 6th. We will go to New England National Park for lunch and return via Wollomombi Gorge, the site of Australia's tallest waterfall (We can't promise you much water though). We will return in time to meet the last flight departing in the afternoon (a Hazelton flight at 5:20 p.m.). Point Lookout at New England National Park is at an elevation of 1600m so you will need to dress warmly for the excursion. For those who are going straight to the airport, please bring your luggage with you to the morning sessions. There will not be time to return to the college.

POSTER SESSION

A poster session will be held on July 4th at 4:00 p.m. Complimentary champagne will be served and bar service will be available. The posters will remain in place and the bar service will continue after dinner.

WORKSHOP

A workshop on the use of computers in teaching and research will be held after dinner on July 4th at 7:30 p.m.

STUDENT PRIZES

As a result of the generous contributions of a number of companies, we will be offering student prizes for outstanding presentations in the following categories:

1) $500 Progen Award for the best post-graduate student oral presentation.

2) $500 Bio-Rad/ Sigma-Aldrich Award for the best post-graduate student poster presentation.

3) $250 Promega Award for the best oral presentation of honours work*.

4) $250 Beckman award for the best poster presentation of honours work*

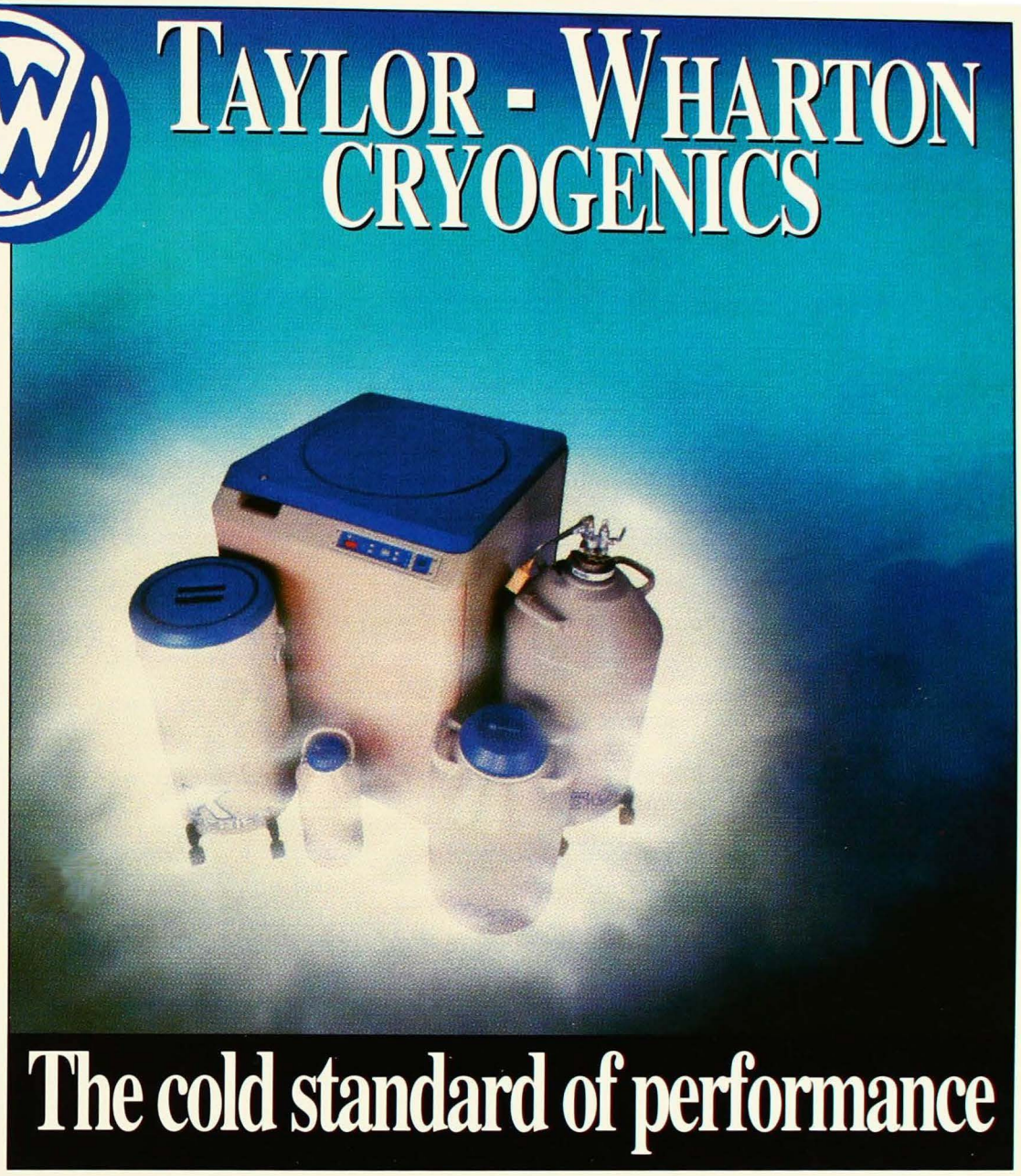
*These awards are for work done as an honours student, not necessarily presented while still an honours student.

The student prizes will be presented at the conference dinner on July 5th.

LOCAL ORGANISING COMMITTEE:

Margaret Katz
Stuart Barker
Lynette McLean
Anatoly Ruvinsky

TAYLOR - WHARTON CRYOGENICS



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TIPS & TUBES

	Catalogue #	Pack Size
1-25 µl	TR-673	10 racks 96 tips ea
	TR-674	288 tips + 1 rack
	TR-674/1000	1000 tips
25–250 µl	TR-697	10 racks 96 tips ea
	TR-698	288 tips + 1 rack

Bulk-Packed Tips		
1–250 µl	RS1017-00-0	1000/bag
100–1000 µl	RS1018-00-0	1000/bag
(blue)	RS1018-00-1	1000/bag
1000–5000 µ	RS1043-05-0	250/bag
Stacked Tray Tips		
1–250 µl	RS1017-01-0	4 x 250
100–1000 µl	RS1018-01-0	4 x 125
(blue)	RS1018-01-1	4 x 125
Individually Wrapped Sterile Tips		
250 µl	RS1017-03-0	500/bag
1000 µl	RS1018-02-0	500/bag

Titer-Pak® I		
1–250 ml	RS1041-00-0	10 x 96 tips
(sterile)	RS1041-01-0	10 x 96 tips
Titer-Pak® II		
100–1000 m	RS1041-00-2	4 x 125 tips
	RS1041-01-2	4 x 125 tips
Titer-Pak® III		
1–250 ml	RS1041-00-3	4 x 250 tips
(sterile)	RS1041-01-3	4 x 250 tips)
Titer-Pak® IV		
1–250 ml	RS1041-00-4	10 x 96 tips
(sterile)	RS1041-01-4	10 x 96 tips

Tip Volume	Pack Size	Catalogue #
20 µl	RS1041-32-5	10 x 96 tips
80 µl	RS1041-34-5	10 x 96 tips
150 µl	RS1041-36-5	10 x 96 tips
1 ml	RS1041-38-5	4 x 125 tips

Positive Displacement Tips

Disposable and autoclavable (under carefully controlled conditions) microsyringe tips to fit the Aerosol-free PCR* pipettes are ionised to minimize static attraction.

The tips have a built-in plunger and can be installed on the PCR* pipette without skin contact. Tips are shipped in racks of 96 with a boxed lid. They are also supplied loose in a bag.

Tips are "Hot Packed" and do not need to be resterilized for most applications.

Standard Pipette Tips

Tips from Robbins Scientific range in size from 1–5000 ml and are precision-moulded of non-wettable polypropylene. They do not warp when autoclaved and are available colourless or colour-coded with non-metallic, non-cytotoxic tints. The tips come bulk-packed (in zip-lock bags), in stacked trays or individually wrapped (sterilized). They are also available in fully enclosed racks (Titer-Paks®), which are available sterilized by γ-irradiation.

These tips fit the following single-channel pipettes:
Eppendorf®, Finnpipette®, Oxford™, Rainin EDP™,Excalibur™, Pipetman®, Socorex

As well as the following multi-channel pipettes:
Costar Octapette® & 12-pette™, Titertek®, Rainin EDP™ and Socorex.

Titer-Pak® Pipette Tips

The Titer-Pak® series, from Robbins Scientific, consists of disposable, fully enclosed racks preloaded with either 96, 125 or 250 pipette tips. The tips, rack and box are fully autoclavable. The vented, telescoping cover protects the tips from dust and dirt. The unique three-piece design ensures that the bottom will not separate from the rack, even after repeated autoclaving. The Titer-Pak® is available sterilized by γ-irradiation.

AeroShield™ Pipette Tips

These tips from Robbins Scientific have a hydrophobic filter which prevents aerosol contamination during pipetting. Each rack of AeroShield™ is individually wrapped and sterilized by γ-irradiation. AeroShield™ tips are RNase free.

* The PCR process is covered by patents issued to the Cetus Corp.

Call toll-free 008 882 555 for a Sample Kit

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Thebarton SA 5031
PO Box 11 Rundle Mall
Adelaide SA 5000

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Facsimile 08 234 2699

Brisbane Office (07) 207 6166
Melbourne Office (03) 428 8883
Sydney Office (02) 887 4438

Bresatec

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MJP-96V ♦ 25 plates

Microtitre plates

Designed specifically for use with the PTC-100-96V Thermal Controller. These polycarbonate V-bottom plates are ideal for setting up multiple reactions *e.g.* PCR*, restriction digests etc.

0.2 ml tubes	RS1044-00-0	125 strips of 8 tubes ea
Caps	RS1044-10-0	300 strips of 8 caps ea
0.2 ml tubes	RS1044-20-0	1000 detached tubes
Tube-tray	RS1044-30-0	box of 20 trays 96 tubes per tray no caps

"Strip Ease®" Tubes and caps 0.2 ml volume

Thin-walled 0.2 ml reaction tubes supplied individually or in strips of 8 tubes. They are designed to be used in the PTC-100-96V Thermal Controller. Caps are available to fit the microfuge tubes.

"Strip Ease®" 0.2 ml Tubes are also available in trays.

This system provides 96 x 0.2 ml tubes preloaded in propylene trays, in a microtitre plate configuration. These racks fit directly on the PTC-100-96V Thermal Controller just like a microtitre plate. MJC-300 caps (above) fit the "Strip-Ease®" tubes.

RS1045-00-0 non-sterile	500 tubes/bag
RS1045-01-0 sterile	500 tubes/bag

Thin Wall 0.6 ml Reaction Tube for PCR* Thermal Controllers

These tubes from Robbins Scientific allow efficient heat transfer and are packaged sterile or nonsterile. Each 0.6 ml polypropylene thin wall tube has an attached Cap-Lok® 2-step seal which opens easily for addition of reagents and closes tightly for boiling and freezing. Tubes are also available in assorted colours.

Microcentrifuge Tubes 0.6 & 1.5 ml

These tubes from Robbins Scientific are precision-moulded from medical-grade, noncytotoxic polypropylene. The tubes feature a two-step cap with a patented Cap-Lok® seal. The first step allows easy, one-hand, nonsplash opening but seals tightly enough for spinning and to prevent leaking. The second Cap-Lok® step is tighter and provides the optimum seal for boiling, freezing, storing or shipping. Also incorporated into the design of the cap is a completely flat, frosted top, which provides an excellent writing surface.

Included in each bag is a Microfuge Tube opener.

The tubes are available in a variety of colours; red, yellow, blue, green, peach & purple.

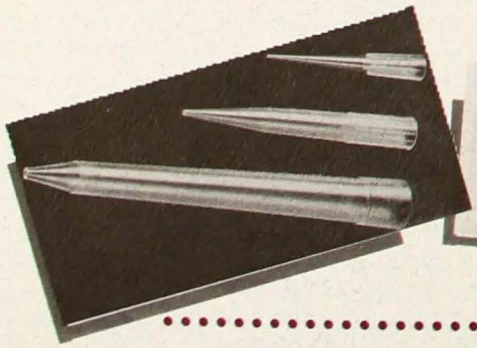
0.6 ml Tubes		
RS1048-00-0	Non-sterile	1000/bag
RS1048-01-0	Sterile	10 x100/bag
1.5 ml Tubes		
RS1012-00-0	Non-sterile	1000/bag
RS1012-01-0	Sterile	10 x 100/bag

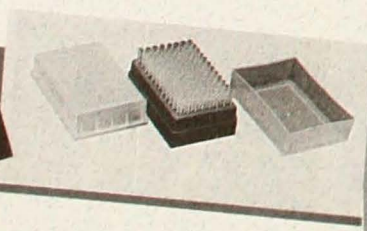
DNA Extraction Tubes (1.5 ml)

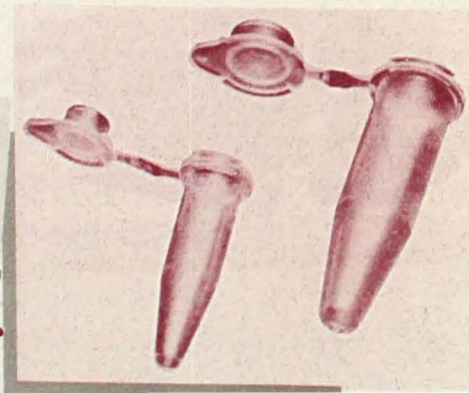
DNA does not usually adhere well to the inside of ordinary microtubes following sedimentation. These microtubes from Robbins Scientific can increase the yield of DNA several-fold over conventional microfuge tubes. A special non-chemical process increases the surface area of the 1.5 ml polypropylene DNA EXTRACTION TUBE, so that nano- and picogram amounts of DNA and RNA can adhere to the tube wall at nominal centrifugal forces.

A Cap Lok® seal is attached to each tube for easy and reliable leak-free application.

RS1046-01-0 ♦	Sterile ♦	5 x 100/bag
RS1046-00-0 ♦	Non-sterile ♦	500/bag







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TRADE REPRESENTATIVES

BECKMAN INSTRUMENTS, Geoffrey Burge and Jackie Smith
BIOTECH INTERNATIONAL
BOEHRINGER MANNHEIM, Brett Kennedy
BRESATEC, Jenny McShane
FSE, Phillip Prather
NEN DUPONT
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Sunderland Theatre

This will be followed by a Function at University House for past and present staff, Honours and graduate students of the Department of Genetics. For further details please contact Cheryl:

Telephone (03) 344 6246, Fax (03) 344 5139.

CONFERENCE PROGRAM

Sunday July 3rd

4:00 p.m.-8:00 p.m. GSA Registration, Drummond College
7:00 p.m.-10:00 p.m. GSA Mixer, Drummond College Seminar Room

Monday July 4th

8:15 a.m. Shuttle bus departs from Drummond College

Venue: **Faculty of Economics, Business and Law, Belshaw Lecture Theatre**
8:30 a.m. Welcome to the University of New England
Professor Bruce Thom, Vice-Chancellor, University of New England

Symposium on Molecular Evolution (Chair: Simon Easteal)

8:45 a.m. *Charles F. Aquadro* (Invited overseas speaker): Selection, recombination, and DNA polymorphism in *Drosophila* (A1).
9:45 a.m. *Chris Collet and Simon Easteal*: Mutation rate uniformity and substitution rate variation within and between mammalian lineages (A2).
10:15 a.m. *Thomas M. Boyce*: Protein structure, functional constraint, and the mode of DNA sequence evolution (A3).

10:45 a.m.-11:15 a.m. **Morning tea/coffee and trade displays**

Concurrent sessions

Warning call
Call at 11:30

Developmental Genetics ES4 Lecture Theatre (Chair: Chris Moran)

11:15 a.m. *Alex Andrianopoulos, William E. Timberlake*: Analysis of the interactions between the developmental regulator *abaA* and its targets in *Aspergillus nidulans* (B1). Finished at 11:35
11:35 a.m. *Warwick Grant, Peter Hunt*: Chemosensory mutants of *Caenorhabditis elegans* (B2).
11:55 a.m. *Paul Whitington, David Merritt*: Which genes specify sensory neuron identity in the *Drosophila* embryo (B3).
12:15 p.m. *Jon Martin, B.T.O. Lee*: A mutation in *Chironomus tentans* Fab. that leads to gynandromorph and mosaic formation (B4).

11-58 start
↓
12:18 finish
12-20 start

12:35

Population/Quantitative Genetics Belshaw Lecture Theatre (Chair: Barrie Latter)

11:15 a.m. *Hamish G. Spencer, R. William Marks*: Estimating the power of the Ewens-Watterson test of neutrality using simulation (C1).
11:35 a.m. *Dianne M. Gleeson*: The population genetics and origin of the sheep blowfly, *Lucilia cuprina*, in New Zealand (C2).
11:55 a.m. *Richard Frankham*: Factors affecting N_e/N ratios in theory and in wildlife: conservation implications (C3).
12:15 a.m. *L. Pradeepa Silva, J.D. Kirton, I.C.A. Martin, C. Moran, F.W Nicholas.* Effect of inbreeding with selection on litter size of Quackenbush-Swiss mice (C4).

12:35-1:50 p.m. **Lunch**

Smith White Student Travel Grant Applicants
Belshaw Lecture Theatre (Chair: Oliver Mayo)

Oral presentations

1:50 p.m. *Nick Campbell*: Outgroup heteroduplex analysis using TGGE: population genetics at the DNA level (D1).

2:10 p.m. *Kelly R. Ewen, Mike Westerman, Margaret E. Matthews*: Construction of evolutionary trees for equine breeds using microsatellite markers (D2).

2:30 p.m. *Rachael L. Murphy, Richard B. Todd, Meryl A. Davis, Michael J. Hynes*: Molecular characterisation of the *facB88* mutation of *Aspergillus nidulans* (D3).

2:50 p.m. *Sharon J. Orford, Jeremy Timmis*: Analysis of gene expression during cotton fibre development (D4).

3:10 p.m. *Gavin D. Recchia, H.W. Stokes, Ruth M. Hall*: Genome Evolution: moving genes by site-specific recombination (D5).

3:30 p.m. *Cheryl Wise, Simon Easteal*: Out of anywhere? Evolution of human mitochondrial (mt)DNA does not reflect the evolutionary history of humans; evidence from a comparative analysis of chimpanzees (D6).

Posters (Displayed at Poster Session)

Charles Claudianos, Hugh D. Campbell: The novel *flightless-I* gene brings together the gelsolin and leucine-rich-repeat gene families and provides a possible marker for the arrival of multicellular life (Poster 6).

Charles Robin, Robyn Russell, John Oakeshott: Evolution of the α esterase gene cluster (Poster 23).

Yan-Hong Wu, John B. Gibson: Differential effect of insertions on the expression of the alcohol dehydrogenase gene in larvae and adults of *Drosophila melanogaster* (Poster 34).

Poster Session

4:00-6:00 p.m. St. Alberts College Junior Common Room. Complimentary champagne. Bar service will be available.

6:00 p.m. **Dinner**, Drummond College.

7:30 p.m. **Computer software workshop**. St. Alberts College Computer Room (Convenor: Jeremy Timmis)

Posters will remain up and bar service will be available until 10 p.m.

Tuesday July 5th

Concurrent sessions

Molecular Genetics ES4 Lecture Theatre (Chair: Michael Hynes)

8:30 a.m. *Julie A. Sharp, Meryl A. Davis, Michael J. Hynes*: A molecular analysis of a *cis*-acting mutation affecting the regulation of the acetamidase gene in *Aspergillus nidulans* (E1).

8:50 a.m. *Saovanee Dharmsthiti, Viji Krishnapillai*: Regulatory signals around the highly conserved biosynthetic *argA* genes of two related *Pseudomonas* bacterial species (E2).

9:10 a.m. *David A. Loebel, Diana Dean, Peter G. Johnston*: Cloning and methylation analysis of the X-linked G6PD gene of wallaroos (*Macropus robustus)* (E3).

9:30 a.m. *Matthew J. Wakefield*: Analysis of predicted RNA secondary structure of the human and mouse *Xist* genes (E4).

9:50 a.m. *Andrew Hugall, Julie Stanton, Craig Moritz*: Significance of two classes of rDNA ITS sequences in parthenogenetic root knot nematodes (genus *Meloidogyne*) (E5).

Molecular Population Genetics Belshaw Lecture Theatre (Chair: Steve McKechnie)

8:30 a.m. *Martin Elphinstone*: Heteroduplex analysis of mitochondrial haplotypes using PCR and temperature gradient gel eletrophoresis (F1).

8:50 a.m. *Fiona Harriss*: An examination of mitochondrial evolution using TGGE analysis: use of Australian *Rattus* as a model taxon (F2).

9:10 a.m. *Susan Fuller*: Rabbit metapopulation structure in south-west Queensland (F3).

9:30 a.m. *Bronwyn A. Williams, Peter R. Baverstock*: The use of the aldolase gene family as potential loci for population and evolution studies (F4).

9:50 a.m. *Shane Lavery*: Molecular population markers for Panaeid prawns (F5).

10:10 - 10:40 a.m. **Morning tea/coffee and trade displays**

Special session
Belshaw Lecture Theatre (Chair: Ross Crozier)

10:40 a.m. *Phil Batterham, Doug McCann*: Australian genetics-a brief history (G1).

11:10 a.m. **M. J. D. White Address**

Oliver Mayo: The genetics of wool quality (G2).

12:10-1:30 p.m. **Lunch**

Concurrent sessions

Molecular Genetics Belshaw Lecture Theatre (Chair: Margaret Katz)

1:30 p.m. *Zhang Weiyi, Martine Yerle, Paul Le Tissier, Chris Moran*: Studies of a Y-linked element in the pig (H1).

1:50 p.m. *Veronica L. Ross, Simon Easteal, Philip Board*: An evolutionary perspective of the glutathione S-transferases (H2). No Show!

2:10 p.m. *Yasmine H.M. Svoboda, Merryl K. Robson, John A. Sved*: P element-induced male recombination can be produced by combining end-deficient elements in *Drosophila melanogaster* (H3).

2:30 p.m. *Cedric E. May*: Crossing over within the nucleolar organizer regions of wheat (H4).

2:50 p.m. *Adam Torkamanzehi*: Transposition-induced mutation: can it be used to generate safer genetic variability? (H5) No show!

Conservation Genetics ES4 Lecture Theatre (Chair: Dick Frankham)

1:30 p.m. *Craig Moritz*: Using information from allele frequency and phylogeny to define units for conservaion and management (I1).

1:50 p.m. *Jessica Worthington Wilmer, Craig Moritz, Les Hall, John Toop*: Extreme population structuring in the threatened Ghost Bat, *Macroderma gigas*: evidence from mitochondrial and nuclear DNA (I2).

2:10 p.m. *Lisa Pope, Craig Moritz*: Population structure of the Yellow-footed rock wallaby inferred from mitochondrial DNA and nuclear microsatellites (I3).

2:30 p.m. *Nancy N. FitzSimmons, Lisa C. Pope, Craig Moritz, Colin J. Limpus*: Male-mediated gene flow in marine turtle populations; the story from microsatellite alleles (I4).

2:50 p.m. *Danquing Zhu, Sandie Degnan, Craig Moritz*: Evolutionary distinctiveness and genetic population structure of the endangered Lake Eacham rainbowfish, *Melanotaenia eachamensis* and its relative *M. splendida* (I5).

Population Genetics/ Phylogenetics ES2 Lecture Theatre (Chair: John McKenzie)

1:30 p.m. *Michaela Srami, Chris Collet*: Molecular systematics of the Australian waterfowl (J1)

1:50 p.m. *Ginny M. Sargent, J.C.S. Fowler, R.T. Wells*: Ancient DNA from *Thylocoleo*, an extinct marsupial: is it a possum or a wombat? (J2)

2:10 p.m. *Steven Cooper, Godfrey Hewitt*: Post-glacial expansion and subdivision of the grasshopper *Chorthippus parallelus* in Europe (J3).

2:30 p.m. *Stephen W. McKechnie, Merrin Spackman*: Large differences in mitochondrial haplotype diversity between closely related *Helicoverpa* moth species (J4).

2:50 p.m. *Lars S. Jermiin, Edgar R. Smith, Ross H. Crozier*: Directional mutation pressure and its interference with estimating DNA sequence divergence (J5).

3:10-3:40 p.m. **Afternoon tea/coffeee and trade displays**

Concurrent sessions

Chromosome Evolution ES4 Lecture Theatre (Chair: Chris Gillies)

3:40 p.m. *Anatoly Ruvinsky*: A new meiotic drive system in mouse chromosome 1 (K1).

4:00 p.m. *David J. Coates, Vicki L. Hamley*: Patterns of genomic change and hybridisation in the *Stylidium caricifolium* species complex (K2). No show!

4:20 p.m. *Michael J. Mahoney, S.C. Donnellan*: A mitochondrial DNA based phylogenetic hypothesis for polyploid speciation in the Australian desert burrowing frogs of the genus *Neobatrachus* (K3).

4:40 p.m. *Margaret Hurst, Dave Shaw*: Rapid and asymmetrical change to the structure of the chromosomal hybrid zone in the Australian grasshopper *Caledia captiva* (K4).

5:00 p.m. *David M. Rowell, A.V. Higgins, N.N. Tait, D.A. Briscoe*: Karyotypic evolution in the Onychophora and properties of chromosomal divergence indices (K5).

Conservation/Human Genetics Belshaw Lecture Theatre (Chair: Neil Murray)

3:40 p.m. *Andrea Taylor, Bill Sherwin, Bob Wayne*: Polymorphism at microsatellite loci in the endangered Northern Hairy-nosed Wombat (*Lasiorhinus krefftii*) (L1).

4:00 p.m. *Margaret Heslewood, Peter Baverstock*: Variation within myoglobin intron 2 in threatened populations of the Gouldian Finch (L2).

4:20 p.m. *Sheila van Holst Pellekaan, M. Frommer, B. Boettcher*: The characterisation of mitochondrial DNA in Australian Aboriginal populations (L3).

4:40 p.m. *June Roberts-Thomson, Barry Boettcher*: VNTR alleles and Australian Aborigines (L4).

5:00 p.m. *Karen E. Humphrey, G.A. Harrison, D.W. Cooper, A.N. Wilton, S.P. Brennecke*: No association found between pre-eclampsia/eclampsia and a HLA-G deletion polymorphism (L5).

5:30 p.m. **Annual General Meeting**
Belshaw Lecture Theatre

7:30 p.m. **GSA Conference Dinner**, Drummond College
Presentation of student awards by Oliver Mayo and trade representatives.

Wednesday July 6th

Joint symposium with Australasian Gene Mapping Workshop

Belshaw Lecture Theatre (Chair: Jay Hetzel)

8:30 a.m. *Chip Aquadro*: The recombinational landscape of the *Drosophila* genome (M1).

9:00 a.m. *David Baillie, Don Riddle, Ann Rose*: The impact of the *C. elegans* genome sequencing project on *C. elegans* genetics (M2).

9:30 a.m. *Lief Andersson*: Evolution of MHC class II polymorphism in artiodactyls (M3).

10:00- 10:30 a.m. **Morning tea/coffee and trade displays.**

Belshaw Lecture Theatre (Chair: Stuart Barker)

10:30 a.m. *Andreas Houben*: Application of microdissection of plant and animal chromosomes (N1).

10:50 a.m. *J.E. Broom, D.F. Hill*: The search for a molecular marker linked to ovine Batten's disease (N2).

11:10 a.m. *Phil Batterham*: How similar are fly genomes? (N3)

11:30 a.m. **Excursion**
Depart for New England National Park and Wollomombi Gorge.
Return via the airport.

Poster session in St Alberts College - 7p.m.

POSTERS

1. *Andrea Taylor, Deryn Alpers, Bill Sherwin*: Use of polymorphic microsatellites for scoring individual-specific genotypes from single hairs in the endangered Northern Hairy-nosed Wombat.
2. *Pascale Besse, Lynne McIntyre, Nils Berding*: Ribosomal DNA variations in *Erianthus*, a wild sugarcane relative.
3. *H.D. Campbell, C. Claudianos, A.B. Kasprzak, J. Hoheisel, K. Chen, J.R. Lupski, I.G. Young, H.G. de Couet, G.L. Gabor Miklos*: Molecular cloning of the human homologue of the *Drosophila flightless-I* gene, involved in gastrulation and muscle degeneration
4. *S.J. Marshall, G.K. Chambers*: Alcohol dehydrogenase and aldehyde dehydrogenase gene variants in the New Zealand population.
5. *S.J. Marshall, G.K. Chambers*: Alcohol dehydrogenase and aldehyde dehydrogenase gene variants in New Zealand alcoholic patients
6. *Charles Claudianos, Hugh D. Campbell*: The novel *flightless-I* gene brings together the gelsolin and leucine-rich-repeat gene families and provides a possible marker for the arrival of multicellular life.
7. *Lyn Cook*: Extraordianry chromosome number variation in the genus *Apiomorpha*.
8. *D.W. Cooper, P.G. Johnston, L.A. Hinds, W. Breed, D.L. Hayman*: The role of the X chromosome in sexual differentiation in marsupials.
9. *Michael Cunningham and Craig Moritz*: MtDNA variation in *Litoria nannotis*, an endangered rainforest frog.
10. *P.D. Driver, P. Trigg, N.D. Murray, I. Mansergh*: Approaches to measuring genetic variation in the Mountain Pygmy-possum (*Burramys parvus*) from field collected fur samples.
11. *P.K. Flynn, A. Masoumi, B.F. Cheetham, M.E. Katz*: Screening the *A. nidulans* chromosome-specific cosmid library.
12. *Kris Freebairn, Janet Yen, John McKenzie*: Genetic and environmental influences on the asymmetry phenotypes of the Australian sheep blowfly, *Lucilia cuprina*.
13. *E. Gale, M. Maugeri, P. Hoeben, P. Timms*: RAPD profiling of koala populations.
14. *Guo Guanglan, Alan Wilton*: Detection of carriers of the disease gene for ceroid lipofucsinosis in dogs.
15. *John S. Harvey, Paul V. Nelson, William F. Carey, C. Phillip Morris*: Detection and characterisation of mutations causing metachromatic leukodystrophy.
16. *Peter Hunt, Warwick Grant*: Dominant ivermectin resistance mutations.
17. *Sadia Kabir, Christopher Taylor, Ken Shepherd, Peter Langridge*: Mapping resistance to the cereals cyst nematodes in rye.
18. *Gawain McColl, Steve W. McKechnie*: Allelic variation at the HSR ω locus in *Drosophila*: implications for gene copy number.
19. *Z.-Z. Chen, Jon Martin, J.W.H. Trueman, P.S. Cranston, B.T.O. Lee*: The haemoglobin VIIB cluster of an Australian *Kiefferulus* species.
20. *S. McCleary, H.B. Allen, M.T. Ivanyi, N.D. Murray*: Use of DNA markers in the conservation management of the Helmeted Honeyeater *Lichenostomus melanops cassidix*.

21. H.D. Perkins, *A. Rimbawanto, G.F. Moran*: Polymorphism in the chloroplast genome of *Acacia mangium*.
22. Fiona M. Pyke, *Philip Batterham and John A. McKenzie*: Diazinon resistance in *Drosophila melanogaster*.
23. Charles Robin, *Robyn Russell, John Oakeshott*: Evolution of the α esterase gene cluster.
24. D.J. Schafer, *P.D. East, M.E. Katz, J.G. Oakeshott*: Structure and molecular evolution of the *Adh* genes of *Drosophila buzzatii*.
25. Deborah C. A. Shearman, *Marianne Frommer*: Sex-specific sequences of *Bactrocera tryoni*.
26. Sarah Sherson, *Chris Cobbett*: Genetic and molecular characterisation of an arabinose metabolism mutant of *Arabidopsis thaliana*.
27. Anna J. Small, *Meryl A. Davis, Michael J. Hynes*: Using *Sccharomyces cerevisiae* to detect *Aspergillus nidulans* nitrogen promoters.
28. *Jane Yeadon*, Amanda Taylor, *David E.A. Catcheside*: *Guest*: a 98 bp inverted-repeat transposable-element from *Neurospora crassa*.
29. Gowrie Thampapillai, *Peter Reeves*. DNA sequence evidence for recombinational repair in the *gnd* locus of *Salmonella enterica*.
30. Richard B. Todd, *Meryl A. Davis, Michael J. Hynes*: Analysis of the *facB* gene of *Aspergillus nidulans*.
31. Chatherine Turney, *Marianne Frommer, John Sved*: Cloning of *mariner*-like transposable elements of the Queensland fruit fly *Bactrocera tryoni*.
32. Rachel Turner, *Alan Wilton, Des Cooper, Shaun Brennecke, Clive Bunn*: Plasminogen activator inhibitor-2. A candidate gene for pre-eclampsia.
33. Patricia A. vanKuyk, *Margaret E. Katz*: *prtB*: an *Aspergillus nidulans* acid protease gene.
34. Yan-Hong Wu, *John B. Gibson*: Differential effect of insertions on the expression of the alcohol dehydrogenase gene in larvae and adults of *Drosophila melanogaster*.
35. Alex Andrianopoulos, *Carlos J. Gimeno, Gerald R. Fink, William E. Timberlake*: Control of *S. cerevisiae* pseudohyphal development by an *A. nidulans* developmental regulator.

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ABSTRACTS

PAPERS IN ORDER OF PRESENTATION
FOLLOWED BY POSTERS

H/nucleotide $= 0.004$ $(0.002 - 0.10)$ range

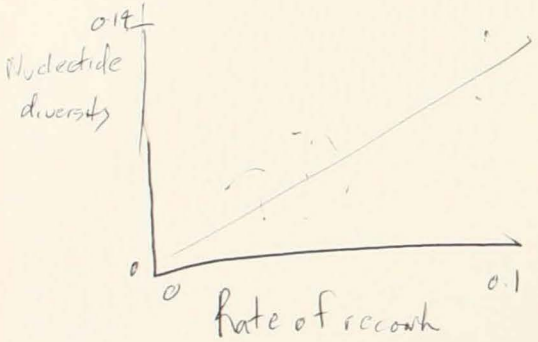
(prob that 2 nucleotides are different at a particular site)

$$H = 4 Ne \mu.$$

$$\text{Divergence} = 2t\mu + 4Ne\mu$$

t = time since speciation.

Selective sweep - fixation of a favoured gene will reduce neighbouring neutral variation
- decouples relationship between levels of variation within and between species



No relationship between % divergence & rate of recombination

? Do melanogaster & simulans have identical patterns of recombination?

Estimate that 1/700 substitutions are driven to ~~selection~~ fixation by selection

Rate of mutation to favoured variants $= 1.4 \times 10^{-12}$

?ACE
-underreplication of heterochromatin

A1

Selection, recombination, and DNA polymorphism in *Drosophila*

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We have recently discovered that levels of naturally occurring DNA variation are positively correlated with recombination rates in *Drosophila melanogaster*. I will discuss recent results from our lab which extend this early result, discuss competing models to explain the pattern, and discuss empirical approaches to distinguish among these models. I will present evidence from an analysis of DNA sequence variation at 15 gene regions distributed across the third chromosome of *D. melanogaster* that demonstrate that the correlation of variation with recombination is robust and is not due simply to the absence of variation in regions of extremely low recombination. With respect to population genetics models predicting such a correlation, examination of the level of sequence divergence between *D. melanogaster* and *D. simulans* and the amount of each gene region transcribed or coding sequence allow the rejection of the hypotheses that levels of mutation or strength of functional constraint cause the observed correlation. The elimination of new deleterious mutations may contribute to the lower levels of variation in regions of suppressed recombination. However, comparison of relative levels of variation on the third chromosome versus the X chromosome suggests that hitchhiking associated with relatively infrequent selective fixation of advantageous mutations plays an important role in the correlation. The frequency distribution of variation in each gene region, however, is not consistent with the simplest selective sweep model. Allozyme variation is not correlated with recombination rate, suggesting a decoupling of evolution at the DNA level and the protein level. The shape of the correlation between variation and recombination allows an estimate of the frequency and strength of selective sweeps needed to produce the observed pattern sampled. I will also discuss possible targets of selection and present evidence of different selective sweeps in different regions of the species range.

Mutation Rate Uniformity and Substitution Rate Variation Within and Between Mammalian Lineages

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Variation in mutation rate, attributed to differences in generation time and metabolic rate, has been invoked under the neutral theory of molecular evolution to account for differences in substitution rate among mammalian lineages. Using marsupials as the outgroup, we show that substitution rates at four-fold degenerate sites and sites in non-coding regions do not vary between the primate and rodent lineages. This implies mutation rate uniformity and argues against the effects of generation time and metabolic rate. In contrast, the substitution rates at non-degenerate sites vary both within and between lineages. This difference in substitution-rate pattern between the two types of site is incompatible with neutral theory but may result from substitutions occurring by fixation of slightly deleterious mutations. Variation in the rate of protein evolution among mammalian lineages appears to be due more to differences in population fixation rates than to biochemical or physiological differences affecting mutation rates.

Support for the theory that most mutations are slightly deleterious comes from the observed distribution of average heterozygosities in animal species. The latter does not follow the distribution predicted by neutral theory or from an overall positive selection coefficient, but rather follows that predicted if the average selection coefficient is weak in magnitude and negative in direction. That most mutations are slightly deleterious is expected if stabilising selection is the predominant evolutionary force. The slightly deleterious mutation theory also has important implications on the determination of genetic minimum population size in conservation biology, these will be discussed.

Nonsynonymous sites - higher substitution rate in rodents

"Protein structure, functional constraint, and the mode of DNA sequence evolution."

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One of the basic tenets of the neutral theory of molecular evolution holds that nucleotide changes at positions that change the amino acid sequence of a gene should be less frequent than changes at silent sites. The presumed fitness cost of changing an amino acid in a protein thus constrains DNA sequence evolution at those sites. In many surveys of nucleotide polymorphism and divergence, this pattern is the rule, and the force responsible labelled functional constraint or purifying selection. But we know that there are portions of many proteins that can change freely in primary sequence without an apparent change in structure or biochemistry. When we see no amino acid variation, is functional constraint always the best explanation? Analysis of the highly polymorphic EST6 and its homologues in *Drosophila*, in light of protein structural data and site directed mutagenesis may help us better understand the nature of functional constraint in enzyme evolution and how DNA sequences evolve under these constraints.

ANALYSIS OF THE INTERACTIONS BETWEEN THE DEVELOPMENTAL REGULATOR *abaA* AND ITS TARGETS IN *Aspergillus nidulans*.

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Asexual development in the ascomycete *Aspergillus nidulans* requires the action of a number of genetically defined regulatory genes of which the *abaA* gene represents the central component in the defined linear regulatory pathway. Loss of function mutations in the *abaA* gene lead to an arrest of development at the primary sterigmata stage (metula) and reiteration of this cell type.

The predicted amino acid sequence of *abaA* contains a new and novel DNA binding motif designated ATTS (also called TEA), which is also present in the mammalian transcription factor *TEF-1* , the *D. melanogaster* developmental regulator *scalloped* and the *S. cerevisiae* Ty1 regulator *TEC1*.

DNA-protein interaction studies have identified AbaA binding sites in the *cis*-acting regulatory regions of a number of developmentally regulated structural genes, as well as the *brlAα* and *wetA* genes which appear to be upstream and downstream regulators of asexual development, respectively. *In vivo* studies using a heterologous system have shown that these binding sites mediate AbaA-dependent transcription activation. A number of these genes are also targets for the *brlA* regulatory gene products.

These results and the predicted regulatory interactions involved in *A. nidulans* development are discussed.

Nice DNA binding assay
— ? recognition of AbaA binding sites - pAAG2 — where did it come from.

pyrimidine
CATTCT ~10 nucleotides TGAATG

— autoregulatory
— binds to its own promoter

Chemosensory Mutants of *Caenorhabditis elegans*.

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The major chemosensory organs of nematodes are a pair of specialised structures at the anterior end of the animal called amphids. They contain a unique class of neurons that are exposed to and presumably sample the external environment. Chemosensory mutants in *C. elegans* fall into two broad classes: those with readily detectable defects in the structure of the amphids, particularly the amphidial neurons, and those where the amphidial neurons appear to be intact but non-functional. The amphidial neuron defect can be readily assayed using lipophilic fluorescent dyes which stain the amphidial neurons in wild type but not mutant worms. In an attempt to rationalise the nomenclature, this class of mutants have been referred to as Dyf (Dye filling defective). During our work on resistance in nematodes to ivermectin (a widely used anti-nematode drug), we noted that many of our Avr (Avermectin resistant) mutants showed severely impaired chemotaxis and that ivermectin interfered strongly with chemotaxis in wild type worms. Further work showed that the major resistance locus is allelic with *che-3*, a major chemotaxis defective locus, and that the chemotaxis/osmotic avoidence mutants which show obvious amphidial defects (ie. are Dyf) are generally also Avr (ie. are AvrDyf). Similarly, the majority of Avr mutants are also Dyf, although there are some loci with Avr nonDyf as well as Avr Dyf alleles and other alleles where the Avr is dominant but the Dyf is not (see abstract by Hunt & Grant). This implies (i) that amphidial neurons are the primary point of entry for ivermectin (ii) that resistance is due to structural defects in these cells and (iii) the requirements for dye and drug entry to the amphids are similar but not identical. This is supported by the observation that *che-3* may encode a novel component of the unique cilia in the amphidial neurons (Grant & Whitington, unpublished). We have also examined several other phenotypes in these mutants and shown that they induce pleiotropic effects on feeding and reproduction, implying that chemoreception is important for these behaviours.

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Which Genes Specify Sensory Neuron Identity in the Drosophila Embryo?

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We are interested in the problem of how sensory neurons in the larva of the fruitfly *Drosophila melanogaster* come to acquire their specific identities during development. The Drosophila embryo is an attractive system in which to investigate the genetic basis for this process because the entire embryonic and larval peripheral nervous system (PNS) has been described at the level of individually identified cells. Several different classes of sense organs (SOs) are present in the larval PNS. Type I SOs, whose neurons have monopolar, ciliated dendrites, include the **e**xternal **s**ense (es) organs and the internal, **ch**ordotonal (ch) organs. Type II SOs, known as **m**ultiple **d**endrite (md) organs because their neurons possess non-ciliated, branched dendrites, have been subdivided into several classes on the basis of dendritic morphology: md-da organs have extensive **d**endritic **a**rborizations; md-td organs have **t**racheal innervating **d**endrites; while md-bd organs have **b**ipolar **d**endrites.

Current models propose that this stereotypic population of sense organs results from a complex series of genetic interactions. In the early embryo, the potential to form sense organs comes about through expression of proneural genes, including the *achaete-scute* complex (ASC) and *atonal*, in segmentally reiterated patches of ectoderm. Proneural gene action causes an individual cell within each ectodermal patch to become a sense organ precursor (SOP), but is currently thought to play no direct role in deciding which type of sense organ this cell will generate. This cell inhibits surrounding cells in the proneural patch from progressing down the neural pathway, through the action of neurogenic genes such as *Notch* and *Delta*. The resulting SOP undergoes further divisions to produce both the sensory neuron and its associated support cells. A further class of genes, the identity selector genes, including *cut* and *pox-neuro*, is thought to be responsible for specifying the identity of the SOP and its progeny.

One of the most important expressions of a sense organ's identity is the pattern of axonal projections made by its sensory neuron in the central nervous system (CNS). We have recently developed a method for staining individual sensory neurons in the embryo of Drosophila and have used it to reveal the central projections made by the complete population of sensory neurons in abdominal segments. Our knowledge of axon projections leads us to a reclassification of sense organ identity. The es organs and ch organs remain as discrete classes: es axons are restricted to a particular region within the CNS, albeit in a somatotopic fashion, while all ch axons project into a discrete longitudinal fascicle. However, we have reclassified the md organs. One subset, which includes most of the md-da and one of the md-td organs, show relatively homogeneous projections, growing into a particular longitudinal fascicle. The remaining md-da, md-td and md-bd organs represent a heterogeneous and separate group in terms of their axon projections.

Remarkably, this axon-based categorisation corresponds precisely with the identity of the proneural gene required for the formation of the sense organs, as indicated by the pattern of SOs found in mutants lacking function of the ASC and/or *atonal* genes. This correlation suggests that proneural genes may play a direct role in specifying the identity of the resulting SO. Under this scheme, an additional proneural gene is needed to differentiate between Type I (es or ch) and Type II (md) SOs. A combination of this hypothetical gene with ASC and/or*atonal* activity would suffice to specify all of the different classes of SOs. We plan to begin a search for this gene by screening for mutant embryos lacking the subclasses of md organs predicted by our model.

A Mutation in *Chironomus tentans* Fab. that leads to Gynandromorph and Mosaic Formation

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Gynandromorphic individuals were observed in a laboratory strain of *Chironomus tentans*, originally from near Winnipeg, Manitoba, Canada. Abnormal individuals display a range of phenotypes related to secondary sexual characteristics, from a slightly masculinized antenna to complete anteroposterior or bilateral gynandromorphism. The continued appearance of such individuals in this strain suggests that mutation is involved, while the occurrence of a range of phenotypes in the offspring of a single mating, suggests that it is possibly a single mutation. However, the mutation appears to have variable penetrance or expressivity since the mutants do not occur in any simple Mendelian ratio. Egg masses producing gynandromorphs or mosaics show a reduced fertility or an increased embryonic lethality, which may be associated with the trait. One of the most common phenotypes is an anteroposterior gynandromorph, i.e. male antennae and a female hypopygium, or the reverse. Individuals with a female abdomen appear to be sterile, but at least some of those with a male abdomen are fertile - if they can be induced to mate, since they exhibit female behaviour. The genetic sex of an individual can be assessed from the genotype for the enzyme malate dehydrogenase 1 (*MDH 1*) which is very closely linked to the primary sex determining locus in this stock. Amongst other things, use of the *MDH* genotype indicates that the hypopygial type of these gynandromorphs is not related to the genetic sex of the individual, as it is in the case of mermithid-parasite-induced intersexes.

More detailed analysis indicates that most specimens are mosaics rather than true gynandromorphs. An hypothesis that can account for most of the phenotypes observed is that the mutation leads to an increase in mitotic recombination, at least in the early embryonic divisions.

? Any Mdh-1 only ?

Estimating the Power of the Ewens-Watterson Test of Neutrality using Simulation

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The neutral hypothesis of evolution argues that the bulk of genetic variation observed at the molecular level in natural populations is selectively neutral. One of the advantages of the neutral hypothesis over the competing selectionist view is its predictive ability. This property has been used to construct a number of statistical tests to detect deviations from neutrality.

The most widely used of these methods is what has become known as the Ewens-Watterson test. This test examines variation at one locus and is applied to a single sample of genes from one population. The null hypothesis under test is the strictly neutral infinite-allele model, under which every mutational event is unique and every allele we see in the standing variation is absolutely neutral. The sample homozygosity, F, is the test statistic. For a sample of n genes of k different kinds (i.e., with k alleles), the probability distribution of F under the null hypothesis can be calculated. For example, with 200 genes of 5 alleles the expected value of F is 0.532, with 95% of the distribution lying between 0.28 and 0.89. In practice, population geneticists find their sample F, compare it with the appropriate interval, and claim evidence for heterotic selection for smaller values and purifying selection for larger values.

Unfortunately, the power of the Ewens-Watterson test can be disturbingly low. For example, we have constructed by simulation a series of 200 populations whose polymorphisms were maintained by selection. Only 8 of these populations, however, could be detected as being non-neutral with a sample size of 200 genes. Increasing the sample size did not improve matters either: 500 genes allowed only 5 detections. This research is discussed in light of recent results arguing that the Ewens-Watterson test is the best possible single-locus, allele-frequency based test.

The population genetics and origin of the sheep blowfly, *Lucilia cuprina*, in New Zealand.

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Lucilia cuprina was first reported in New Zealand in 1988. Maggot samples received from farmers during 1988-89 flystrike season confirmed the presence of this fly throughout most regions of the North Island. First records from the South Island were in February 1990. It's progress southwards has been tracked through a large trapping effort to the latest report in February 1994 at the mouth of the Rakaia River, mid-Canterbury. This is the most southerly report of this species anywhere in the world.

The arrival and subsequent spread of this pest in New Zealand has raised many questions which include; Where did the New Zealand population originate from? How long has it been here in New Zealand? and What is the level of genetic variation? This talk will discuss the variety of genetic approaches used towards answering these questions and how that data is to be used in an integrated pest management control program.

FACTORS AFFECTING N_e/N RATIOS IN THEORY AND IN WILDLIFE: CONSERVATION IMPLICATIONS

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The genetic effects of finite population size are predicted to depend on the effective size (N_e), rather than the census size (N). Consequently, the ratio of N_e/N is a critical parameter for wildlife management. Unequal sex-ratios, variance in family sizes, fluctuations in numbers over generations and selection are all predicted to affect the N_e/N ratio. Our experimental evaluations of the predicted effects of unequal sex-ratios, variance in family sizes and fluctuations in number over generations have validated theoretical predictions.

Until recently N_e/N ratios have been considered to lie in the range of 0.5-0.8 (Falconer 1989). A review of N_e/N ratios indicates that there is very wide variation in the ratio, with a value of 10^{-6} being reported. Species with high fecundity (and presumably higher variance in family sizes) have significantly lower N_e/N ratios than species with low fecundity. In species with low fecundity, there is a trend for lower ratios in species with harem mating systems. There is no direct information available on the importance of fluctuation in population size on N_e/N ratios in wildlife, but information on long term effective population sizes suggest that it causes important reductions. If deleterious genetic effects are to be minimised, populations sizes in wildlife need to be greater than previously recognised, especially in highly fecund species.

EFFECT OF INBREEDING WITH SELECTION ON LITTER SIZE OF QUACKENBUSH-SWISS MICE

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Quackenbush-Swiss(QS) mice are a highly prolific strain of mice with average litter size of 16.7 pups born alive. This strain has been maintained as an outbred population where the inbreeding was deliberately minimised. Some selection has been applied for increased litter size. Seven inbred lines (IQSs) have been created from QS by 20 generations of full-sib mating with strong selection for increased litter size.

Litter size information from these two populations of mice has been used to evaluate the effect of slow unintentional and rapid intentional inbreeding.

Genetic parameters of litter size for the QS mice were estimated using a repeatability animal model with the derivative free maximum likelihood approach. Since the inbreeding was done along with selection, the effect of inbreeding is confounded with selection. Fitting an animal model considering the level of inbreeding of the animal as a fixed effect enabled this confounding to be disentangled. Accordingly, the opposing effects of both inbreeding and selection could be quantified under circumstances of slow and rapid inbreeding.

As expected, inbreeding depression was higher in rapid inbreeding than in slow inbreeding. In IQS lines, the inbreeding depression was 0.51 pups per 10% increase in inbreeding coefficient, whereas in the QS population, it was 0.34 pups per 10% increase in inbreeding coefficient. Selection response was 0.12 and 0.31 pups per generation for QS and IQS respectively.

Comparable analyses were carried out with seven inbred lines separately. Despite the fact that they all originated from the same base population, each line has responded to selection and inbreeding somewhat differently. However, the overall trend was similar except for one line which became extinct at generation 8. At the end of 20 generations of inbreeding, the average litter sizes of existing lines ranged from 9.1 to 15.3 pups born alive.

Outgroup Heteroduplex Analysis using TGGE: Population Genetics at the DNA Sequence Level.

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Recently developed denaturing gel electrophoretic techniques (including TGGE) have the potential to detect differences between PCR products as little of as one base pair. Their ability to provide the resolution of DNA sequencing for a fraction of the time, effort and expense point to them as the logical successor to allozyme electrophoresis as the method of choice for population geneticists. Successful examples of their use for the bulk screening of samples needed for population genetics are hard to find, however.

Here I present a novel alternative to the standard TGGE/Heteroduplex Analysis protocol that I have found hugely increases sensitivity and is easily applicable to large numbers of samples. The approach relies on using amplified DNA from a closely related outgroup to the target species as a reference sample. PCR products of the chosen locus, from all individuals to be screened, are subjected to heteroduplexing with the reference. When run on an optimised temperature gradient gel, different alleles/haplotypes are resolved by their characteristic double-banded heteroduplex pattern.

Using this technique, I can routinely detect differences of as small as one base change in a 433 base pair fragment of Control region mitochondrial DNA from *Melomys cervinipes* (an Australian rodent). The idea can easily be (and is being) extended to nuclear loci. The theoretical basis of the empirically determined increased sensitivity of TGGE/HA when using an outgroup reference sample is unclear at this stage though I suggest a couple of possible explanations here.

The enormous strength of this quick and simple screening technique is that when coupled with targeted DNA sequencing it provides both frequencies of alleles/haplotypes and their phylogenies. This allows a sophisticated analysis of population genetic processes using both classical gene frequency analyses and coalescence theory based techniques – not possible using allozyme data.

Try using sheep/goat/pig? as outgroup for haplotype analysis of cattle.

Construction of evolutionary trees for equine breeds using microsatellite markers.

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Modern *Equus caballus* breeds originate from three ancient horse types:
1/ The Tarpan (or wild horse of Europe) which is believed to be the ancestor of the pony breeds of today;
2/ The "cold-blooded" European horse (Forest Horses) which is likely to be the ancestor of draft breeds; and
3/ The "hot-blooded" Oriental horse which gave rise to the racing and riding horse.

These horse types are easily distinguishable by appearance. Protein markers have been used to establish genetic relationships. The older breeds are more distinct while the modern breeds show closer relationships reflecting their common ancestry (Blokhuis and Buis, 1979).

Recently, Bowcock *et al.*, (1994) has constructed evolutionary trees of human populations by using a large number (30) of polymorphic microsatellites. Microsatellites are di-,tri, tetra- etc., nucleotide repeat polymorphic loci that are inherited. Microsatellites are currently used at the Victorian Institute of Animal Science for DNA fingerprint parent identification and sire registration on various species (eg. horses, cattle, dogs and ostriches). Horse microsatellites are being used to type a large number of loci for each horse from the three different horse types: heavy type horse breeds, light type horse breeds and pony type horse breeds. Using the method described by Bowcock *et al.*, (1994) microsatellite markers will be used to construct trees reflecting the known discrete *Equus caballus* populations and their evolutionary relationships.

Blokhuis, H.J. & Buis, R.C. (1979). *Anim.Blood.Grps biochem. Genet.* **10**:27-38.
Bowcock, A.M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J.R. & Cavalli-Sforza, L.L. (1994). *Nature* **368**:455-457.

Molecular Characterisation of the *facB88* Mutation of *Aspergillus nidulans*.

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The acetamidase-encoding *amdS* gene of *Aspergillus nidulans* allows the utilisation of acetamide as both a sole carbon and nitrogen source. As well as being subject to carbon and nitrogen metabolite repression, *amdS* is regulated by a number of specific induction controls[1]. One such specific regulator is *facB* which mediates acetate induction of *amdS* and genes required for acetate metabolism[2].

The *facB88* reciprocal translocation results in high level, constitutive expression (superactivation) of *amdS*. The translocation breakpoints lie within *facB* on chromosome VIII and a previously unidentified gene on chromosome IV, designated *amdX*. The two novel recombinant genes resulting from the translocation have been cloned (M.J. Hynes, unpublished). The 5'*facB*-3'*amdX* clone alone is sufficient for *amdS* superactivation and sequence analysis revealed that it contains the DNA binding domain of *facB* fused to a second DNA binding domain encoded by *amdX*. Mutagenesis, internal deletions and truncations of this clone followed by transformation studies are providing clues as to why this recombinant gene encodes such a potent activator of transcription.

To elucidate the function of *amdX* the gene has been cloned and sequenced. A gene knockout experiment is being attempted to determine the phenotype of an *amdX* null strain.

In vitro DNA binding studies using the AmdX DNA binding domain expressed as a fusion protein in *E. coli* have demonstrated that AmdX can bind specifically to a region within the *amdS* promoter. It is therefore likely that *amdX* is a native regulator of *amdS*.

References:
1. Hynes, M.J. and Davis, M.A. (1986) The *amdS* gene of *Aspergillus nidulans*: control by multiple regulatory signals. BioEssays **5**: 123-128.
2. Hynes, M.J. (1977) Induction of the acetamidase of *Aspergillus nidulans* by acetate metabolism. J. Bacteriol. **131**: 770-775.

ANALYSIS OF GENE EXPRESSION DURING COTTON FIBRE DEVELOPMENT

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The commercial cotton fibre is a product of Malvaceous plants of the species *Gossypium hirsutum* L. Cotton fibres are differentiated from single cells of the outer epidermis of ovules and originate at, or soon after, anthesis (1). Cotton fibre differentiation, characterised by a precise and synchronous growth and uncomplicated by cell division, is a suitable experimental system in which to study cellular and developmental events.

The fibre length largely determines the quality of the resulting spun thread. In view of this, the study of factors involved in controlling the extent of fibre growth is agriculturally important. A differential screening experiment was designed to identify mRNAs that are preferentially expressed in fibres. A fibre cDNA library was constructed in λZAP®II and 25,000 recombinant plaques were screened with labelled cDNAs from both cotton fibre and leaf. Four clones appeared to be fibre-specific and six clones appeared to have elevated expression in fibre tissue. In addition, many plaques did not hybridise to either probe but contained inserts tentatively ascribed to rare mRNAs (2). The tissue specificity and temporal expression of these three classes of clone have been investigated. Several clones have been sequenced in their entirety. A cDNA clone showing significant homology to plant translation elongation factors has been selected for further characterisation.

In addition, studies have shown that ribosomal RNA metabolism may be related to fibre development (3;4). There are many levels at which the accumulation of RNA may be controlled, and these are currently under investigation. Cotton varieties differing in their fibre lengths have been successfully cultivated and their lengths verified. Nucleolar sizes were measured in young fibres and correlated with final fibre length. The number of rRNA genes in each variety was measured by quantitative hybridisation to genomic DNA samples in comparison with known loadings of a cloned rDNA repeat unit.

1. Anderson, D.B. and Kerr, T. (1938). Ind. Eng. Biochem. 30: 48-54.
2. Hodge, R., Wyatt, P., Draper, J. and Scott, R. (1992). Plant J. 2: 257-260.
3. Peeters, M-C., Voets, S., Wijsmans, J. and DeLanghe, E. (1988). Annal. Bot. 62: 377-382.
4. Ramsay, J.C. and Berlin, J.D. (1976). Bot. Gaz. 137: 11-19.

Genome Evolution: Moving Genes by Site-Specific Recombination

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Gene cassettes are a novel class of mobile elements found in gram-negative bacteria which consist of only a gene coding region, usually an antibiotic resistance gene, and a specific recombination site, known as a 59-base element. Cassettes can be found as free closed circular molecules or inserted at a specific site within integrons. Integrons are the genetic elements which encode the DNA integrase responsible for cassette movement. The integrase catalyses both the insertion of cassettes into integrons and their excision. Recently we have demonstrated that the integron integrase is able to catalyse, at low frequency, recombination events between a 59-base element and several secondary recombination sites with the consensus sequence GNT. Recombination events of this type represent a potential mechanism for the movement of integron-associated gene cassettes to non-specific locations and hence for the evolution of bacterial genomes, and their plasmids and transposons, by gene acquisition. Evidence for the movement of a gene cassette to a secondary site in the wild has been obtained. A cassette which encodes an *aadB* gene conferring resistance to gentamicin and tobramicin, and is normally associated with integrons, has been found in a clinically isolated plasmid that does not contain an integron. The *aadB* cassette is inserted in a plasmid identical to the broad host range plasmid RSF1010. DNA sequencing demonstrated the presence of the complete cassette downstream of a known RSF1010 promoter and in the correct orientation for gene expression. By constructing models it is possible to conclude that insertion was via a single integrase-mediated recombination event between a free circular cassette and a secondary site with the sequence GAT. Deletion of the *aadB* cassette from this non-specific location could not be detected indicating that such insertions are likely to be stable.

Out of Anywhere?: Evolution of human mitochondrial (mt)DNA does not reflect the evolutionary history of humans; evidence from a comparative analysis of chimpanzees.

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The coalescence time of all human mitochondrial (mt) genomes is approximately 200 ka. There also appears to be more mt genome variation in Africa than in Asia or Europe. These observations have been interpreted as indicating an origin of modern humans (*Homo sapiens*) in Africa approximately 200 ka ago with subsequent expansion into Asia and Europe, with displacement of the previously resident *H. erectus* on all three continents.

This interpretation assumes selective neutrality of the entire (clonal) mt genome. The hypothesis of selective neutrality can be tested by comparing nuclear and mitochondrial genome diversity in humans and other related species. We present mt genome sequences and microsatellite allelic distributions for a large sample of unrelated chimpanzees. Comparison of these data with the equivalent data from humans shows that whereas humans have much greater levels of nuclear genome diversity than chimpanzees, they have much lower levels of mt genome diversity. The relative extent of nuclear and mt genome genome diversity in chimpanzees is similar to that seen in other primates.

These results suggest that the nuclear and mitochondrial genomes of humans have different evolutionary histories. Since the nuclear genome diversity estimate is based on many, independent loci whereas the mt genome is, in effect, one locus, it would seem that it is the mt genome result that is anomalous . The anomaly is explained by a mitochondrial genotype that arose about 200 ka ago and was then spread by natural selection throughout the human species. This interpretation implies that the spread of mt genomes was not associated with a spread of people. The new mt genome spread through existing populations that still exist and which originated from a common ancestor at a much earlier time.

A molecular analysis of a *cis*-acting mutation affecting the regulation of the acetamidase gene in *Aspergillus nidulans*.

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The acetamidase gene (*amdS*) of *Aspergillus nidulans* encodes an acetamidase which enables utilisation of acetamide as a sole nitrogen and carbon source. The *amdS* gene is subject to complex multiple control by a number of *trans*-acting regulatory genes. These regulatory genes mediate induction and repression of *amdS* expression in response to specific environmental stimuli. One positive acting regulatory gene, *areA*, regulates *amdS* in response to nitrogen limitation. AreA belongs to the family of GATA proteins identified in humans, mice, chickens *Xenopus*, *Caenorhabditis*, *Drosophila* and fungi. These proteins display high affinity binding to a sequence motif conforming to the consensus T/A(GATA)A/G.

A mutation (*amdI38*)has been identified adjacent and 5' to the *amdS* structural region which leads to an increase in AreA mediated *amdS* expression. The sequence was cloned by construction of a genomic mini-library and screened with a probe containing the *amdS* coding region. Sequence analysis has revealed a single base pair change at position -233 relative to the start point of translation creating a new GATAA sequence in addition to the five native potential GATAA sequences in the *amdS* promoter region.

amdS::lacZ reporter constructs were created to assay *in vivo* effects of the *amdI38* mutation on its own and in conjunction with another *in vitro* generated mutation which abolished a native *areA* DNA-binding site at -133. These constructs were introduced into *A.nidulans* by a two-step gene replacement. Analysis of these strains has indicated that *amdI38* leads to elevated *amdS* expression in the presence of the other *areA* binding sites. Mutation of the -133 GATAA reduces *amdS* expression and the *amdI38* mutation can supress the loss of this native site.

REGULATORY SIGNALS AROUND THE HIGHLY CONSERVED BIOSYNTHETIC *argA* GENES OF TWO RELATED *Pseudomonas* BACTERIAL SPECIES

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The biosynthetic and isofunctional *argA* genes, encoding N-acetyl glutamate synthase, of *Pseudomonas aeruginosa* and *P. putida* are very highly conserved (1). We have examined the DNA sequences of this pair of genes for potential regulatory signals such as promoters, ribosome binding sites (Shine-Dalgarno sequences) and transcription termination signals by computer analysis against consensus sequences in databases. By comparison with functional promoters of other *Pseudomonas* genes (2) the 5' end of *P. aeruginosa argA* had a single potential RNA polymerase σ^{70} recognizable sequence (-35/-10) whereas *P. putida argA* had two such sequences. With respect to sequences recognizable by the σ^{54} co-factor of RNA polymerase (GG-10 bp-GC) (-24/-12) two such sequences were found for *P. aeruginosa argA* but none for *P. putida argA*. The Shine-Dalgarno sequence -AGG- was found in both genes at the expected 5-12 bp 5' of the translational start codon. Analysis of sequences downstream of the termination codons revealed potential stem-loop structures for transcriptional termination of mRNA. Although the calculated free energy of these structures were within the range expected for termination they were nevertheless not typical when compared with that of rho-independent or rho-dependent terminators of other genes.

Of particular importance in this analysis was the identification of two potential σ^{54} recognizing promoters in the *argA* gene of *P. aeruginosa*. This is unusual as such promoters have not previously been identified in housekeeping genes (e.g. those involved in amino acid biosynthesis). This and the other characteristics of the potential regulating sequences need to be confirmed for their functionality by the identification of the transcriptional start sites by e.g. S1 mapping and by site directed mutagenesis.

1. Dharmsthiti, S. & Krishnapillai, V. (1993). J. Genet. 72:1-14.
2. Deretic, V. *et al* (1989). Bio/Tech 7:1249-1254.

Cloning and Methylation Analysis of the X-linked G6PD gene of Wallaroos (*Macropus robustus*)

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In marsupials, the paternally derived X chromosome is always inactivated in female somatic cells, and the degree of inactivation is tissue and locus specific. Random X inactivation occurs in eutherians, loci subject to inactivation are completely silenced.

CpG islands in promoter regions of X-linked loci of eutherian mammals are more methylated on the inactive X chromosome than the active X. The reverse methylation pattern is often observed in the body of the gene and in downstream sequences[1].

There are no sex differences in methylation in a CpG island in the first intron of G6PD of the Virginia Opossum, but the reverse methylation pattern was observed in the body of the gene[2]. This methylation pattern is also present in the HPRT gene of wallaroos[3]. The active X of wallaroos appears to be more methylated overall[4]. It has been suggested that since sex differences in methylation have only been found outside promoter regions, methylation plays no part in marsupial X inactivation[2]. However in the Virginia opossum, inactivation at the G6PD locus is incomplete. It would be informative to study the methylation status of the promoter region of a marsupial X-linked gene in which no expression of the paternal allele is observed.

We have isolated genomic and cDNA clones spanning the full-length of the wallaroo G6PD gene by library screening and PCR. The non-conserved 5' untranslated region of the wallaroo gene was obtained by RACE-PCR and used to locate the promoter region within a genomic clone. Like the eutherian G6PD genes, the upstream region of wallaroo G6PD contains a CpG island, although the island in the wallaroo appears considerably smaller than in humans. We are currently assessing the methylation status of the CpG island on the active and inactive X chromosomes. Preliminary results indicate that there are no sex-related differences at HpaII sites in this region. However, further analysis is necessary as it may be that methylation of only a few CpG dinucleotides is required for the maintenance of X inactivation.

1.Riggs, A. (1990) Aust. J. Zool. 37:.419-441.
2. Kaslow, D.C. and Migeon, B.R. (1987). Proc. Nat. Acad. Sci. 84:6210-6214.
3. Piper, A.A., Bennett, E.M., Noyce, L., Swanton, M.K. and Cooper, D.W. (1993) Somat. Cell. Mol. Genet. 19:141-159.
4. Loebel, D.A. and Johnston, P.G. (1993) Chromosoma 102:81-87.

Analysis of Predicted RNA secondary structure of the Human and Mouse *Xist* genes

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One of the two X chromosomes in somatic cells of female mammals becomes cytologically heterochromatic and genetically inactive early in embryogenesis. The cis-limited inactivation seems to be controlled by an inactivation centre, and a gene, *Xist*, has been cloned which may have this role.

The product of the *Xist* gene is a 17kb and 14kb transcript in human and mouse respectively. Despite this length there is no conserved ORF between human and mouse. Combined with the observation that the *Xist* RNA is nuclear localized and remains associated with the inactive X chromosome for most of the cell cycle it is suggested that the product of the *Xist* gene is a functional RNA.(Brown *et al* 1991, Brockdorff *et al* 1991)

Previous sequence analysis has shown there is a conserved direct repeat which has been detected in all eutherian mammals tested.(Hendrick *et al* 1993) This domain has homology to a domain in the *Xlsitr* in *Xenopus* which is involved in RNA localization in Oocyte and some homology to the *Biciod* RNA localization domain in *Drosophila*.(Kloc *et al* 1993) In order to further analyse the homology between this region and attempt to identify other regions of homology I have undertaken computer modelling of RNA secondary structure for the entire human and mouse genes using the Mfold minimum energy determination program (Zuker 1989). Due to technical constraints this analysis was performed in 3.5kb overlapping segments. The resulting structures were then compared using the Newtree program (Lee and Zuker 1991) and a pattern matching algorithm (Wakefield-unpublished) to detect conserved secondary structure.

Brown *et al* 1991,Nature 351:329-331.
Brockdorff *et al* 1991, Nature 349:82-84.
Hendrick *et al* 1993, Hum Molec Genet 2:664-672.
Kloc *et al* 1993, Science 262:1712-1714.
Zuker 1989, Science 244:48-52.
Lee and Zuker 1991, J Biomol Struct Dyn 8:1027-1044.

Mouse 14kb RNA
Human 17kb

Aims to Clone marsupial Xist - used as an outgroup for analyses

Significance of two classes of rDNA ITS sequences in parthenogenetic root knot nematodes (genus *Meloidogyne*).

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The plant parasitic nematode genus *Meloidogyne* contains some obligate mitotic parthenogenetic forms. These have a more extended host range than sexual types and infect a wide range of crops. We have previously shown that mtDNA haplotypes correlate with nuclear esterase markers, suggesting the parthenogenetic lineages arose separately from distinct but closely related females.

To test for additional congruence between nuclear and mitochondrial genes we have analysed by sequencing cloned PCR product some 60 or so nuclear ribosomal gene ITS (internal transcribed spacer plus 5.8s rDNA) from nine mtDNA haplotype groups. The phylogenetic structure among these ITS sequences essentially mirrors that of the mtDNA except that each of the parthenogenetic haplotypes have a second class of ITS about 10% divergent.

The presence of two allele 'families' could be due to i) amplification of paralogous pseudogenes, or ii) hybridization in the ancestory of the parthenogens. The two sexual individuals so far examined have only one type of ITS sequence, favouring the hybridization hypothesis.

Heteroduplex Analysis of Mitochondrial Haplotypes using PCR and Temperature Gradient Gel Electrophoresis.

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Analysis of population structure using mitochondrial DNA (mtDNA) has traditionally been time consuming (DNA sequencing) or low in resolving power (restriction site analysis). The advent of the polymerase chain reaction (PCR) has facilitated both approaches, but the ability to screen large numbers of individuals quickly, simply and with high resolution is completely satisfied by neither. I report here on our use of Temperature Gradient Gel Electrophoresis (TGGE) for screening PCR-amplified mtDNA with the potential to resolve haplotypes differing by a single nucleotide and the ability to screen large numbers of individuals in a matter of hours.

An Examination of Mitochondrial Evolution Using TGGE Analysis: Use of Australian *Rattus* as a model taxon.

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To fully exploit the wealth of information arising from recent technological advances in molecular biology we need to develop an empirical framework which is both robust and generally applicable. This would enable us to interpret the significance of new data and calibrate levels of variability within and between different taxa.

The Australian *Rattus* are an extremely well characterised group with an ideal hierarchical structure. Detailed analyses of chromosomal and allozyme evolution, interspecies hybrid fertility and morphometeric studies need only be complemented by DNA studies to make this an ideal model taxon.

This study focused on the evolution of the D-loop region of Mitochondrial DNA; a rapidly evolving loci, ideal for population studies. Variation within the D-loop was examined heirarchically by sequencing one individual from each species, subspecies, and each population (see figure 1).

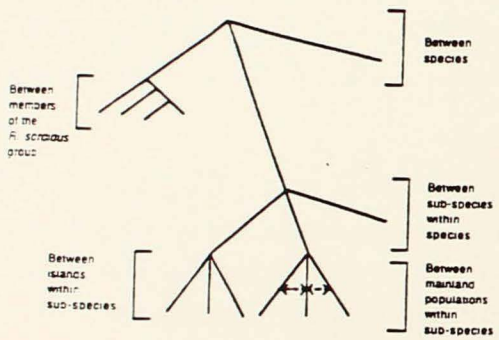

FIG 1 : Diagrammatic illustration of the hierarchical structure of the relationships among Australian *Rattus*. The broken lines indicate gene flow between taxa.

The populations studied occur on a number of isolated islands off the coast of South Australia and belong to the subspecies *R. fuscipes greyii* . These island populations were chosen because their extreme isolation, and relatively long time since separation from the mainland (14,000 to 6,500 years), provided an ideal system in which to study the micro-evolution of the D-loop. Screening of D-loop variation within the island populations was performed using Temperature Gradient Gel Electrophoresis (TGGE). Once calibrated, TGGE heteroduplex analysis is a quick and easy method for screening large populations whilst maintaining a high degree of resolution.

If outgroup is too distant, detection of substitutions declines. Best results with ~~Melomys~~ Rattus leucopus? – 40 substitutions in 400bp fragment.

Rabbit Metapopulation Structure in South-West Queensland.

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Current rabbit control measures in western Queensland are based on the management of individual warrens (eg. baiting of a warren system, release of myxomatosis at key warrens). Effective control will rely on the integrity of the warren as a local population unit and the degree of isolation of each unit, and therefore, it is necessary to define the level of scale, local or regional, at which rabbit population structure should be considered. Genetic markers obtained using allozyme electrophoresis and temperature gradient gel electrophoresis (TGGE) have been used to characterise the degree of structuring between rabbit populations, at two levels of geographic scale, in south-west Queensland.

At the local level, at distances up to 50 km, the allozyme data revealed no significant differences in allele frequency, no significant differentiation between sites (F_{st} values < 0.007, Rogers' genetic distance values < 0.06) and no isolation by distance or environmental discontinuity effects. Therefore, on a local scale rabbit populations appear to exhibit high levels of gene flow over large geographic areas. At a more regional scale, with sites separated by distances up to 1200 km, preliminary TGGE results suggest that no significant population structuring exists over a wide (1000 km^2) area in south-west Queensland. However, at one site in the far south-east (Mitchell) significant differentiation was detected. Allozyme data supports this finding.

These data suggest that current management strategies for rabbit populations in south-west Queensland may be inefficient if undertaken at a local scale because extensive gene flow occurs over large distances. Preliminary results imply that two different metapopulation systems may be active in the study area in western Queensland.

The Use of the Aldolase Gene Family as Potential Loci for Population and Evolution Studies.

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In order to efficiently use DNA polymorphisms as genetic markers, it is becoming increasingly necessary to have a foundation for comparison between loci. We are looking to establish a suite of genes which can be used for lower order phylogenetics and population genetics. Regions of interest are those which are highly polymorphic but are flanked by highly conserved regions amenable to the design of universal primers for PCR-based techniques.

Introns are expected to be highly polymorphic and hence be useful as genetic markers. The aldolase gene family consists of three functional genes (A, B and C) in mammals, all containing introns flanked by conserved regions. In a number of mammals, including humans and rats, an intronless pseudogene related to aldolase A has also been observed.

The Australian *Rattus* provide an ideal model species for this study as they are a well characterised group. Both chromosome and allozyme evolution data is available at the species, subspecies and population levels. Utilising primers which simulateously amplified multiple members of the aldolase gene family, we investigate the potential value of these loci for use in both population and phylogeny studies, using *Rattus* as the model taxa.

MOLECULAR POPULATION MARKERS FOR PENAEID PRAWNS

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The catch of wild Penaeid prawns in Australia is worth $250 million per year. In the battle to maintain sustainable harvesting, fisheries managers require basic information on the identification and definition of discrete prawn stocks. Genetic markers are powerful tools for detecting population subdivision and gene flow. However, they are also conservative in detecting the levels of subdivision and migration in which managers are interested, particularly in the marine realm. Rather than being simply an interesting extension of techniques, the development of more sensitive markers is thus critical in answering the sorts of questions that fisheries managers are asking. Both mitochondrial and nuclear DNA markers are being investigated in three species of Penaeid prawn. Using PCR techniques, promising population markers have been found by sequencing portions of the mitochondrial cytochrome oxidase I gene, and by examining VNTR variation in nuclear micro-satellites.

Penaeus esculentus — no allozyme variation over geographical range
merguiensis
plebejus - Eastern King Prawn

Mt DNA - P. esculentus - clear geographical subdivision of 3 haplotypes - western, northern, eastern (Moreton Bay)

- P. merguiensis - 1 haplotype only on west
- 3 other haplotypes in east

- P. plebejus - 8 haplotypes, but no clear pattern of geographical differentiation

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Microsatellite markers - large numbers of $(CA)_n$ repeats with long repeat length & high variability.
- primers are species specific !!

AUSTRALIAN GENETICS: A BRIEF HISTORY

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Since colonization Australia has had a productive history of endeavour in plant and animal breeding. However fundamental genetics was late in becoming established. Before the 1950s,there was no separate department of genetics in any university in the country. Reasons for the delay include geographical isolation, Australian and British colonial science policy and the lack of a 'critical mass' of researchers. Through the efforts of Ian Clunies Ross and the CSIRO, several prominent scientists were induced to come from overseas to set up a framework for an Australian-based genetics community. These founders have had a profound impact upon the foci set and the standards maintained by genetics researchers in this country. The educational structures set in place have been responsible for high quality genetics graduates being produced at a number of universities.

Reference

McCann, D.A. and P. Batterham (1993). Australian genetics: a brief history. Genetica 90: 81 - 114.

The Genetics of Wool Quality

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Wool quality is defined in terms of traits measured objectively (eg, fibre diameter distribution, tensile strength, staple length, amount of contamination) and subjectively (eg, colour, handle, style, weather damage, staple confirmation). There is no overall integrative measure that can be described as wool quality. The genetics of quality is investigated and applied to traditional quantitative methods, results of which will be presented.

Other approaches to the genetics of quality are beginning to be made. Their prospects will be discussed.

Studies of a Y-linked element in the pig.

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A cosmid clone isolated from a commercial porcine genomic library as part of a gene mapping program at the University of Sydney has been shown to detect both X-linked and Y-linked RFLPs in European breed of pigs. The Y-linked fragment is absent from male Chinese Meishan pigs. Fluorescent *in situ* hybridisation has shown that the element is present in the pseudoautosomal region of the X and Y, the telomere of the Y chromosome of European but not Chinese pigs, the centromere of chromosome 3, the centromere and telomere of chromosome 6 and the telomere of chromosomes 11 and 12.

The element is about 3 kb in length and the sequence has been substantially determined. It has no homology with any sequence in the Genbank database and in particular is not related to a family of porcine Y-linked sequences previously described. Preliminary analysis has revealed several long ORFs. Given the widespread and polymorphic distribution of the element, it will be very interesting to determine its function and mode of movement.

Check re NOR loci !!

Ribosomal RNA genes are necessary for X+Y pairing in Drosophila.

AN EVOLUTIONARY PERSPECTIVE OF THE GLUTATHIONE *S*-TRANSFERASES

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The glutathione S-transferases (GSTs) are a large multi-functional enzyme family. An array of these enzymes occurs in most aerobic organisms and are generally considered to serve in the dual roles of intracellular detoxication and as storage and transport proteins. Levels of expression vary between tissues, developmental stages and individuals. Certain forms of GST have elevated levels of expression in mammalian tumour cells, and have been associated with drug resistaence. GSTs have also been associated with an increased risk of cancer, in particular, an increased frequency of the GSTM1 deficiency has been found in lung cancer patients, stomach and colon adenocarcinomas and skin cancers. On the basis of biochemical and immunological characterisation, the cytosolic GSTs in mammals are divided into at least 4 evolutionary classes, termed Alpha, Mu, Pi and Theta. Additionally, definition of mammalian GST classes has included amino acid sequence similarities of at least 50%. However, between classes the similarity is thought to be only of the order of 30%.

In view of the recently available evidence from crystallographic studies that have shown three dimensional structural similarities among representative enzymes from each of the Alpha, Mu and Pi classes and of the increasing number of GST sequences available, this study was initiated to gain a greater understanding of the evolutionary relationships of the GSTs.

Multiple alignment of the GST sequences, incorporating evidence from the recent studies of 3D structure, has provided the basis for an evolutionary analysis. An early duplication of an ancestral GST gene and subsequent radiation of GSTs both within and between species is indicated. This duplication apparently occurred prior to the plant/animal split as sequences from animals appear in both sectors of the tree. Evidence also supports a pre-vertebrate origin for the Alpha, Mu and Pi classes which were originally defined in mammalian species.

Comparison among the human Mu class sequences indicates complex evolutionary relationships suggesting multiple gene conversion events have occured.

P ELEMENT-INDUCED MALE RECOMBINATION CAN BE PRODUCED BY COMBINING END-DEFICIENT ELEMENTS IN *DROSOPHILA MELANOGASTER*

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The P elements of *Drosophila melanogaster* are the best characterised eukaryotic transposon, however many of their molecular mechanisms have yet to be elucidated. P elements have been shown to be a cause of the "hybrid dysgenesis" syndrome, characterised by high mutation rates, gonadal sterility, segregation disorder, and male recombination. It is known that incomplete P elements at exactly homologous sites can produce up to 20% male recombination within the chromosome arm on which they are located, whereas single elements only induce about 1% recombination.(Sved et al., Mol. Gen. Genet., 1991) We have used this system to test the effects of deficient P element ends on male recombination.

We used the procedure outlined by Johnson-Schlitz and Engels (Mol. Cell Biol., 1993) of screening for derivatives following mobilisation of a particular P element insertion. The P[CaSpeR] element insertion at 50C was used and screened for deletions. Of the 64 chromosomes lines produced, a number were found to have deficiencies in either end of the element, while others had internal deletions with both ends remaining intact. Here we discuss recent results in which two elements at homologous sites with opposite ends deficient combine to induce the same high levels of recombination as produced by elements with complete ends. Furthermore, each end-deficient element is unable to induce male recombination on its own or in conjunction with another element deficient for the same P element end. This leads to a model in which the combination of ends from different P elements can function normally in excision and insertion resulting in recombinant chromosomes.

We have also investigated the effects of combining end-deficient elements with the complete P[CaSpeR] element. High levels of recombination resulted, and results in this case were consistent with the model of excision followed by repair from the region of the end-deleted element. Although distinct, these models are not mutually exclusive. We will discuss the relationship between the models and circumstances which may allow one mechanism to dominate over another.

Crossing over within the nucleolar organizer regions of wheat

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In hexaploid wheat (*Triticum aestivum* L. em. Thell.), there are two major *Nor* loci, *Nor-B1* and *Nor-B2*, located at the junction between the short arm and satellite of chromosomes 1B and 6B, respectively. Each locus contains from hundreds to thousands of copies of rRNA gene sequences separated by lengths of spacer DNA. While the rRNA genes are highly conserved in sequence (and contain sections that are recognizable from the Archaea to Bacteria to Eukaryotes), the intervening non-transcribed spacer DNA sequences are comprised of varying numbers of short subrepeat DNA sequences, polymerase I promoters, and transcription initiation and termination signals. Because these spacers show such variability in sequence and length, they can be used to identify allelic variants of the *Nor* loci.

In wheat, a total of eight alleles of the *Nor-B1* locus on chromosome 1B, and twenty alleles of the *Nor-B2* locus on chromosome 6B have so far been identified. These variants are recognized by the presence within them of differing proportions of differing lengths of intervening spacer DNA fragments. The spacers differ in length by multiples of 135 bp, indicating a simple decrease or increase in the number of subrepeats of that length within different spacers. At the *Nor-B2* locus, individual spacer fragments contain from 5 to 35 subrepeat units and at the *Nor-B1* locus, spacers containing from 11 to 17 subrepeats are present. It is thought that these differences are due to DNA strand misalignment during replication. However, although the mean number is slightly more than two, up to 10 differing length spacers have been found within a single locus (e.g. *Nor-B2b)*. These show the presence of differing numbers of rRNA repeat units of differing lengths resulting in a different number of rRNA genes within each variant. Such changes in the number of different rDNA repeat sequences in different alleles are clearly caused by the occurrence of crossing over within the *Nor* locus itself. While alleles are normally inherited as specific loci, the formation of allelic variants by crossing over between arrays of differing length rRNA repeat units is a not uncommon event during the evolution and breeding of wheat - as will be illustrated from a study of Chinese and Australian wheats.

TRANSPOSITION-INDUCED MUTATION: CAN IT BE USED TO GENERATE SAFER GENETIC VARIABILITY?

(Some lessons from Drosophila)

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Results of several short term selection experiments, with Drosophila, in which effects of contemporaneous bursts of P- transposition on quantitative traits in selected lines are examined, are reviewed. Substantial selection responses attributable to transpositional mutation were observed in the experiments which were properly designed.

Furthermore, an X-linked major mutation appeared in one of the selection lines. The mutation, later called tonock (tnk), causes a large reduction in abdominal bristle number. Further analysis showed that: tonock complements a scute mutation and a deletion, it reverts back to the wild-type phenotype at a frequency of 6.8 % in dysgenic crosses, and maps approximately 0.4 cM proximal to the yellow locus. There were two new P-element insertions, revealed by in situ analysis, near the tip of the X chromosome, suggesting the 2A-C site as the possible location of the mutation. The occurrence of the mutation in a line which was originally derived from a non-dysgenic cross confirms temporal destabilisation of the P cytotype in such lines. This questions the validity of these lines as transposition negative controls. High levels of fluctuating asymmetry for abdominal bristle number, in the tonock, indicates that the mutation creates developmental stress for this character.

The phenomenon of transposition is discussed with respect to its likely application in animal breeding and/or conservation, as a potential safer source for induction of genetic variability than conventional mutagens, where depletion of genetic variation is a problem.

Using information from allele frequency and phylogeny to define units for conservation and management

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Genetic information can be used in two ways to define units for conservation and management. One is to define "Evolutionary Significant Units" (ESUs), the conservation goal being to identify populations that represent a substantial component of the species' evolutionary legacy. The other is to define "Management units" (MUs), demographically-closed populations that should be the basis for population monitoring and manipulation. These two applications differ markedly in concept and practice. ESUs are recognised primarily to contribute to conservation strategy and the setting of priorities, both long-term management issues. Information on both allele phylogeny and frequency should be used to define an ESU, a possible qualitative criterion being monophyly for mitochondrial DNA alleles and significant divergence of nuclear allele frequencies. Although such a definition may be overly restrictive in some cases, it has the advantage of being based soundly on theory and avoiding the issue of "how much divergence is enough?" that plagues quantitative criteria. MUs are delineated in order to assist with the short-term management of the components of an ESU. These are best defined by allele frequency data, the criterion for an MU being significant divergence of allele frequencies for mtDNA alleles, nuclear alleles or both. The distinction between ESUs and MUs will be illustrated with examples including kangaroos, elephant seals, marine turtles and coconut crabs.

Extreme population structuring in the threatened Ghost Bat, *Macroderma gigas* : evidence from mitochondrial and nuclear DNA.

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The Ghost Bat, *Macroderma gigas* , has undergone a major range contraction and is currently restricted around a few, highly disjunct maternity sites. The amount and distribution of mitochondrial DNA (mtDNA) variation within extant populations has been used to assess levels of current and historical maternal gene flow between those populations. An approximately 330bp fragment of mtDNA spanning a hypervariable area of the control region, was amplified and sequenced using 22 individuals from four current Ghost Bat populations. The mean sequence diversity of 4.5% between populations was six times higher than that within populations (0.68%) and alleles within populations were found to be monophyletic. Restriction enzyme analysis of amplified products from an additional 100 individuals revealed fixed allelic differences in the distribution of control region genotypes between the four populations. The amount and distribution of nuclear DNA variation has also been examined by screening all samples from the four populations across six variable microsatellite loci. It is suggested that this extreme genetic subdivision makes each population an independent entity and is a consequence of long-term female philopatry.

Population structure of the Yellow-footed rock-wallaby inferred from mitochondrial DNA and nuclear microsatellites

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The yellow-footed rock-wallaby, *Petrogale xanthopus* (Gray, 1854), is considered to be potentially vulnerable (Kennedy, 1992). The occurrence of this wallaby on isolated rocky outcrops is thought to restrict dispersal between populations, but the extent to which that occurs is unknown.

Tests for genetic differences between populations were performed using both sequence divergence estimates provided by mitochondrial DNA sequence analysis, and the distribution and frequencies of microsatellite and mitochondrial alleles among four populations. This is one of the first uses of microsatellites for this purpose. The populations analysed were selected to test for genetic differentiation at different geographic scales.The proportion of genetic variation distributed among populations of the yellow-footed rock-wallaby was high in comparison to other similarly distributed species. Sequence divergence between Queensland and South Australian populations was high, ten times that found within Queensland. This suggested that these two populations were phylogenetically distinct, supporting the classification of these two groups as separate evolutionary entities.Variation in mitochondrial allele frequencies indicated significant, but recent divergence between populations separated by 70 km. High levels of homogeneity and shared mtDNA alleles between the two populations studied separated by 10 km reduced the power to test for restricted dispersal.

The distribution and frequencies of microsatellite alleles indicated that each population studied, even the two populations within Idalia National Park, was genetically distinct. Trapping position data and behavioural observations were used to establish potential breeding or family groups within a population. Analysis of these groups showed little evidence of structuring. This supported the analysis of populations at the colony level and also indicated that the effects of breeding tactics on genetic structuring, if present, were minor.

These results suggest that dispersal between colonies is infrequent over ecological time and may be influenced by both habitat and behaviour. Lack of subdivision within the colony studied indicated panmixia, thus discrete colonies, even those joined by hospitable habitat, can be considered as single units.

Male-mediated gene flow in marine turtle populations; the story from microsatellite alleles

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Most of what we know about marine turtle genetics and population divergence comes from studies of mtDNA which has been an appropriate focus for the conservation of nesting populations. But to answer broader questions of gene flow and for an understanding of male migratory behaviour, we have identified marine turtle microsatellites and analysed their variability across populations. We designed six sets of polymerase chain reaction (PCR) primers from three species of marine turtles and tested them across the two extant marine turtle families, thought to have diverged 100-150 million years ago. We found an extreme conservation of flanking sequence that allowed amplification across all marine turtles, and a persistence of polymorphism in most cases (even in a freshwater turtle). Using these primers, we have compared microsatellite allele divergence to previous data on mtDNA haplotype variation among marine turtle populations in Australia. In particular, we have focused on 8 populations of green turtles from three regions; Western Australia, Gulf of Carpentaria, Northern Great Barrier Reef and Southern Great Barrier, for which we have extensive mtDNA data. Results from these studies will be presented indicating a more complex story than identified by a previous comparison of global variation in nuclear and mtDNA sequences.

Evolutionary distinctiveness and genetic population structure of the endangered Lake Eacham rainbowfish, *Melanotaenia eachamensis* and its relative *M. splendida*

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Abstract

The Lake Eacham rainbowfish is thought to be extinct in the wild and is the focus of a captive breeding program. However the distinctiveness of *M. eachamensis* from the more widely distributed *M. splendida splendida* has been questioned. We have used a combination of mitochondrial DNA sequences and RFLP data to analysis the genetic structure of captive *M. eachamensis* 's stocks and its relationship to *M. splendida splendida* from the Atherton Tableland and elsewhere. Phylogenetic analysis of sequences revealed that *M. eachamensis* mtDNA is very distinct from that of most *M.splendida splendida* (all populations but Tinaroo, Euromoo and several small creeks in Atherton Tableland) and is closely related to that from a western subspecies, *M. splendida australis*. This suggests that *M. eachamensis* represents an evolutionary lineage distinct from eastern *M. splendida*. More extensive surveys using RFLP markers identified from the sequences revealed the "eachamensis-type" mtDNA in several populations of fish, some phenotypically resembling *eachamensis*, but most with *splendida* phenotypes. This suggests a history of hybridization between the eastern *splendida* and western *eachamensis* lineages, perhaps following headwater capture of the Barron River. We are also examining the distribution of nuclear polymorphisms in relation to mtDNA genotypes to further test the genetic composition of natural populations resembling *eachamensis* and to test for disequilibrium with mtDNA markers. The relevance of these data to conservation priorities and future management of the Lake Eacham rainbow fish will be discussed.

Molecular Systematics of the Australian Waterfowl

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A consensus classification for the waterfowl (order Anseriformes) has never been reached. There have been many revisions of the relationships within the order including those of the monotypic Australian genera. The Southern Hemisphere anseriforms comprise a large number of monotypic, endemic species which have traditionally been linked to the established genera and tribes of the Northern Hemisphere. More recently, however, with the recognition of endemic Australian radiations of marsupial mammals and passerine birds, the affinities of the six monotypic Australian genera of anseriforms have been questioned. In particular, whether they are more closely related to one another, or whether some or all of these monotypic genera have closer affinities with the Northern Hemisphere genera. Classification of the taxonomic relationships of the aberrant Australian endemic species may also provide confirmation or refutation of the recently advanced hypothesis of a Southern Hemisphere origin for the Anseriformes.

A 307bp fragment of the mitochondrial cytochrome *b* gene of the 19 native Australian anseriforms and four Northern Hemisphere species was enzymatically amplified by PCR and manually sequenced. The Chicken (*Gallus gallus*) and Muscovy Duck (*Cairinia moschata*) cytochrome *b* sequences were obtained from Genbank.

The patterns of evolutionary dynamics within the cytochrome *b* gene of Anseriformes appear to conform to those reported in studies of avian and other vertebrate mtDNA. A new phylogenetic classification for the Anseriformes is proposed. The phylogenetic trees generated in this study indicate that the monotypic Australian genera, the pygmy-geese and the swans and geese are members of the subfamily Anserinae which appears to represent a Southern Hemisphere radiation. Within the Anserinae, the Cape Barren Goose and Freckled Duck link most closely with each other, the Pink-eared Duck appears to be closely related to the true geese, the Musk Duck is a sister taxa to the Pink-eared Duck and may be less closely related to the *Oxyura* than previously thought and the Magpie Goose is the most divergent member of the Anseriformes included in this study. The Maned Duck and the remaining native Australian anseriforms are members of the established European genera and tribes of waterfowl. These species probably represent a secondary radiation of recent Northern Hemisphere invaders of Australia. Finally, the data provides some support for the theory of a Southern origin for the Anseriformes.

ANCIENT DNA FROM *THYLACOLEO*, AN EXTINCT MARSUPIAL: IS IT A POSSUM OR A WOMBAT?

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Thylacoleo carnifex, the Marsupial Lion, became extinct about 20 thousand years ago. It is an extinct member of an Australian marsupial group named diprotodontian marsupials. The extant members of the diprotodontian marsupials include possums, kangaroos, koalas and wombats. The positioning of *Thylacoleo* amongst the diprotodontians is conjectural. This is due to its conflicting morphological characters which have proven to be a great challenge to palaeontologists. The tooth formula and jaw musculature of thylacoleonids bears a great resemblance to phalangeroids (possums), as does the foot structure. Conversely, the structure of the tympanic bulla of *T. carnifex* consists of the same bone as vombatoids (the Koala and wombats), and not phalangeroids.

An independent technique with which to assess the relationships of *Thylacoleo* is necessary to solve this problem. In recent years it has become apparent that fragmented DNA molecules survive the ravages of time and are retrievable from exceptionally preserved specimens. DNA sequences covering a specific site of the mitochondrial cytochrome *b* sequence of diprotodontian marsupials differentiates vombatoids apart from phalangeroids. DNA sequence of this site of *Thylacoleo* DNA will provide support for its phylogenetic placement amongst diprotodontian marsupials.

DNA has been successfully extracted and amplified from fossil *Thylacoleo* bones excavated from Victoria Fossil Cave, Naracoorte, S.A., aged between 50 thousand to 150 thousand years old.

Post-glacial expansion and subdivision of the grasshopper *Chorthippus parallelus* in Europe.

by Steven Cooper† and Godfrey Hewitt

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Data from fossil pollen and insect distributions suggest that during the last ice age (18,000 BP), many species that are now common and widespread over Europe may have survived only in sheltered refugia in southern parts of Iberia, Italy and the Balkans. In these isolated regions they would have evolved in allopatry over many thousand years, accumulating independent genetic differences, and then following climate amelioration expanded northwards when suitable habitats opened up again. Such cycles of range contraction and expansion, and allopatric evolution associated with ice ages are likely to play a significant role in the process of divergence and speciation.

Using the grasshopper *Chorthippus parallelus* as a model species, these hypotheses were examined by investigating the spatial distribution of an anonymous 300 bp non-coding nuclear DNA marker sequence (Cpnl-1). DNA sequences were obtained, using a Pharmacia ALF auto-sequencer, from over 300 grasshoppers from populations located across the species range in Europe. Automatic and manual processing of sequence chromatograms revealed 71 Cpnl-1 haplotypes. Phylogenetic analyses using distance and parsimony procedures and estimation of genetic differentiation (K_{st} values) between populations provide evidence for subdivision of *C.parallelus* into at least 5 geographic regions corresponding to major refugial regions in Europe. In addition, these results indicate that the French form of *C.parallelus* originated following range expansion of the grasshopper across northern Europe from a Balkan refugium.

These results emphasize the important role played by major climatic changes in the maintenance and generation of biodiversity.

Large differences in mitochondrial haplotype diversity between closely related *Helicoverpa* moth species

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The native budworm, *Helicoverpa punctigera*, and the bollworm, *Helicoverpa armigera*, have been sampled Australia-wide for DNA sequence variation over 250bp of the control-loop, or A+T-rich region, of mitochondrial DNA (mtDNA). Despite high levels of homology of this sequence between the species, and similar movement and breeding patterns, very different levels of genetic variation were detected. In *H.punctigera* 14 haplotypes occurred over 96 individuals, one common type predominated, and the effective number of 'alleles' was 1.7. Any two haplotypes, on average, were very similar in sequence, differing by only 1.1 bases of the 250 sequenced. In the less vagile species, *H.armigera*, 33 types were detected over 113 individuals, three or four types were common throughout the species range and the effective number of 'alleles' was 9.9. Two average haplotypes differed by 2.3 bases. We discuss possible causes of these differences, including differences in mutation rate, differences in effective population size and a recent selective sweep of a favourable mtDNA mutation through the species with low diversity, the native budworm.

Directional mutation pressure and its interference with estimating DNA sequence divergence

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THE nucleotide composition in metazoan mitochondrial DNA (mtDNA) is generally A+T biased. This is believed to be due to A+T pressure acting over several hundred million years[1]. Using mtDNA as data, studies of the rate of evolution and of the phylogeny among metazoan taxa often apply the one[2]– and two[3]–parameter substitution models. These models assume an unbiased base composition and thus may be unsuitable for the analysis of mtDNA. However, the degree to which the biased base composition affects estimates of DNA sequence divergence is as yet unknown.

Two new models for estimation of DNA sequence divergence are presented. The models extend the existing one– and two–parameter models to circumstances where the DNA is A+T or G+C biased. The new models are compared with the old models[2,3], and it is concluded that the latter overestimate DNA sequence divergence when the DNA is A+T or G+C biased. The one–parameter model[2] can produce overestimates of the rate of nucleotide substitutions per site of up to 50% whereas those produced by the two–parameter model[3] can reach infinity. We therefore conclude that directional mutation pressure will bias estimates of evolutionary distance and, where species are markedly heterogenous with respect to base composition, will lead to topological errors in phylogenies inferred using DNA distance methods. In addition, the result has a bearing on the molecular clock theory.

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1 Jermiin LS, Graur D, Lowe RM, Crozier RH (1994a) Analysis of directional mutation pressure and nucleotide content in mitochondrial cytochrome *b* genes. J Mol Evol 39:000–000 (in press)

2 Jukes TH, Cantor CR (1969) Evolution of protein molecules. pp 21–132. In: Munro HN (ed) Mammalian protein metabolism III. Academic press, New York, pp 21–132

3 Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120

A new meiotic drive system in mouse chromosome 1.

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Aberrant chromosome 1 carrying an inverted fragment with two amplified homogeneously staining regions was isolated from wild populations of *Mus musculus*. Meiotic drive causing a preferential passage of this chromosome was demonstrated for heterozygous females. Genetic analysis allowed to identify a two-component system conditioning a deviation form equal segregation. The system consists of a distorter and responder, which is associated with the inserted region. The distorter is located on chromosome 1 distally to responder, between *In* and *Pep-3* genes, and acts when in *trans* position. Polymorphism of the distorters is expressed as a variation in their effect on meiotic drive level in the laboratory strains and in wild populations. The specific features of the aberrant chromosome include increased mortality of homozygotes and decreased fertility of homozygous females. The obtained data indicate that chromatid segregation in heterozygous females depends upon which sperm enters the oocyte before the second division. The drive is powerful when sperm cells bear normal chromosome 1, and segregation normalises, during MII, when it is sperm bearing aberrant chromosome 1. Computer simulations of the dynamics of aberrant chromosome shown possibility of its spreading and stable genotypic equilibrium. The frequencies determined by computer analysis agree well with those obtained for studied natural populations. The evolutionary pathway for the origin and fixation of the aberrant chromosome 1 in natural populations are considered.

Distortion present in blastocysts.

Homozygous ♀ ~10% fertility

Homozygous ♂ & ♀ ~40% viability.

Distortion bearing sperm suppress the distortion phenomenon in heterozygous ♀♀.

PATTERNS OF GENOMIC CHANGE AND HYBRIDISATION IN THE *STYLIDIUM CARICIFOLIUM* SPECIES COMPLEX.

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S. caricifolium complex taxa are diagnostically differentiated by a range of chromosome re-arrangements, are generally well differentiated allozymically and exhibit unusually high levels of allelic diversity. Within taxa significant levels of allozyme divergence are found between adjacent populations and major temporal shifts in allele frequencies can occur within populations over a few years.

The two most closely related taxa *S. affine* and *S. caricifolium* have extensive north/south distributions and are known to come into close parapatry or sympatry in three regions. In the northern region where the taxa are sympatric there is little overlap in flowering time, and chromosomal and allozyme data indicate limited hybridization and introgression. In contrast, in the southern region there is substantial overlap in flowering time, reduced allozyme divergence among allopatric populations between taxa and the data indicates that hybridization is extensive and ongoing . The contrasting patterns in the two regions generate various hypotheses with two alternatives worth particular consideration. In the first increasing genomic divergence between the two species occurs in a south-north direction and this is reflected in the increased adaptive divergence of the species in the northern populations relative to the southern populations. In the alternative, hybridisation has occurred in a north-south direction. This might imply that sympatry in the north is older than in the south, and the level of adaptive divergence of the species in the north is greater because of the greater level of genomic divergence which might follow selection minimizing hybridisation during prolonged sympatry.

In the third region a group of *S. affine* like populations are located adjacent to *S. caricifolium* and appear to be of hybrid origin . Unique allelic combinations suggest that novel mutational capabilities may be associated with this taxon's apparent hybrid origin. Similarly, the morphologically, chromosomally and allozymically distinct Wilroy taxon suggests that adaptively novel genomes may be generated in small isolated populations which are subjected to elevated levels of inbreeding.

A mitochondrial DNA based phylogenetic hypothesis for polyploid speciation in the Australian desert burrowing frogs of the genus *Neobatrachus*.

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Speciation by polyploidy is rare in animals, yet, in vertebrates, there is a disproportionate concentration of polyploid species in anuran amphibians. The Australian desert burrowing frogs of the genus *Neobatrachus* are a complex of diploid and tetraploid frogs whose evolutionary histories have not been resolved. Importantly the mechanisms of origin of the tetraploids (allopolyploid or autopolyploid) have not been resolved by studies of chromosomes or allozymes. In this genus six species are diploid and four tetraploids, and several cryptic diploid-tetraploid species pairs are recognised. Furthermore several diploid and tetraploid taxa are known to hybridize in various combinations. To examine these questions, we sequenced a portion of the cytochrome *b* gene of the mitochondrial DNA to determine phylogenetic relationships among the species. The mtDNA phylogeny was used to examine the specific question: Did the tetraploid species, have a single origin from one lineage, or multiple independent origins from two or more lineages? Two populations of each species were included with the exception of the following species where only one population was studied, *N. albipes* (2n), *N. pelobatoides* (2n), and *N. kunapalari* (4n), and species from two genera placed in the same subfamily were used for outgroup comparison. For polyploids we sampled one population as far away from any diploid population as possible and the second adjacent to neighbouring or sympatric diploids, to test the possibility of directional hybridisation. At least two specimens from each population were studied. Preliminary data from 250 bp of this gene were analysed phylogenetically using maximum parsimony. A consensus cladogram of minimum length trees shows three lineages, 1) *N. albibes* (2n), 2) *Heleioporus psammophilus* (one of the predicted outgroups) - *N. aquilonius* (4n, Pt Headland population) and *N. kunapalari* (4n), and, 3) the remaining diploids and tetraploids. Relationships within lineage two were not resolved, and within lineage three the tetraploids were monphyletic and the sister taxa to the diploids. These relationships were not resolved. It appears that polyploidy has arisen at least twice in *Neobatrachus*. The placement of *H. psammophilus* raises the of question whether one of the polyploid events has invloved intergeneric hybridisation? There is no clear indication which of the diploid species of *Neobatrachus* was involved in the origin of the polyploid species. It is apparent that estimating a robust mtDNA phylogeny will require further data. In addition it would be desirable to have a phylogeny of the subfamily Limnodynastinae, or at the very least for other species of *Heleioporus*.

Rapid and Asymmetrical Change to the Structure of the Chromosomal Hybrid Zone in the Australian grasshopper *Caledia captiva.*

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The hybrid zone between the Moreton and Torresian taxa of *Caledia captiva* is extremely narrow and is defined by a series of chromosomal rearrangement differences that involve all members of the genome (2n = 22 + XX/XO). The transition from a Moreton metacentric to a Torresian acrocentric genome occurs over a distance of only 800 metres.

Despite persistent and high levels of hybridisation , the introgression of any chromosomal rearrangements beyond the boundaries of the zone is non-existent. In complete contrast, other molecular and biochemical markers (mtDNA and rDNA RFLPs and allozyme variants) that diagnostically distinguish the Moreton taxon, are found in Torresian populations up to 400km to the north of the zone's current location.

Analyses of past biogeographical and current climatic patterns in the area have led us to propose that (a) the molecular and biochemical markers have been left behind as the hybrid zone has moved south during periods of climatic change and (b) the current location of the narrow parapatric boundary is maintained by rainfall and temperature seasonality gradients. The construction of bioclimatic envelopes for each of the taxa using a BIOCLIMATE PREDICTION SYSTEM has revealed distinctively different envelopes for each taxa, the boundaries of which overlap in the region of the hybrid zone. Laboratory and field experiments have shown that seasonality in both rainfall and temperature are the major limiting factors in embryonic development and survival.

In 1991, the region occupied by the hybrid zone was subjected to unusually dry conditions, which predictably, should favour the Torresian taxon. An examination and analysis of the chromosomal structure of the hybrid zone has revealed major changes in its composition when compared with equivalent analyses carried out in 1977, 1983 and 1985. The 1991 data reveal a highly significant and asymmetrical migration of Torresian individuals across the zone into predominantly Moreton populations.The frequency of Torresian individuals indicates that the immigrant population is approximately equal in size to the resident Moreton population. Such a high level of asymmetrical migration would have profound effects upon the hybrid zone in future generations. An analysis of the zone in 1992 and 1993, one and two generations after the migration event, has shown that the migrants have established themselves, reproduced and dramatically changed the chromosomal structure of the zone.

The details of these rapid and asymmetrical changes in the chromosomal composition of the zone will be presented and discussed.

Karyotypic Evolution in the Onychophora, and Properties of Chromosomal Divergence Indices.

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An extensive chromosomal radiation has occurred in the Australian Onychophora, consistent with the electrophoretic diversity previously documented. On the basis of karyotypic patterns, seven distinct groupings can be distinguished, which differ from one another by fixed gene differences of 70% or more (based on 20 electrophoretic loci). These groupings can be further subdivided into over 30 distinguishable karyomorphs.

Within some of these groups it is possible to quantify differences in chromosomal size distribution among populations and construct distance matrices. These chromosomal distances are strongly correlated with genetic distance measures based on electrophoretic data, but overestimate divergence among genetically very similar populations and underestimate divergence among very distantly related populations.

The behaviour of the chromosomal divergence index may reflect replicative slippage resulting in detectable size differences among chromosomes, or drift among genes responsible for chromosomal condensation.

?? index when 2n differs ??

Polymorphism at microsatellite loci in the endangered Northern Hairy-nosed Wombat (*Lasiorhinus krefftii*).

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We have investigated the utility of hypervariable microsatellite loci to measure genetic variability remaining in the Northern Hairy-nosed Wombat, one of Australia's rarest mammals. This species suffered a dramatic range and population reduction over the past 120 years and now exists as a single colony of about 70 individuals at Epping Forest National Park, central Queensland. Our preliminary research on multilocus DNA fingerprints did not reveal informative variation in this population, so we chose to examine variation in microsatellite repeats, a class of loci known to be highly polymorphic in mammals. To assess the suitability of various wombat populations as a reference for comparisons of genetic variability and subdivision we further analysed mitochondrial DNA cytochrome *b* sequence, using phylogenetic methods. Our results show that appreciable levels of variation still exist in the Epping Forest colony although it has only 41% of the heterozygosity shown in a population of a closely-related species.

From museum specimens collected in 1884, we also assessed microsatellite variation in an extinct population of the Northern Hairy-nosed Wombat, from Deniliquin, New South Wales, 2000 kilometres south of the extant population. The apparent loss of variation in the Epping Forest colony is consistent with an extremely small effective population size throughout their 120 year decline.

VARIATION WITHIN MYOGLOBIN INTRON 2 IN THREATENED POPULATIONS OF THE GOULDIAN FINCH

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A dramatic range contraction since the 1970s has placed the Gouldian Finch(*Erythrura gouldiae*) on the list of endangered species. In order to design an effective management plan for those finches remaining it is necessary to know if the apparent geographic isolation of populations has been accompanied by genetic differentiation.

To detect genetic differentiation between recently separated populations it is necessary to target rapidly evolving segments of DNA for analysis, such as the introns of nuclear genes. Such products amplified using the Polymerase Chain Reaction(PCR) can be sequenced to detect changes but this is a slow and expensive process for population level screening. Temperature Gradient Gel Electrophoresis (TGGE) detects changes in DNA that alter its thermal behaviour. Large numbers of individuals can be readily screened for the presence of particular alleles using this technique.

In this study the second intron of the nuclear myoglobin gene was amplified for 183 Gouldian finches from four locations using PCR. The products were screened by TGGE using heteroduplex analysis. 8 alleles were identified amongst all the birds screened and representatives carrying each allele were cycle sequenced. In this study we found no detectable differences in allelic frequency at the myoglobin intron between the sampled populations.

THE CHARACTERISATION OF MITOCHONDRIAL DNA IN AUSTRALIAN ABORIGINAL POPULATIONS.

SM van Holst Pellekaan, M Frommer, B Boettcher.
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Mitochondrial DNA studies of living populations have been useful in elucidating questions regarding ancestral groups and possible migration patterns throughout the world. A few Australian Aboriginal samples have been included in wider studies but not examined intensively for possible homo/heterogeneity within Australia which, together with archaeological and linguistic data, may be informative about the founding populations of this continent.

Blood samples have been collected with informed consent involving extensive consultation, from the Darling River region of NSW and from Yuendumu in central Australia. A 600bp segment from the hypervariable region of the mitochondrial D-loop has been amplified using PCR. Internal primers are being used in cycle sequencing methods to determine the sequence of the most informative areas and to identify the types of variation contained therein.

VNTR ALLELES AND AUSTRALIAN ABORIGINES

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Variable Number of Tandem Repeats (VNTR), or minisatellites, are highly polymorphic alleles and have many applications in genetic research. VNTRs are used widely in population studies, forensic work, as well as in the study of human hereditary disease and in gene mapping. In this study the Cellmark VNTR probes MS1, g3, MS8, MS31, MS43a and the c-Harvey *ras* I (HRAS) VNTR were used to determine the frequency distribution for each of these VNTRs alleles in DNA extracted from whole blood samples of 110 full-blood Australian aborigines from a central Australian tribe. The frequencies of each of the VNTR alleles in the Australian aborigines were compared with those of Caucasians. Different frequency distributions were observed between the two populations, especially with g3, MS43a and HRAS. The results are discussed with reference to forensic implications and also population differences in terms of the separation of the two races over 150,000 years ago. Possible mechanisms involved in the generation of new alleles are discussed.

NO ASSOCIATION FOUND BETWEEN PRE-ECLAMPSIA / ECLAMPSIA AND A HLA-G DELETION POLYMORPHISM

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The highly polymorphic class I HLA-A, -B, -C genes are not expressed in placental trophoblast cells. Instead, another HLA molecule, HLA-G, has been detected and its expression is largely confined to the placenta. Because HLA-G also shows limited polymorphism at the protein level it has been proposed that HLA-G may play a fundamental role in blocking a maternal immune response against the placenta and may be important in ensuring a successful pregnancy. We have recently described a deletion polymorphism in the 3' UTR of the gene (Harrison, *et al.* 1993). Pre-eclampsia/ eclampsia (PE/E), a disorder of pregnancy, is believed to be a result of abnormal trophoblastic implantation. Although the function of HLA-G in placental cells is still unknown, it could be considered as a candidate gene for PE. Previous studies have shown that there is no linkage between maternally expressed susceptibility genes and the HLA region, but the fetal involvement still needs to be considered. The results of an investigation of the HLA-G polymorphism in pedigrees affected with PE/E will be reported. The data do not indicate any relationship between the disorder and this polymorphism.

This work was supported by the National Heart Foundation.

Harrison G.A., Humphrey, K.E., Jakobsen I.B. and Cooper D.W. (1993) A 14 bp deletion polymorphism in the HLA-G gene. *Human Molecular Genetics* **2**, 2200.

The recombinational landscape of the *Drosophila* genome

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The distribution of recombination events within and across the chromosomes of *D. melanogaster* is not uniform. I will discuss our recent efforts to develop a high resolution map of the distribution of recombination across the *Drosophila* genome. We have estimated regional rates of recombination per unit physical length for 146 X-chromosome, 71 second-chromosome, and 85 third-chromosome reference coordinate genes that are mapped to the nearest 0.1 centiMorgan, and that are localized to a cytological interval no larger than 5 cytological bands on the polytene chromosome physical map. Coefficients of exchange, in units of centiMorgans per cytological band, are calculated for all pairs of reference loci spanning intervals of 20 to 50 cytological bands across the three major chromosomes of *D. melanogaster*. We have also applied our standardized method to the inferred DNA content map of Sorsa. The method proves to be highly robust with respect to the pattern of variation in recombination rates. Molecular genetic studies of several genomic regions are consistent with the predictions for both high and low recombination. Recombination is suppressed near the centromeres of all three chromosomes and the telomeres of X, 2L, and 3L, yet appears to remain high near the telomeres of 2R and 3R. Substantial heterogeneity in recombination rates is also documented across each chromosome. I will also discuss available data for *D. simulans* which demonstrates a reduced level of centromeric and telomeric suppression of recombination in this species.

The Impact of the *C. elegans* Genome Sequencing Project on *C. elegans* Genetics.

David Baillie, Don Riddle and Ann Rose

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The *Caenorhabditis elegans* genome sequencing project is well underway. The sequencing consortium, headed by John Sulston at the Sanger Centre in Cambridge, UK, and by Robert Waterston at Washington University in St. Louis, USA, has generated about 10 million nucleotides of sequence on chromosome III. In addition, about two thousand cDNAs have been partially sequenced and positioned on the physical map of the genome. In order to correlate the DNA sequence information with biological function, we have been developing a genetic toolkit. The toolkit consists of a set of balancers (crossover suppressors) that span the genome of *C. elegans*. In addition, we are generating sets of overlapping deficiencies. The balancers and deficiencies are being used to isolate and characterise lethal point mutations in essential genes. These lethal mutations provide a genetic handle on a large number of previously unidentified genes required for the normal development and maintenance of the organism.

Having available the entire sequence of a chromosome, as we now do for chromosome III, dramatically changes the way we are doing genetic analysis. For example, on the left half of the chromosome we have produced a set of 170 lethal alleles that define 83 essential genes within the sequenced portion of the chromosome. Our goal is to assign each of the essential genes to a specific coding element as identified by sequence analysis. We are using two strategies. The first approach makes use of the deficiencies from the toolkit. The lethals are being mapped with respect to deficiencies by complementation testing and the deficiencies are being mapped with respect to the physical map by PCR. Probes for the PCR reactions are designed after examination of the genomic sequence. The second approach makes use of cosmids from the physical mapping project. We are constructing a set of transgenic animals containing cosmids and a dominant selectable marker, *rol-6*. These transgenic fragments are then used as small (about 35,000 bp) duplications to genetically map the lethal mutations by rescuing the phenotype. Each cosmid carries the information for about eight genes on average. Our initial results indicate that we have about one lethal rescued per cosmid over the extent of the sequenced chromosome.

The next step will be the assignment of a specific region of the cosmid to a specific essential gene. This is greatly facilitated by the availability of a restriction map that can be derived from the cosmid sequence. Specific plasmid subclones of appropriate coding sequences can then be directly tested for their ability to complement the lethal phenotype. All of the strains and information generated by this work are made available to the research community via the Caenorhabditis Genetics Centre and the acedb database.

This work has been funded by the NIH NCRR (USA) and CGAT (Canada).

Evolution and significance of MHC class II polymorphism in artiodactyls

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The major histocompatibility complex (MHC) class I and class II genes encode membrane glycoproteins with a central role in the initiation of an immune response by presenting peptides to T cells of the immune system. We have characterized the organization and genetic polymorphism of MHC class II genes in cattle by RFLP and sequence analysis. The DQA, DQB and DRB3 genes are extremely polymorphic in cattle. These loci show the hallmarks of MHC polymorphism, like the presence of large number of alleles and many amino acid substitutions between alleles. The degree of polymorphism at cattle DQ and DR loci are comparable to the one in humans which is notable considering the large difference in effective population size between the two species. Evidence that the class II polymorphism in cattle is maintained by natural selection is provided by observations of (1) a highly significant excess of nonsynonymous over synonymous substitutions in the exon encoding the peptide binding domain, (2) a deviation in the allele frequency distribution from the one expected for selectively neutral alleles and (3) a deficit of MHC homozygotes in a sample of Swedish breeding bulls. Our knowledge on the level of MHC polymorphism is restricted to a limited number of species. To improve this we are currently analysing the homologue of the most polymorphic class II locus in cattle, DRB3, in other artiodactyls. We have observed restricted DRB polymorphism in the moose since SSCP (Single Strand Conformation Polymorphism) and sequence analyses have only revealed 7 DRB alleles among animals representing two continents, Europe and North-America. Moreover, the polymorphism is restricted to involve only 8 nonsynonymous and a single synonymous substitution. We propose that the moose lost most of its DRB polymorphism in a population bottleneck which must predate the divergence of the European and American subspecies more than 10,000 years BP. The limited polymorphism observed is most likely functionally significant since all amino acid substitutions are non-conservative and occur at peptide binding sites. Positive selection for replacement substitutions and intraexonic recombination have contributed to the reconstitution of MHC polymorphism in the moose after a putative bottleneck. The comparative data on class II polymorphism in artiodactyls will be discussed in relation to the possible cause and selection mechanism promoting MHC diversity.

Application of Microdissection of Plant and Animal Chromosomes

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Precise isolation of defined chromosome regions is possible by microdissection with glass needles or in combination with a laser microbeam, provided the target chromosome is morphologically distinguishable in mitotic or meiotic metaphase plates. A few chromosomes are sufficient for microcloning of chromosome-specific DNA. The amplification of DNA sequences from isolated chromosomes is achieved by digestion of chromosomal DNA using frequently cutting restriction enzymes and ligation of cleaved fragments to synthetic oligonucleotides, which serve as primers for PCR or, alternatively, degenerate oligonucleotide primed (DOP) PCR. PCR products offer suitable probes for FISH and RFLP mapping and may be cloned directly. Furthermore, isolated chromosomes are suitable for the physical mapping of short or low copy DNA sequences on chromosomes. For this purpose, PCR is done with sequence specific primers and chromosome specific DNA as a template to identify the chromosome carrying a specific sequence. The use of defined translocation chromosomes may refine the resolution of this method to a subchromosomal level.

Telenius, 1992
DOP-PCR

The Search for a Molecular Marker Linked to Ovine Batten's Disease

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Department of Biochemistry, University of Otago, Dunedin, New Zealand

The neuronal ceroid lipofuscinoses (Batten's disease) are a group of hereditary neurodegenerative disorders mainly affecting children. Symptoms include progressive blindness, seizures and dementia, and eventually premature death. The disorders are classified into three major types (CLN1, CLN2 and CLN3) based on age of onset and variation in symptoms; all three are inherited as autosomal recessive traits. An ovine model characterised by Prof. Robert Jolly and co-workers at Massey University resembles CLN3, (juvenile neuronal ceroid lipofuscinosis).

Genetic linkage studies of human families have shown that all three major types are non-allelic; the CLN3 gene has been mapped to human chromosome 16p11. Since functional or syntenic gene clusters tend to be conserved across mammalian species, and human gene probes frequently hybridise effectively to sheep DNA, we are using gene probes from human 16p in conjunction with DNA samples from defined sheep pedigrees to test the hypothesis that ovine Batten's disease is equivalent to the human disease CLN3.

A number of gene probes mapped to human chromosome 16p have been obtained and checked for homology to sheep DNA. These probes detect a total of 11 useful RFLPs in sheep, which are being mapped in sheep using the International Mapping Flock, a three generation flock developed specifically for linkage mapping. To date we have established the presence of a linkage group in sheep consisting of 5 genes from human 16p together with several gene probes from elsewhere in the human genome, and three sheep specific microsatellites. These probes are currently being tested for linkage to ovine Batten's disease.

lysosomal storage of subunit c of mitochondrial ATPase
- plus other proteins, phospholipids

	Human location
CLN1	1p32
CLN2	13q31-32
CLN3	16p11

Sheep disease - like CLN3 - but not associated with series of syntenic markers from human 16
- brain atrophy.
- apparent rearrangement in the candidate region
- locus may have been translocated elsewhere.

97-LK

HOW SIMILAR ARE FLY GENOMES?

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The vinegar fly, *Drosophila melanogaster,* has been studied as a model genetic organism. Mutations in more than half of the genes in the genome have been detected and characterized. As a precursor to sequencing, most of the genome has been isolated in contiguous units consisting of λ, cosmid, P1 and YAC clones. Cloned *Drosophila* genes have been used as probes to isolate homologues from a variety of species including mammals.

The transfer of resources and information from the *Drosophila* model to other insects has great potential. The research of Foster and colleagues indicates that the linkage map of *D. melanogaster* can be aligned with those of other dipteran insects. In the Australian sheep blowfly, *Lucilia cuprina*, there are chromosomes corresponding to each of the chromosome arms of *D. melanogaster*. Building on this foundation the comparitive genetic analysis of these two genomes can be extended using cloned *D. melanogaster* genes to:-

(1) map their *L. cuprina* homologues genetically with RFLP markers.

(2) isolate homologous *L. cuprina* clones which can be used to localize genes via chromosomal *in situ* hybridization.

Examples of these types of genetic analysis will be presented.

Reference

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

6,786 genes mapped in Drosophila
80 in L cuprina

~10% of genome sequenced.
- YAC, Plac cosmid contigs spanning genome,

Variation in intron length in Lucilia between homologues due to interspersed repeats

POSTER 1

Use of polymorphic microsatellites for scoring individual-specific genotypes from single hairs in the endangered Northern Hairy-nosed Wombat.

Andrea Taylor, Deryn Alpers and Bill Sherwin

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The rare and endangered Northern Hairy-nosed Wombat (*Lasiorhinus krefftii*) survives as a single population of approximately 70 individuals at Epping Forest National Park, central Queensland. Conservation strategies to date have included exclusion of cattle from the Park, as well as a program of multidisciplinary research into the little-understood biology of the species and its habitat. One aspect of this research involves the characterisation and quantification of genetic variation remaining in the colony. Nine polymorphic microsatellite loci have been identified from wombat genomic libraries[1], revealing individual-specific genotypes amongst the 30 wombats so far examined, with a combined potential for around 2,600 different genotypes. Since microsatellite genotypes are scored by PCR analysis and therefore require minimal input DNA they are ideal genetic markers for the identification of individuals from single hairs collected at burrow mouths. This will provide managers with a non-invasive method for regularly censusing the population as well as elucidating some aspects of wombat behaviour. We present here the results of our preliminary hair collection, storage and DNA extraction trials, including comparisons with genotypes scored in blood from known individuals.

1 Taylor, A.C., Sherwin, W.B. and Wayne, R.K. (1994) Genetic variation of microsatellite loci in a bottlenecked species: the Northern Hairy-nosed Wombat. *Molecular Ecology* **3(4)**.

POSTER 2

RIBOSOMAL DNA VARIATIONS IN *ERIANTHUS*, A WILD SUGARCANE RELATIVE.

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Erianthus is being used in sugarcane breeding programs as a source of *Pachymetra* resistance and drought and flooding tolerance. *Erianthus*-specific markers are being isolated to enable us to evaluate the Australian *Erianthus* spp. germplasm collection and to follow introgressed chromosomes in intergeneric hybrids. We have initially focussed on the ribosomal DNA (18s+26s RNA genes, and 5s RNA genes). A total of 53 *Erianthus* individuals were studied, representing 6 different species. Fifteen *Saccharum* representatives (*S. spontaneum* and *S. officinarum*) were also studied as sugarcane references. Two genus-specific markers were revealed: ribosomal DNA units (18s+26s rRNA genes) show an *Erianthus* specific additional *Bam*HI site in the IGS, and 5s rDNA units are of a different repeat length in *Erianthus* (450 bp) and *Saccharum* (350 bp). Within the *Erianthus* genus, species-specific restriction site variations were also revealed in the rDNA units and gave new information on the classification and relationships between the different *Erianthus* species. Moreover, on the basis of restriction mapping of the rDNA units, this study has also allowed us to compare the different *Erianthus* species to *Saccharum* and related genera such as sorghum and maize. The usefulness and application of these markers in breeding programs is discussed.

Molecular Cloning of the Human Homologue of the *Drosophila flightless-I* Gene, Involved in Gastrulation and Muscle Degeneration

H. D. Campbell[1], C. Claudianos[2,3], A.B. Kasprzak[1], J. Hoheisel[4], K. Chen[5], J. R. Lupski[5], I.G.Young[6], H.G. de Couet[7] and G.L. Gabor Miklos[1]

[1]Research School of Biological Sciences, ANU, ACT, [2]Division of Botany and Zoology, ANU, ACT, [3]Division of Entomology, CSIRO, ACT, [4]German Cancer Research Centre, Heidelberg, [5]Baylor College of Medicine, Houston, Texas, [6]John Curtin School of Medical Research, ANU, ACT, [7]Zoology Department, University of Hawaii, Honolulu.

Severe mutations in the *Drosophila melanogaster flightless-I* gene are lethal, resulting in incomplete cellularization during early embryogenesis, with subsequent abnormalities in mesoderm invagination and in gastrulation. The flightlessness of flies carrying weak mutations correlates with ultrastructural abnormalities in the indirect flight muscles. We have determined the sequence of *fliI* cDNAs, enabling prediction of the amino acid sequence of the 1256 residue protein. Database searches and subsequent analysis showed that the protein consists of two domains. The C-terminal region shows significant homology to gelsolin and related actin-binding proteins, whereas the N-terminal region consists of 16 copies of a leucine-rich-repeat (LRR) of a type known to be involved in protein-protein interactions in other systems.

Database searching also revealed a close similarity to LRR sequences encoded by *C. elegans* cosmid B0303. We isolated corresponding *C. elegans* cDNAs using a synthetic probe based on B0303 sequence. The nucleotide sequence of the cDNA predicted a protein of 1257 amino acids with 49% identity to the *Drosophila* protein.

We have cloned a homologous human cDNA using the polymerase chain reaction with nested sets of degenerate oligonucleotide primers based on conserved regions of the *C. elegans* and *D. melanogaster* proteins. The human protein of 1268 residues is 58% identical to the *Drosophila* protein. The presence of a highly conserved homologue in nematodes, flies and humans is indicative of a fundamental role for this protein in many metazoans. We have determined the chromosomal map position of the human *fliI* homologue by fluorescence in situ hybridization, and are examining possible correlations with human genetic disorders that map nearby. Recently, we have identified human homologues of genes flanking the *fliI* gene in *Drosophila*, and intend to characterise these novel genes and examine their human chromosomal map positions as well.

POSTER 4

ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE GENE VARIANTS IN THE NEW ZEALAND POPULATION. Marshall, S.J. and Chambers, G.K., School of Biological Sciences, Victoria University, P.O. Box 600, Wellington, New Zealand.

Ethyl alcohol is a xenobiotic commonly encountered by human subjects. The principle route of detoxication is metabolism by the liver enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Variant forms of both enzymes are known, and genetically controlled variation in ALDH and/or ADH activity is believed to have a protective role against alcoholism (Thomasson *et al.*, 1991). Allelic variants at the ADH 2 locus have been studied by use of DNA amplification by the Polymerase Chain Reaction (PCR) followed by direct sequencing of single stranded products. Significant variation at the ADH 2 locus has been found in three Polynesian populations; New Zealand Maori, Cook Island Maori and Western Samoan (ADH 2-2 frequencies: N.Z. Maori: 0.53, C.I. Maori: 0.55, W. Samoan: 0.45). The high frequency of ADH 2-2 alleles is also found in Orientals, indicative that Maori and other Polynesians have Asian ancestors. Variation at the ALDH 2 locus has also been examined. ALDH 2-2 variants were observed in Oriental subjects (0.21) but not in Polynesian or Caucasian subjects. Genetic drift is proposed to be responsible for the absence of ALDH 2-2 in Polynesian populations.

Marshall, S., O'Brien, D., Wheeler, K., Hermans, I.F., Chambers, G.K., Robinson, G.M. and Stace, N. (1994) N.Z. Med. J. (in press)

Thomasson, H.R., Edenberg, H.J., Crabb, D.W., Mai, X.-L., Jerome, R.E., Li, T.-K., Wang, S.-P., Lin, Y.-T., Lu, R.-B. and Yin, S.-J. (1991) Am. J. Hum. Genet. 48:677-681

POSTER 5

ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE GENE VARIANTS IN NEW ZEALAND ALCOHOLIC PATIENTS. Marshall, S.J. and Chambers, G.K. School of Biological Sciences, Victoria University, P.O. Box 600, Wellington, New Zealand.

A study of the New Zealand Maori population has found an alcohol dehydrogenase (ADH) isozyme variant, the Oriental or "atypical" form of ADH, ADH 2-2, to a high frequency (Marshall *et al.*, 1994). Thomasson *et al* (1991) have reported that the ADH 2-2 isozyme is found in reduced levels in Oriental alcoholics, and they proposed a protective role for this isozyme against alcoholism. We have determined the ADH 2 and ALDH 2 genotypes for young male New Zealand Maori and Caucasian alcoholic patients. No difference was found between the Caucasian alcoholics (ADH 2-2 = 0.03) and the general Caucasian population (ADH 2-2 = 0.03). Maori alcoholic patients showed a significantly (χ^2, $p < 0.001$) reduced frequency (0.09) of the ADH 2-2 alelle when compared with the New Zealand Maori population (ADH 2-2 = 0.53). The ALDH 2-2 alelle was not observed in either the Caucasian or the Maori population. This result suggests that ADH 2-2 is protective against alcoholism in the Maori population of New Zealand.

Marshall, S., O'Brien, D., Wheeler, K., Hermans, I.F., Chambers, G.K., Robinson, G.M. and Stace, N. (1994) N.Z. Med. J. (in press)

Thomasson, H.R., Edenberg, H.J., Crabb, D.W., Mai, X.-L., Jerome, R.E., Li, T.-K., Wang, S.-P., Lin, Y.-T., Lu, R.-B. and Yin, S.-J. (1991) Am. J. Hum. Genet. 48:677-681

POSTER 6

The Novel *flightless-I* Gene Brings Together the Gelsolin and Leucine-Rich-Repeat Gene Families and Provides a Possible Marker for the Arrival of Multicelluar Life.

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The *Drosophila melanogaster* gene *flightless-I*, involved in gastrulation and muscle degeneration, has *Caenorhabditis elegans* and human homologues. In these highly conserved genes with putative vital function, two previously known gene families have been brought together, families encoding the actin-binding proteins related to gelsolin and the leucine-rich-repeat group of proteins involved in protein-protein interactions. Both these gene families exhibit characteristics of molecular changes involving replication slippage and exon shuffling events. These events have been traced in a phylogenetic study to help account for the structural and functional evolution of *flightless-I* and related proteins.

Phylogenetic comparison of 17 amino acid sequences of 8 protein types shows the putative evolutionary relationships of flightless proteins and other gelsolin like proteins. Conversely, comparison of 24 amino acid sequences of leucine-rich-repeat proteins including the flightless proteins indicates that flightless is a member of a structurally related group of these proteins. Included in the flightless cluster are yeast adenylate cyclases, and a receptor-like protein kinase from the plant species *Arabidopsis thaliana* suggesting the possibility that cell membrane associated ligands for this group could be related. It is hypothesized that an evolutionary lineage from an ancestral monomeric gelsolin precursor protein has undergone at least four independent gene reorganization events to account for the structural diversity of the extant family of gelsolin related proteins. Gene duplication events amongst related gelsolin sequences are now confirmed at the very begining of multicellular life, possibly having evolved over 1.5 billion years ago.

EXTRAORDINARY CHROMOSOME NUMBER VARIATION IN THE GENUS *APIOMORPHA*

Lyn Cook

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Apiomorpha (Hemiptera: Coccoidea: Eriococcidae) is a genus of scale insect the females of which induce spectacular galls on eucalypts. Currently there are 40 species of *Apiomorpha* described morphologically, with all but one endemic to Australia.

Karyotypic analysis of 33 of the described species and two undescribed species of *Apiomorpha* revealed a chromosome number range from 2n = 4 to 2n = 152, a 38-fold difference. This range exceeds that published for the whole of the Coccoidea (range 2n = 4 to 2n = 84). Among animals, only the ant genus *Myrmecia* is reported to exhibit a greater range with a 42-fold difference (2n = 2 to 2n = 84) (Imai *et al.*, 1990, *Psyche* 97: 133-140).

THE ROLE OF THE X CHROMOSOME IN SEXUAL DIFFERENTIATION IN MARSUPIALS

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[3]Department of Anatomy and Histology, University of Adelaide, SA, 5005,
[4]Department of Genetics, University of Adelaide, SA, 5005

An intersex is an animal which has a mixture of male and female sexual characteristics. It is sterile, as opposed to an hermaphrodite which is able to reproduce as both a male and a female. To date karyotypic and phenotypic details have been published for 11 different marsupial intersexes. In this presentation, we describe another five.

The data indicate that gonadal sex is largely determined by the Y. However, by contrast with eutherian ("placental") mammals, a second, normal pathway for differentiation of certain sexual characteristics exists. Bilaterally mosaic animals with a hemi-pouch on one side and a hemi-scrotum on the other provide striking evidence for the hypothesis that the pouch and the scrotum are strict developmental alternatives in marsupials. The karyotypic data indicate that the pouch versus scrotum dimorphism is controlled by the X rather than the Y. XXY kangaroos are males with no scrotum and a pouch. By contrast XO animals have a scrotum and no pouch. The possibility exists that X-inactivation arose in marsupials as a means of sex differentiation rather than as a system of dosage compensation. There is also some evidence that body size might be influenced by the X in marsupials. In considering the implications of the marsupial intersex data, it should be borne in mind that the X of marsupials and the block of genes in human Xq have so far been found to be homologous. It also needs to be remembered that marsupials have paternal X-inactivation, with weak expression of paternally derived alleles in some tissues for PGK1 and GPD.

POSTER 9

MtDNA variation in *Litoria nannotis*, an endangered rainforest frog

Michael Cunningham and Craig Moritz

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Populations of many frogs endemic to the wet tropical rainforests of Northern Queensland have declined over much of their rainge. In some species, such as the Waterfall Frog, *Litoria nannotis*, upland populations have largely disappeared yet foothill populations are still found throughout the wet tropics. Other rainforest animals from this area have shown strong spatial structure in the distribution of genetic diversity, often with a large genetic disjunction between the Atherton Tableland and Daintree areas. We present a survey of mtDNA variation at Cytochrome-b in *Litoria nannotis*, define evolutionary significant units in this frog and discuss the application of these data to the management of this endangered species.

Approaches to measuring genetic variation in the Mountain Pygmy-possum (*Burramys parvus*) from field collected fur samples.

Driver, P.D.[1], Trigg, P.[2], Murray, N.D.[2] and Mansergh, I.[3]

Burramys parvus exists in two isolated populations (both ca. 1000 individuals) along the Great Divide in Australia. One population occurs in NSW and there is a more fragmented population in Victoria. This fragmentation leads to a risk of loss of genetic variability and also local extinction of isolated sub-populations. Management of *B. parvus* that is informed by empirical genetic studies is therefore required.

This project utilises fur collection from wild populations as a relatively non-invasive technique for collecting DNA. The polymerase chain reaction (PCR) is then used to amplify DNA extracted from the fur. A number of sites in the mitochondrial and nuclear DNA of *B. parvus* have been used in order to detect different levels of variation.

Our research goals are to assess:

-the degree of genetic difference between the New South Wales and the Victorian populations,

-the degree of genetic variation within the Victorian population,

-whether the amount of genetic variation within the Victorian sub-populations is related to their sizes, as measured by long-term ecological monitoring. The size of the population necessary for long-term viability and ability to adapt to environmental change may be estimated from this relationship.

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POSTER 11

SCREENING THE *A.nidulans* CHROMOSOME-SPECIFIC COSMID LIBRARY.

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Department of Animal Science[1] & the Department of Biochemistry, Microbiology & Nutrition.[2] University of New England. NSW. 2351.

The filamentous fungus, *A.nidulans,* has a number of systems of wide domain regulation which control the synthesis of enzymes involved in the metabolism of nitrogen, carbon, sulphur & phosphorus nutrients. A group of these enzymes, the extracellular proteases, are secreted when the preferred carbon, nitrogen or sulphur source is limiting in the growth media.

Two mutant strains with abnormal extracellular protease levels have been isolated in this laboratory. One strain, carrying a mutation designated *xprE1,* is defective in the production of extracellular proteases under nitrogen or carbon starvation but not under sulphur starvation growth conditions. Another strain, carrying the *xprF1* mutation, exhibits raised levels of extracellular proteases.

We decided to isolate the *xprE* & *xprF* genes using an *A.nidulans* chromosome-specific cosmid library. Since direct selection of transformants which exhibit wild-type levels of extracellular proteases was not possible, we undertook a method of co-transformation using the plasmids *pMOO6* & *pAN22*. These plasmids carry the selectable markers *argB* & *prn* respectively. The transformants were then screened for wild-type extracellular protease levels.

We have been successful in generating wild-type co-transformants to the strain carrying the *xprF1* mutation allowing us to isolate the cosmid clone which appears to carry the *xprF* gene.

Unfortunately, we have been so far unable to clone the *xprE* gene, however, we have generated a new mutant which has high levels of extracellular proteases. If we can show that it's phenotype is due to plasmid insertion, plasmid rescue will be undertaken & flanking sequences used as probes to screen an *A.nidulans* lambda library.

GENETIC AND ENVIRONMENTAL INFLUENCES ON THE ASYMMETRY PHENOTYPE OF THE AUSTRALIAN SHEEP BLOWFLY, *LUCILIA CUPRINA*.

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Departures from symmetry have been used in field and laboratory studies to indicate developmental perturbation, however the asymmetry phenotype has seldom been partitioned into genetic, environmental and genotype x environment interactive components. The diazinon resistance system (Rop-1 locus) of *L.cuprina* offers an opportunity to assess the relative importance of these effects as the genetic basis of asymmetry is defined. Resistant flies(Rop-1/Rop-1 and Rop-1/Rop-1$^+$) have elevated levels of asymmetry relative to susceptible flies (Rop-1$^+$/Rop-1$^+$). In the presence of a dominant modifier (M) the asymmetry levels of resistant phenotypes return to levels of susceptible phenotypes.

In the results presented here the influence of the environmental variables temperature, larval density and insecticide concentration on the asymmetry phenotype of each of the modifier and Rop-1 genotypes were considered. Asymmetry scores were independent of temperature and density for +/+;Rop-1/Rop-1 and +/+; Rop-1/Rop-1$^+$ genotypes. However U shaped responses were observed for all other genotypes with asymmetry levels being significantly elevated when development occurred above or below standard temperatures (27^{o}C) and densities (100 larvae per food unit). For insecticide concentration there was a positive linear association between asymmetry scores and concentration for susceptible phenotypes. Resistant phenotypes (Rop-1/Rop-1 and Rop-1/Rop-1$^+$) displayed a non-linear response.

Therefore the asymmetry phenotype is influenced by genetic and environmental effects, the response depending on a specific genotype in a particular environment. The results suggest extreme caution is needed when interpreting field data based solely on phenotypes.

RAPD PROFILING OF KOALA POPULATIONS

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Many populations of Australian koalas are presently suffering habitat reduction as a result of continued urban development. This is causing readjustments in social structure due to crowding. Subsequently, there is a direct reduction in genetic diversity of these populations, which may result in reproductive problems, decreased survival or increased susceptibility to chlamydial disease significantly increasing the koala's death rate and decreasing its birth rate beyond safe levels. Koala populations, especially in south-east Australia, have suffered large fluctuations due to local extinctions and an extensive program of relocations and restocking. Thousands of the relocated animals derive from populations on islands in Westernport Bay, which originate from small numbers of founders and have subsequently suffered bottlenecks. Consequently, genetic variation may be severely reduced in the south-eastern populations. In comparison, Queensland populations do not appear to have suffered the same bottlenecks and theoretically should have a larger gene pool. Genetic diversity is also affected by inbreeding, which may occur in captive populations.

This project focuses on the development of a rapid, reliable DNA profiling technique to study the genetic variation between koala populations (Queensland and Victorian) and individuals within a population. We chose to use the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) as a novel approach to profiling koala populations. We have access to four koala populations; (i) Mutdapilly, Queensland (N White); (ii) Gold Coast, Queensland (C Pieters); French Island and Stoney Rises, Victoria (W Sherwin). DNA was extracted from whole blood samples using a high salt method. PCR and electrophoresis conditions were optimised for the highest yield of distinguishable bands and best band separation and resolution. RAPD-PCR of koala DNA generated 10-20 bands with a size range of 0.4-3.0 kb. While there were several common bands across all four populations, it was still possible to differentiate between them. RAPD profiles were sufficiently unique to distinguish between individuals within a population. Preliminary investigations indicate that the RAPD-PCR technique produces reliable profiles from koala DNA. The RAPD-PCR technology currently being developed will be useful: (i) for estimating the degree of genetic variation between koala populations; (ii) for investigations into the degree of inbreeding within populations; (iii) for possible designation of future sub-species; (iv) to assist with captive breeding programs; (v) for parentage determination; (vi) to assist in local relocation programs and provide advice on the most genetically suitable koalas to send to overseas zoos.

This project was supported by Australian Koala Foundation and Lone Pine Koala Sanctuary.

POSTER 14

DETECTION OF CARRIERS OF THE DISEASE GENE FOR CEROID LIPOFUCSINOSIS IN DOGS

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* *Student presentation*

Ceroid lipofuscinosis (CL) is a simple recessive disease trait which is common in border collie dogs in Australia. It is a storage disease in which the subunit c of ATP synthase accumulates in the lysosomes, eventually causing nerve damage which affects vision, temperament, behaviour and coordination in the animal from about 18 months of age. The disease appears to very similar to Batten's disease in human and CL in sheep. The underlying biochemical defect in these diseases is unknown. We presently have samples from over 100 dogs including several known carriers and a couple of affected dogs and are continuing to collect samples.

CL in border collies is widespread in NSW and Victoria due to inbreeding and the high use of some champion dogs that were carriers. At least 12 litters have produced affected dogs with 22 of 79 offspring being affected. There are 24 known carriers alive. All cases can be traced back to a single dog imported in the 1950s so there will probably not be any heterogeneity of the disease and there will be strong linkage disequilibrium between CL and surrounding genes.

A gene for Batten's disease in human has been localised to chromosome 16p12. Probes for a large number of genes and anonymous DNA sequences in the region have been developed in the human, as well as dinucleotide repeat loci. We will look for RFLPs in dogs using these human genes as probes on Southern blots of dog DNA to see if the dog homologues are linked to the CL gene in dogs. As part of a dog genome map project a large number of simple sequence repeats (SSR) polymorphisms have been isolated and characterised. We will use these published dinucleotide repeat markers for genetic mapping in dog, to look for loci which are linked to CL in dogs. The RFLPs will help determine whether CL in border collies is caused by the same gene as Batten's disease in man. Any linked polymorphic loci will allow pre-symptomatic diagnosis and carrier detection for CL.

Detection and Characterisation of Mutations Causing Metachromatic Leukodystrophy.

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Metachromatic Leukodystrophy (MLD) is an autosomal recessive lysosomal storage disorder caused by a deficiency of the enzyme Arylsulfatase A (ARSA). The eight ARSA exons and adjacent intron boundaries from 11 patients with MLD were PCR amplified in seven discrete reactions. Amplified products were analysed for the presence of sequence alterations by single stranded conformation polymorphism analysis and subsequent direct sequencing of PCR products.

Nine putative disease causing sequence alterations were identified in this study, including a deletion, a splicing mutation, one nonsense and six missense mutations.

Six MLD patients were all found to be homozygous for a C to T transition in exon IV that resulted in the substitution of a highly conserved threonine residue at amino acid 274 with a methionine ($T_{274}M$). The six patients representing four families were all of Lebanese descent, and all were known to be the result of consanguineous marriages.

The altered amino acid is rigidly conserved among ten related sulfatase enzymes, therefore it is most likely that the resultant protein will have little or no enzyme activity. This is consistent with the very low ARSA activity measured in these patients and their uniformly severe clinical presentation.

DOMINANT IVERMECTIN RESISTANCE MUTATIONS.

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Dominant ivermectin (IVM) resistance genes in *Caenorhabditis elegans* are very rare. After screening 1.2 million F1 genomes in an ethylmethane sulfonate mutagenesis screen we identified no dominant mutations for ivermectin resistance at 5 ng/mL (in NGM agar) ; wildtype worms are sensitive to 2.5 ng/mL but not 1.25 ng/mL. A much larger screen eventually provided us with 4 strains carrying dominant mutations (alleles nr272, nr2344, nr2389 & nr2477): heterozygotes and homozygotes are resistant to 10 ng/mL IVM. All four alleles are X-linked; nr2389 maps between the visible markers *dpy-7* and *unc-6*, whereas the other three alleles map to the right of *unc-18*. Although all four mutations have an amphid dye filling defective (Dyf) phenotype as homozygotes, the Dyf phenotype is incompletely dominant in nr272, nr2344 and nr2477, and dominance is increased by growth of heterozygotes on IVM. The Dyf phenotype is completely recessive in nr2389 and is not influenced by growth conditions.

Another mutagenesis screen has been conducted to identify suppressors of the IVM resistance conferred by nr2477. Sixteen strains have been isolated by mutagenizing nr2477 and looking for reduced resistance in the F2 offspring. The ivermectin resistance status of the resultant strains which are nr2477/*sup-?* doubles varies from slightly resistant (growth at 3ng/ml but not at 5) through Wt (growth at 1.25 ng/mL but not at 3) to super sensitive (no growth on agar containing 1.25 ng/mL IVM). All of the nr2477/*sup-?* strains are Dyf, and many are Dpy. All of the *sup* alleles are recessive, as the *sup*/+, nr2477/+ animals are resistant to IVM at 5ng/mL.

In summary therefore we have four dominant resistance alleles at (at least) two loci on linkage group X. All four are Dyf as homozygotes but some are nonDyf (or are incompletely Dyf) as heterozygotes. Suppressors of varying strength have been generated for the nr2477 allele and although all of these effect IVM resistance, none effect dye-filling (i.e. *sup*/*sup*, nr2477/nr2477 animals are Dyf). In addition some suppressors are Dpy.

Some of the questions we are pursuing further are:

1) Many Dyf mutations at previously defined loci impart IVM resistance (see Grant and Hunt - this meeting) new alleles at these same loci, isolated in screens for resistance to IVM only, are sometimes nonDyf. Similarly, resistant heterozygotes of some dominant resistance alleles are nonDyf; and conversely, non-IVM resistant, Dyf worms can be made using suppressors. What is the link between these phenotypes?

2) What is the mechanism behind suppression of IVM resistance by the sup alleles we have generated, especially the Dpy *sups*.

POSTER 17

Mapping Resistance to the Cereals Cyst Nematodes in Rye

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ABSTRACT

The Cereals cyst nematode, *Heterodera avenae* Wollenweber, cause serious damages to cereal crops in Australia, mainly in South Australia and some districts of Victoria. Resistance to Australian pathotype has been identified in triticale line T701-4-6 and localised to the long arm of rye chromosome 6 (6R). Efforts has been made to transfer the resistance gene into wheat. DNA markers linked to inherited traits are quite useful for gene transfer in plant breeding programs. The present project aims to develop a chromosomal map of rye chromosome 6 (6R) and the identification of molecular markers linked to the *Cre r* locus, using cDNA clones from barley and oat and wheat genomic DNA libraries. For this purpose a "6R" test-cross mapping population was established. F1 progeny derived from the cross between the wheat variety Chinese Spring carrying chromosome 6R from Imperial rye as an addition and Chinese Spring carrying a 6R substitution from T701-4-6 were back crossed to Chinese Spring. Wheat plants monosomic for 6R chromosome were selected using the GOT isozyme marker. RFLP markers have been identified linked to *Cre r* locus on the long arm of 6R. A short arm mapping study is underway to provide a comprehensive genetic map of the entire 6R chromosome.

Allelic variation at the HSR ω locus in *Drosophila* : implications for gene copy number.

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In a survey of natural allelic variation at major heat shock protein (hsp) loci of *Drosophila melanogaster* interesting variation has been detected at the hsr ω locus, that encodes the ω heat shock RNAs. This proposed single copy gene differs from other hsp genes as no translated product has been detected, yet heat shock produces greatly elevated RNA levels. The techniques of polymerase chain reaction in conjunction with denaturing gradient gel electrophoresis were used to separate nucleotide sequence variants among inbred lines. Two single and one multi-banding pattern were observed, each breeding true within its line. The genetic basis of this variation is examined here, including the possibility that this gene exists in multiple copies among the lines investigated.

The haemoglobin VIIB cluster of an Australian *Kiefferulus* species

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The haemoglobins of *Chironomus* are relatively well known at the level of amino acid sequence and, in an increasing number of cases, at the DNA sequence level. However, as yet no study has been made at either level of the haemoglobins of other genera.
We have investigated the structure and gene sequence in a 14.5kb segment of the haemoglobin VIIB cluster of an undescribed *Kiefferulus* species known as '*K. cornishi*'. The clone of this region, obtained by homology to a clone from the *C. piger* cluster, hybridizes to the same band of *Chironomus* polytene chromosomes as does the *C. piger* clone, and both clones also hybridize to the same band on arm IR of *Kiefferulus* species. The *Kiefferulus* cluster shows many similarities to that of *Chironomus*. We may not have the whole cluster but have identified eight genes, of which seven appear to be functional. The genes have been named KC HbVIIB-a to -g to indicate that, while they show homology to the *Chironomus* HbVIIB genes, they are not homologous to the individual genes in that genus.
Amino acid sequence homologies between the putative proteins from the *Kiefferulus* genes show 77-100% identity, and when compared to the amino acid sequences of the *C. thummi* or *C.piger* proteins, 75-86% identity. The highest similarity is between CTT HbVIIB-7 and KC HbVIIB-e. KC HbVIIB-f shows deletions within the open reading frame so that, while there is still high identity at the DNA level, the open reading frame has a stop codon one amino acid beyond the end of the leader sequence of the putative protein. Therefore it is unlikely that this gene is functional. There is no homolog of the unique CTP Hb-Y gene in the *Kiefferulus* genome, or apparently in those of *C. tentans* or the Australian *Chironomus* species, suggesting that this gene may be confined to a particular *Chironomus* lineage.
The data suggest that the ancestral cluster contained fewer genes. Subsequent to the separation of these genera both clusters have increased in size, possibly by unequal crossing over, and by the incorporation of the Hb-Y gene into the *Chironomus* cluster. The extension of the study of the haemoglobin genes to other genera thus offers insights into the evolution of these genes and the clusters they seem to produce, as well as into the relationships between the genera.

McCLEARY,S., H.B. ALLEN, M.T. IVANYI AND N.D.MURRAY*. School of Genetics and Human Variation, La Trobe University, Bundoora, 3083, Australia.

USE OF DNA MARKERS IN THE CONSERVATION MANAGEMENT OF THE HELMETED HONEYEATER *Lichenostomus melanops cassidix*.

The helmeted honeyeater *Lichenostomus melanops cassidix* is a distinctive and endangered subspecies within the Yellow-tufted Honeyeater complex of south-eastern Australia. The only remaining wild population consists of about twenty breeding pairs. To aid in the management of wild and captive populations we have developed "DNA-fingerprinting" and a PCR-based restriction analysis of the mitochondrial D-loop region.

The minisatellite *per* probe has identified a single major locus with twelve alleles. This has been valuable in monitoring genetic diversity and determining parentage in the wild population, where some infidelities have been identified and aspects of both social and population structure clarified. In contrast, a restriction analysis of the D-loop (800 base pairs) in all remaining maternal lineages has so far revealed no genetic variation.

These findings confirm the importance of a multi-targetted approach to the measurement of genomic diversity in conservation genetics.

Polymorphism in the chloroplast genome of *Acacia mangium*

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Acacia mangium is a tropical acacia with a rather disjunct natural distribution ranging from the Moluccan Islands in the north-west, to Townsville in the south-east. It has recently become extremely important in SE Asia as a fast-growing plantation tree for paper pulp. However, the original source material for these plantation populations was derived from a very narrow genetic base with sub-optimal growth characteristics. There is therefore a need to develop genetic markers to assist in the implementation of improved breeding strategies.

Previous studies using isozymes detected only low levels of genetic diversity within this species, so isozyme markers would be of little use in breeding programs. We are therefore developing a number of types of DNA markers, from both the chloroplast and nuclear genomes, for use in population analysis and breeding programs.

As a first step towards this goal, we have surveyed the level and types of polymorphism in the chloroplast genome from natural populations of *A. mangium* . This was done using five individuals from each of ten populations from throughout its natural range, digesting each with ten different restriction enzymes, and hybridizing with a total of thirteen (ten petunia and three tobacco) heterologous chloroplast probes which cover the entitre chloroplast genome.

A total of seven polymorphisms were detected: three were site loss or gain mutations and four were insertion/deletion mutations of 80-400 bp. Only two of these mutations (both insertion/deletions events) occured in Australian material, being largely fixed in populations from the southern-most part of the range (Townsville-Cairns region). Six of the seven mutations occured in populations from the southern lowlands of western Papua New Guinea and eastern Irian Jaya, but were all of limited distribution within these populations. No variants were found in the Moluccan Island or Cape York populations.

The southern lowland region of Papua New Guinea also produced most of the genetic variability as determined by isozyme markers, suggesting that this region may be the epicentre of evolution for *A. mangium*.

Diazinon resistance in *Drosophila melanogaster.*

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In *Lucilia cuprina* one mechanism is almost solely responsible for conferring resistance to the organophosphorous insecticide, diazinon. An altered carboxylesterase, E3, encoded by the *Rop-1* gene has been found in both laboratory and field isolated resistant strains. Theoretically there are several mechanisms through which resistance to organophosphorous chemicals can arise (e.g. Acetylcholinesterase, Mixed Function Oxidases, and Glutathione-S-Transferases). Among the many known resistant species these resistant mechanisms occur with equal frequency. Thus the results in the *L. cuprina* system become even more intriguing: why does the *Rop-1* mutation keep occurring? It is possible that within a species a particular option will be favoured. The option chosen seems to a certain extent, to be determined by the selective pressure to which the organism is exposed. To further examine this notion diazinon resistance was studied in *Drosophila melanogaster*. *D. melanogaster* is not a pest species and is often indirectly exposed to insecticides, thus the selective pressures may be different to those seen in *L. cuprina*. Thirteen natural populations were sampled from along the east coast of Australia and tested for diazinon resistance. Resistance to diazinon was observed in all populations with resistance levels ranging from high (2% mortality) to relatively low (84.2% mortality). There was no evidence of a cline between resistance and latitude. Genetic analysis of these resistant populations indicates there may in fact be several genes responsible for conferring resistance to diazinon in *D. melanogaster*, a situation at variance with the observations in *L. cuprina* .

Evolution of the α esterase gene cluster

Charles Robin, Robyn Russell and John Oakeshott.

A large and functionally diverse group of hydrolytic enzymes share a common structure called the 'α/β hydrolase fold'. Presumably, all these enzymes have evolved from a single primordial α/β hydrolase domain, by a process of gene duplication and divergence. Included in this group are the *Drosophila* esterases Est23, Est9, *ali* and *Mce* which map to a cluster of eleven genes, known as the α esterase cluster. We are interested in the gene duplication events that have occurred during the evolution of the cluster, and the effects of these and subsequent divergence, upon the function of the esterases.

We use sequence data to reconstruct the phylogeny of the α cluster. Perhaps the most intriguing finding with respect to this phylogeny is the distribution of five intron sites, which indicate that a large amount of intron change has occurred throughout the clusters evolution. We then interpret the divergence of the α esterases on a structural model derived from the crystal structure of *Torpedo californica* acetyl choline esterase. The variation in residues lining the active site gorge and contributing to the substrate binding loops indicates a functional shift between esterases of the cluster. Ultimately we aim to identify residues responsible for known *in vitro* substrate preferences by using kinetic studies of baculovirus expressed proteins, and perhaps *in vitro* mutagenesis.

STRUCTURE AND MOLECULAR EVOLUTION OF THE *Adh* GENES OF *Drosophila buzzatii*

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Gel electrophoresis of proteins of *D.buzzatii* show multiple bands of alcohol dehydrogenase activity suggestive of a gene duplication of the *Adh* gene in this species.(Oakeshott *et al*.1982) This is in contrast with most species of *Drosophila* which have a single *Adh* gene transcribed from two promoters. (Benjayati *et al*.1983)

We have cloned the *Adh* gene region in *D.buzzatii* from a genomic DNA library using the *D.melanogaster Adh* gene as a probe. Southern blot and sequence analysis reveal that there are three genes located within an eight kilobase region. This *Adh* gene structure is similar to other *Drosophila* species within the mulleri subgroup.(Fischer and Maniatis,1985) The upstream gene is a pseudogene (*Adh-P*), the middle gene *Adh-2* and the downstream gene *Adh-1*.

Comparisons of 5 ' flanking and coding regions of the *Adh* genes of *D.buzzatii* and other species show that

(1) The *D.buzzatii Adh-1* and *Adh-2* genes are more similar in both coding and non-coding regions than either gene compared to *Adh-P*.

(2)The 5' flanking regions of *D.buzzatii Adh* genes are more similar to their orthologous homologues in other species than their paralogous homologues.

(3) The *Adh* coding regions of Hawaiian *Drosophila* are more similar to the *D.buzzatii Adh* coding regions than *Adh* genes from the subgenus Sophophora.

(4) The *D.buzzatii Adh-1* gene is more similar to the *D.buzzatii Adh-2* gene than *Adh-1* genes from other species.

In general these observations support Atkinson's model of the evolution of *Adh* gene structure. (Atkinson *et al*.1988) It is likely the three gene structure present in *D.buzzatii* was derived from an ancestor with a single gene. The evolution of the three gene structure is likely to have involved three separate events; two gene duplications and a deletion inactivating the upstream gene. In *D.buzzatii* there is also evidence for gene conversion events between the *Adh-1* and *Adh-2* genes.

REFERENCES.

Atkinson P.W, L.E. Mills, W.T. Starmer and D.T. Sullivan. *Genetics* 120:713-723

Benjayati C., N.Spoerel, H. Haymerle and M.Ashburner. *Cell* 33:125-133

Fischer J.A. and T. Maniatis *Nucleic Acids Res.* 13:6899-6915

Oakeshott J.G., G.Chambers, P.East, J.Gibson and J.S.F. Barker. *Aust. J. Biol. Sci.* 35:73-84

SEX-SPECIFIC SEQUENCES OF *Bactrocera tryoni*

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The Queensland fruit fly, *Bactrocera tryoni* , (formerly *Dacus tryoni*, family Tephritidae) is known to infest most types of commercially grown fruit and methods of biological control such as sterile insect release (SIT) are being researched. The degree of success of SIT relies in part on a knowledge of the genetics of the organism and an ability to manipulate the genome and alter the sex ratios.

The genetics of *B. tryoni* have not been well studied unlike other dipteran species such as *Drosophila melanogaster, Musca domestica, Lucilia cuprina* and a closer relative *Ceratitis capitata*. Studies of these and other dipteran species have identified numerous sex-specific genes. Sex-specific genes may be defined as those genes whose products are expressed either differentially or only in one sex (such as the genes that determine sex) as well as those associated with sex-limited functions such as egg or sperm production.

Two approaches were taken in the isolation of sex specific sequences. Degenerate PCR primers were made to regions of greatest identity between the chorion gene of *Drosophila melanogaster*, *D. virilis* and *C. capitata*. A 262bp product was isolated, cloned and sequenced. Translation of this region shows a high degree of identity between *B. tryoni* and *C. capitata*. with only one amino acid difference found out of 85.

The second approach used the RAPD technique with 10mer primers and extracts of male and female genomic DNA. A male-specific fragment of around 420bp was isolated, cloned and sequenced. Southern blotting is underway to determine the specificity and copy number of this region.

POSTER 26

GENETIC AND MOLECULAR CHARACTERISATION OF AN ARABINOSE METABOLISM MUTANT OF *ARABIDOPSIS THALIANA*.

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Monosaccharide precursors of cell wall polysaccharide biosynthesis are the nucleoside diphosphate sugars (NDP-sugars). Such activated sugar donors are derived largely from the photosynthetic product fructose-6-phosphate via a series of carbohydrate interconversion reactions, however an additional pathway operates for some sugars whereby free monosaccharide can be converted to the corresponding NDP-sugar via a phosphorylated intermediate. L-arabinose is an example of one such sugar.

Genetic and biochemical evidence indicates that the *ARA1* gene product plays an important role in the metabolism of L-arabinose. Individuals homozygous for the *ara1-1* mutant allele are, unlike the wildtype, sensitive to exogenous L-arabinose at concentrations greater than 3mM, show an 85 to 90% reduction in the ability to incorporate radioactively labelled L-arabinose into ethanol-insoluble polysaccharide material and possess 10% of the wildtype level of arabinose kinase activity.

A suppressor mutant has been isolated from an *ara1-1* population exposed to gamma radiation. This revertant grows normally on 30mM L-arabinose and metabolises this sugar at near wildtype levels. Preliminary molecular analysis indicates the presence of a complex chromosomal rearrangement in the predicted *ARA1* region on chromosome 4 of the *Arabidopsis* linkage map. Detailed genetic and molecular characterisation of this mutant will be described as an aid to cloning the *ARA1* gene by complementation.

POSTER 27

Using *Saccharomyces cerevisiae* to detect *Aspergillus nidulans* nitrogen regulated promoters

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In *Aspergillus nidulans*, the positively acting protein AreA activates the expression of many genes encoding nitrogen utilization enzymes in the absence of easily metabolized nitrogen sources such as glutamine or ammonium. The genes *GLN3* in *Saccharomyces cerevisiae* and *nit2* in *Neurospora crassa* encode products which similarly are required for gene activation under conditions of limiting nitrogen. All three predicted proteins are members of the GATA family of DNA binding proteins, containing a zinc finger of the type described as recognizing the sequence 5'A/TGATAA/G3'.

A library containing *A. nidulans* sequences of average size 256bp inserted immediately before a *CYC1-lacZ* fusion in a yeast vector was transformed into yeast. Approximately 30,000 transformants were screened for increased *lacZ* expression on a variety of limiting nitrogen sources compared to expression on ammonium. Thus far three clones have been found which exhibit this phenotype in wildtype but not *gln3* yeast strains.

These clones have been rescued from yeast, and the *A. nidulans* inserts of two clones have been sequenced. Consistent with expectations based on NIT2 studies that a high affinity GLN3 binding site would contain multiple GATA sequences, both inserts contain several GATA motifs. Current work involves isolating further GLN3 responsive elements to compare possible binding site sequences, and examining the effects of these sequences in *A. nidulans* .

Guest: A 98 BP INVERTED-REPEAT TRANSPOSABLE-ELEMENT FROM *Neurospora crassa*.

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The DNA sequence 3′ of *histidine-3* in *Neurospora crassa*, in the region that contains the recombinator *cog*, diverges by 8% between the St Lawrence 74*A* and Yale laboratory strains which have different provenance from wild collections. The largest of three insertions present only in St Lawrence is flanked by a 3 bp direct repeat, has an inverted repeat structure and shares features with several transposable elements. At 98 bp, it may be the smallest eukaryotic transposable element yet found. Southern transfers probed with sequences from the terminal inverted repeat show the *Neurospora* genome to contain multiple iterations of the element. PCR amplification of *Neurospora* genomic DNA with a single primer comprising 26 bp of the TIR gave products of discrete sizes ranging from 100 bp to about 1100 bp, suggesting that the element isolated (*Guest*) may be a deletion derivative of a family of larger transposable elements.

DNA sequence evidence for recombinational repair in the *gnd* locus of *Salmonella enterica*

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New South Wales 2006.

The *gnd* gene of *Salmonella enterica* which codes for a metabolic enzyme, 6-phosphogluconate dehydrogenase is located immediately 3' to the highly polymorphic O antigen coding locus *rfb*. Another polymorphic locus *cld* is located at a distance of around 2kb, 3' to the *gnd* gene. The product of *cld* gene has been shown to regulate the chain length of the O antigen. The DNA sequence analysis of *gnd* locus of *S. enterica* has shown that chromosomal rearrangements happening in the adjacent loci, *rfb* and *cld* occassionally extend into either ends of the *gnd* gene. Three different recombination junctions associated with large segment transfers have been identified in the *gnd* gene of *S. enterica*. Despite being located in such a potentially disruptive region, the nucleotide diversity and the overall genetic variation in the *gnd* locus of *S. enterica* is not different to that of other loci coding for housekeeping enzymes in this species such as the *Gap*A and *mdh* loci. The DNA sequence analysis also shows a strong association of the dam methylase target sequence 5' GATC 3' at the locations previously identified as hot spots for chi stimulated site specific recombinations. The consistent association of the 5' GATC 3' sites at these recombination junctions, together with the conserved nature of the coding region up to these junctions show that mismatch repair by the mut HLS pathway could be preserving the genetic variation of this gene at a level similar to other housekeeping genes in *S. enterica*.

POSTER 30

Analysis of the *facB* gene of *Aspergillus nidulans*.

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The *facB* gene of *Aspergillus nidulans* encodes an activator which mediates acetate induction of the *amdS* gene (encoding acetamidase) and genes required for acetate metabolism *via* the glyoxylate bypass. Cloning and sequence analysis of *facB* revealed a $Zn(II)_2Cys_6$ DNA binding cluster, a putative leucine zipper-like dimerization motif and potential acidic activation domains (H.M. Martin, S. Sapats, J.A. Sharp, M.E. Katz, M.A. Davis and M.J. Hynes, unpublished).

DNA binding studies are being used to investigate the regulatory function of *facB*. The FacB protein has been expressed in *Escherichia coli* as a Maltose Binding Protein fusion. This fusion has been used in mobility shift assays to demonstrate that FacB is a DNA binding protein that binds to specific *amdS* promoter sequences.

In vitro mutagenesis has been used to alter specific residues in the DNA binding domain of FacB. A *facB* allele containing a mutated $Zn(II)_2Cys_6$ cluster fails to complement a *facB* null mutant for growth on acetate. Thus, the DNA binding cluster is essential for FacB function. A fusion protein containing a mutated zinc cluster has been expressed in *E. coli* to confirm that DNA binding activity is abolished. Transformation analysis has indicated that this mutant *facB* allele interferes with growth on acetate in wildtype and leaky *facB* mutant strains. Deletion constructs of the mutant allele are being used to localise the region of the protein responsible for this effect.

CLONING OF *MARINER*-LIKE TRANSPOSABLE ELEMENTS OF THE QUEENSLAND FRUIT FLY *BACTROCERA TRYONI*

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DNA sequences related to the transposable element *mariner* have been found to be abundant in the genome of the Queensland fruit fly, *Bactrocera tryoni*. A 480bp PCR fragment related to the transposase coding region of *mariner* was amplified from *B. tryoni* genomic DNA using degenerate oligonucleotide primers. The fragment was cloned and 21 random clones sequenced. Sequence variants could be grouped into two of the five subfamilies of *mariner*-like elements, the "honey bee" and "horn fly" subfamilies, as classified by Robertson and MacLeod (Insect Molecular Biology, 1993). However, none contained a complete open reading frame. All cloned sequences had either small deletions or additions resulting in frameshifts and stop codons, indicating that none would act as an active transposase source.

Evidence suggesting that *mariner*-like elements are very numerous in the *B. tryoni* genome include the following: (1) No two of the 21 PCR fragment sequences were identical. (2) Southern blotting and hybridization with a mariner-like DNA probe resulted in banding patterns characteristic of high copy number sequences. (3) Genomic library screening yielded a large number of hybridizing lambda clones.

It is not yet known if any of these sequences are bordered by intact inverted repeat ends or correspond to potentially functional transposable elements. To investigate this further, genomic library clones are currently being characterized.

POSTER 32

PLASMINOGEN ACTIVATOR INHIBITOR-2. A CANDIDATE GENE FOR PRE-ECLAMPSIA

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* Honours student presentation

Pre-eclampsia (PE) is a disease of first pregnancy which has been shown to be highly heritable. Plasminogen activator inhibitor-2 (PAI-2) on chromosome 18 has been suggested as a possible candidate gene for PE as it is expressed only in pregnancy.

PAI-2 specifically inhibits urokinase plasminogen activator which converts the inactive proteolytic enzyme plasminogen into the active plasmin. PAI-2 is only found during pregnancy where there is a dramatic increase in circulating levels, and its major source is the placenta. In PE PAI-2 levels are greatly reduced possibly causing the increase in fibrinolytic activity associated with the disease.

The mode of inheritance of PE is unknown. Two models of inheritance will be analysed in family studies looking for linkage: a recessive model, and a dominant model with fifty percent penetrance. In both cases the defective gene is assumed to be acting in the mother rather than the foetus. Genetic variability at PAI-2 is low so CA repeat microsatellites from the same region of chromosome 18 will be examined. LOD scores will be used to look for linkage between the disease gene and the markers.

prtB: an *Aspergillus nidulans* acid protease gene.

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Due to the economic importance of the hydrolytic enzymes produced by fillamentous fungi, *Aspergillus* proteases have become an area of great interest. Early studies showed that the four broad classes of endo-proteases (alkaline, thiol, acid & neutral) were produced by *Aspergillus* species (Cohen 1977). Work on *A. nidulans* (Cohen 1973) confirmed the existence of neutral and alkaline proteases in this species. Unfortunately the methods used were unsuitable for the detection of thiol or acid proteases, therefore it was unknown if proteases of these classes were also produced by *A. nidulans*.

In *A. nidulans* the production of extracellular proteases occurs in response to nutrient limitation for carbon, nitrogen, or sulphur, making this system suitable for the study of gene regulation. Acid proteases have been cloned from the closely related species *Aspergillus niger* var. *awamori* (Berka et al 1990) and *Aspergillus oryzae* (Gomi et al 1993). With the intent of examining the regulatory region, the structural protease gene *prtB*, has been cloned using a heterologous probe. A PCR generated 1063bp region of the *pepA* (acid protease) gene from *A.niger* was used to isolate *prtB* from a lambda library of genomic *A. nidulans* DNA. It is hoped that by studying the regulatory region of *prtB*, we will be able to determine the regions of DNA involved in carbon, nitrogen, and sulphur regulation in this system. Molecular analysis of this gene is currently in progress.

References:

Berka RM et al (1990).Molecular cloning and detection of the gene encoding aspergillopepsin A from *Aspergillus awamori*. Gene 86: 153-162

Cohen BL (1973). The neutral and alkaline proteases of *Aspergillus nidulans*. J. Gen. Microbiol. 77: 521-528

Cohen BL (1977)
The proteases of *Aspergilli*
In "Genetics and Physiology of *Aspergillus*" pp 281-292
JE Smith and JA Pateman eds. Academic Press London

Gomi K et al (1993). Cloning and nucleotide sequence of the acid protease-encoding gene (*pepA*) from *Aspergillus oryzae*. Biosci, Biotech. Biochem 57(7): 1095-1100

Differential effect of insertions on the expression of the alcohol dehydrogenase gene in larvae and adults of*Drosophila melanogaster*

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In *Drosophia melanogaster* the expression of the single *alcohol dehydrogenase* gene is controlled by two promoters. The larval promoter is used principally in late embryos and all larval stages and at a low level in adults, whilst the adult promoter is active transiently in embryos, at a moderate level in third instar larvae and at a high level in adults. We have analysed two naturally occurring variant *Adh* alleles that are characterised by abnormally low ADH activity in adults, but normal levels in third instar larvae. A variant with a similar phenotype was reported by Thompson et al (1977) and partially analysed by Schott et al (1988).

Molecular analyses have shown that in one of the two variant *Adh* alleles there is a 1.15kb defective P element insertion 5bp 5' from the adult promoter. In the second variant allele there is a 2.4 kb defective *hobo* element inserted at exactly the same site. Northern analyses have shown that the insertions cause a reduction in *Adh* mRNA in adults flies. The 2.4 kb *hobo* insertion is unstable in somatic cells at 20°C; the deletion products will be described.

1. Thompson, Ashburner and Woodruff (1977) Nature 270:363
2. Schott, East and Paigen (1988) Genetics 119:631

CONTROL OF *S. cerevisiae* PSEUDOHYPHAL DEVELOPMENT BY AN *A. nidulans* DEVELOPMENTAL REGULATOR.

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Control of conidiophore development in *Aspergillus nidulans* requires the action of a number of sequentially expressed regulatory genes. These genes, denoted *brlA*, *abaA* and *wetA*, define the core regulatory pathway and have been shown to activate numerous morphogenetic genes. Mutations in these genes leads to an arrest in development at specific stages with a concomitant loss in expression of particular sets of morphogenetic genes.

The *abaA* gene represents the central regulatory component of this linear pathway and encodes a transcription activator with an ATTS/TEA DNA binding domain. Expression of *abaA* is activated by the upstream *brlA* regulatory gene. The AbaA product positively feedback regulates the expression of *brlA* and itself, thereby fixing the pathway in an activated state and leading to developmental commitment. It also activates the expression of the downstream *wetA* gene and a number of morphogenetic genes.

Expression of *abaA* in diploid *S. cerevisiae* cells under nitrogen-starvation conditions induces pseudohyphal development, similar to the effect observed upon overexpression of the *S. cerevisiae PHD1* gene. The *PHD1* promoter contains four AbaA binding sites which have been shown to bind AbaA *in vitro*. Therefore, it is likely that *abaA* activates the *PHD1* gene in *S. cerevisiae*. However, expression of *abaA* in a *phd1* mutant under the same conditions still leads to pseudohyphal development suggesting that other activities important for this dimorphic switch are also under *abaA* control. Furthermore, the *PHD1* gene shares homology with the *A. nidulans stuA* gene, which is also involved in the regulation of conidiophore development. These results suggest that pathways regulating conidiophore development in ascomycetes and pseudohyphal growth in yeast may be related.