CHRIS MORAN

# 42ND ANNUAL CONFERENCE


## Canberra 4-8 July 1995

# PROGRAM & ABSTRACTS

# GENETICS SOCIETY OF AUSTRALIA

## 42nd Annual Meeting
## Australian National University, Canberra. July 1995

## CONFERENCE INFORMATION FOR REGISTRANTS

### ACCOMMODATION

Accommodation is at Burgmann College. The Manning Clark Lecture Theatres are situated next to the ANU Union Buildings, 10 minutes walk from the college and 2 minutes walk from tea, coffee, poster and trade displays in Melville Hall. Eating facilities at the ANU Union include an excellent Asian Bistro, Steak Bar, Bakery, Coffee Shop and Sandwich Bar. The University Union Bar is situated downstairs.

### PARKING

You will receive an orange ticket with your registration papers indicating that you are attending the conference. This will allow you to park in any of the blue permit parking areas on campus, provided it is in clear view on your dashboard.

### BRIAN'S BUS

Brian's Bus operates between several campus locations and the Civic area. Timetable details can be obtained at Burgmann College.

### REGISTRATION

At Burgmann College 4.00 - 7.00pm on Tuesday 4 July and in the foyer of the Manning Clark Lecture Theatre Complex from the morning of Wednesday 5 July.

### POSTERS

The poster session will be held in Melville Hall, where morning and afternoon tea will be served daily. Posters can be put up any time from Wednesday morning onwards. Please remove all posters after the Friday evening poster session

### MIXER

A mixer will be held at Burgmann College 7.00 - 10.00pm on Tuesday 4 July. Finger food will be served and the bar will be open.

## MEALS

Lunch may be obtained at a variety of locations on campus. These include :

Students Union - Sullivans Restaurant (1st Floor)
- Asian Bistro (1st Floor)
- Snack Bar (Sandwiches and fast food - Ground floor)
- Salwa's (Lebanese takeaway)

The Gods - Coffee Lounge and Italian Food (Arts Theatre complex)

Vivaldi's Restaurant (Arts Theatre complex)

All are within the general area of the lecture theatres. In addition, Canberra Civic area has plenty of restaurants and clubs catering for a wide range of tastes. The O'Connor shopping centre is less than a kilometre away, on the corner of Macpherson St and David St, O'Connor. This centre boasts two Vietnamese restaurants, two Italian restaurants, a vegetarian restaurant and a takeaway, as well as a bakery and supermarket. The Canberra North Bowling Club on McCaughey St has a quiet, relaxed atmosphere and the lowest priced bar in walking distance.

## ANNUAL DINNER

The Annual Dinner will be held on Thursday 6 July, 7.30 - 11.00pm in the Students' Union Building (First Floor). The dinner will consist of an Asian banquet (12 dishes (including vegetarian dishes ) wine and soft drinks. Dr Max Whitten is our invited guest speaker.

## VISIT TO THE NATIONAL BOTANIC GARDENS

For those who are coming to lunch after the conference, we will be meeting at Kookaburra's Restaurant in the National Botanic Gardens on Saturday 8 July at 1.00pm. The gardens are situated on the side of Black Mountain, five minutes walk from Burgmann College on the opposite side of Clunies-Ross Drive. The National Botanic Gardens specialises in native flora from all parts of Australia, and is a great place for birdwatching or general relaxation.

## USEFUL TELEPHONE NUMBERS

| | |
|---|---|
| Aerial Taxi Cabs | 285 9222 |
| Vivaldi's Restaurant | 257 2718 |
| Gods Cafe and Bar | 248 5538 |
| Health Service | 249 3598/4098 |

## SOME "GOOD VALUE" RESTAURANTS CLOSE TO THE ANU

| | |
|---|---|
| **Cafe Tasca** (Portuguese Cuisine)<br>9 Lonsdale St. Braddon (BYO) | 257 2852 |
| **Tu Do Vietnamese Restaurant**<br>O'Connor Shops (BYO*) | 248 6030 |
| **Siamese Kitchen** (Thai cuisine)<br>14 Lonsdale St., Braddon (BYO) | 248 8802 |
| **Great Wall Chinese Restaurant**<br>113 Marcus Clarke St (Licensed) | 247 5423 |
| **Anatolia Turkish Provincial Cuisine**<br>Cnr Bunda & Mort St. (Licensed) | 257 1100 |
| **Shalimar Indian Restaurant**<br>9 Tasman House Marcus Clarke St<br>(Licensed/BYO) | 249 6784 |
| **Delicateating** (Italian cuisine)<br>O'Connor Shops (Licensed/BYO*) | 2471314 |
| **Vietnam Restaurant**<br>O'Connor Shops (BYO*) | 2487093 |

* the O'Connor supermarket has a good selection of wines and soft drinks

## CONFERENCE T-SHIRTS

The conference T-shirts feature a coloured logo designed by Dr Dick Barwick. The logo contains the chromosomes of a hybrid *Macropus* , mitochondrial DNA and flowers of Mendel's peas. It is an extremely attractive design and, at $20 - $26, well worth the price. The T-shirts are be available in Small, Medium, Large, XL and XXL in bleached or unbleached cotton with long or short sleeves. Orders received before the June 20th have been filled and can be paid for and collected at the registration desk. Further orders will be accepted at the conference, but an additional charge for postage will be necessary.

## STUDENT PRIZES

As a result of the generous contributions of several companies, the Society will be offering student prizes for outstanding poster and paper presentations. In addition, the Research School of Biological Sciences has offered a prize of $250 for the most outstanding poster/paper presentation by an honours or postgraduate student at the conference. Where possible prizes will be awarded at the Dinner on Thursday evening, however timetabling constraints necessitate the later awarding on some prizes.

1. **Amrad Pharmacia Biotech Award** for the best post-graduate student oral presentation - $250.

2. **Biotech International Award** for the best post-graduate student poster presentation - $250.

3. **Promega Award** for the best honours* oral presentation - $250

4. **Sigma-Aldrich Award** for the best honours* poster presentation - $200

5. **Research School of Biological Sciences Prize** for the most outstanding paper/poster presentation at the conference - $250

( * for work done as an honours student, not necessarily presented while still an honours student).

**Local Organising Committee:**

Dave Shaw

Tony Pryor

Dave Rowell

Cathy Stewart-Moore

Robyn Russell

Angela Higgins

The other members of the committee are particularly grateful to Dave Shaw who made the greatest contribution to the organisation of the conference. Dave's work is especially appreciated given that the wasn't even able to attend.

## SUSTAINING MEMBERS

Sigma Aldrich Pty. Ltd.

Promega Corporation

Beckman Instruments

Boehringer Mannheim Australia

Biotech International

Oxford University Press

Genesearch Pty. Ltd.

Gelman Sciences

Radiometer Pacific

Taylor-Wharton (Australia) Pty. Ltd.

Applied Biosystems Division (Perkin Elmer)

Scitech Pty. Ltd.

DuPont (Australia) Ltd.

Blackwell Science Pty. Ltd.

Bresatec Ltd.

Amersham Australia Pty. Ltd.

Selby Scientific Ltd.

Gordon & Breach

Daintree Industries

AMRAD Pharmacia Biotech

Integrated Sciences

Dynal Pty Ltd

# CONFERENCE PROGRAMME

## TUESDAY 4th JULY

4.00pm - 9.00pm GSA Registration, Burgmann College

7.00pm - 10.00pm GSA Mixer, Burgmann College

## WEDNESDAY 5th JULY

**Manning Clark Lecture Theatre 1**

8.30am Welcome to the Australian National University
**Professor Sue Serjeantson,** Deputy Vice-Chancellor, Australian National University.

9.00am Plenary Lecture by **Professor Rob Nicholls**, Case Western Reserve University, Cleveland, Ohio. " Imprinted genes, phenotypes and mechanisms of imprinting".

Chairperson : **Jenny Graves**

10.00am **Morning Coffee/Tea in Melville Hall**

**SYMPOSIUM ON HUMAN EVOLUTION**
**Manning Clark Lecture Theatre 1**
**Chairperson : Jenny Graves**

10.30am **Simon Easteal** " Molecular evidence for the timing of human evolution"

11.20am **Colin Groves** " The earliest fossil representatives of the human line."

11.50am **Cheryl Wise** "Recent coalescence of human mitochondrial genomes results from natural selection and not the movement of people out of Africa."

12.20pm **Greg Adcock**, E.S.Dennis, T.H.Loy, A.J.Peacock and A.G.Thorne "Extracting the past: the analysis of DNA variation in prehistoric aboriginal Australians."

12.50 - 2.00pm **Lunch**

**Concurrent Session**

**SMITH WHITE TRAVEL GRANT PAPERS**
**Manning Clark Lecture Theatre 2**
**Chairperson : Alan Wilton**

2.00pm **David J. Clancy** and Ary A. Hoffmann "Evolution of the endosymbiont *Wolbachia* , Drosophilid hosts and infection of a novel host *Drosophila serrata.*"

2.20pm **Craig R. Primmer**, Anders Pape Moller and Hans Ellegren "A wide-range survey of cross-species micro satellite amplification in birds."

2.40pm **Mary Morgan-Richards** "Variation in the rate of chromosome evolution within the New Zealand weta (Orthoptera : Stenopalmatidae)."

**Concurrent Session**

**HUMAN EVOLUTION**
**Manning Clark Lecture Theatre 1**
**Chairperson : Simon Easteal**

2.00pm **Sheila van Holst Pellekaan**, Marianne Frommer and Barry Boettcher "Mitochondrial D-loop diversity in Australian Aborigines."

2.20pm **Stephen A. Wilcox**, Megan J. Smithwick and Jennifer A. Graves "The evolution of genomic imprinting in mammals."

2.40pm **Amanda B.Spurdle** and Trefor Jenkins " The origins of the Lemba "Black Jews" of Southern Africa: Evidence from Y chromosome studies."

3.00 - 3.30pm **Afternoon Tea - Melville Hall**

**Concurrent Session**

**SMITH WHITE TRAVEL AWARDS (contd)**
**Manning Clark Lecture Theatre 2**
**Chairperson : Alan Wilton**

3.30pm **Coral G. Warr** and Leonard E. Kelly "Gating of light-sensitive ion channels in *Drosophila melanogaster* ."

3.50pm **Yan-Hong Wu** and John B. Gibson "Defective *hobo* insertion 5' to the distal start site of the alcohol dehydrogenase gene in *Drosophila melanogaster* affects only adult ADH activity."

4.10pm **S. Bartoszewski** and J.B.Gibson "The second intron controls expression of the *Gpdh* gene in *D. melanogaster* ."

4.30pm **C. Robin**, R.J.Russell and J.G. Oakeshott "Gene duplication and divergence in the $\alpha$ esterase cluster of *Drosophila melanogaster.* "

4.50pm **Lynn Jones**, Helena Richardson and Rob Saint "*Cyclin E* transcriptional regulation during *Drosophila* development."

5.10pm **Lyn Cook** "Rapid and extraordinary chromosomal evolution in an Australian Eriococcid."

**Concurrent Session**

**GENETICS OF NATIVE FAUNA AND FLORA**
**Manning Clark Lecture Theatre 1**
**Chairperson : Dick Frankham**

3.30pm **Bronwyn A. Houlden**, Philip England and William B.Sherwin "Hypervariable microsatellite loci reveal low levels of variation in bottlenecked koala populations from south eastern Australia."

3.50pm **David J. Coates** and Vicki L. Hamley " Population genetic structure, genomic change and hybridisation in the *Stylidium caricifolium* species complex."

4.10pm **Tamzin Donald,** Carolyn Leach, Tricia Franks and Jeremy Timmis "B chromosome sequence organisation in an Australian native plant species."

4.30pm M. Byrne, T.L.Parrish and **G.F. Moran** "Comparison of diversity in the nuclear and chloroplast genomes of *Eucalyptus nitens* ."

4.50pm **Axel Janke,** Neil J. Gemmell and Arndt von Haeseler " Structure, function and evolution of the mammalian mitochondrial genome."

5.10pm **Karen B. Firestone** and William B. Sherwin "Taxonomy of Quolls."

**THURSDAY 6th JULY**

**SYMPOSIUM ON MULTIGENE FAMILIES**
**Manning Clark Lecture Theatre 1**
**Chairperson : John Oakeshott**

9.00am **J.L.VandeBerg**, J.E. Hixson, C.M. Kammerer, D.L. Rainwater and J.W. MacCluer." Genes and gene families that affect plasma lipoprotein phenotypes and susceptibility to atherosclerosis."

10.00am **Morning Coffee/Tea - Melville Hall**

**MULTIGENE FAMILIES (CONTD)**
**Manning Clark Lecture Theatre 1**
**Chairperson : John Oakeshott**

10.30am **Peter R. Schofield** "The ligand-gated ion channel receptor superfamily : heritable mutations in the glycine receptor help to define structural domains of the receptor."

11.00am **David Ollis** "Dienelatone hydrolase : an $\alpha/\beta$ hydrolase fold enzyme."

11.30am **T.M.Boyce,** R.D.Newcomb, K.Y.Smyth, C. Robin, C.Claudianos, P.M. Campbell, K. Medveczky, R.J. Russell.and J.G. Oakeshott."Evolution of a multigene family : esterases caught in the act."

12.00noon **Margaret L. Delbridge** and J.A.M.Graves " Cloning and characterisation of Marsupial spermatogenesis genes."

**Concurrent Session**

**PHYLOGENETIC ANALYSIS**
**Manning Clark Lecture Theatre 2**
**Chairperson : Ross Crozier**

10.30am **J.S.F. Barker**, S.G. Tan, D.J.S. Hetzel, C.H. Lau, T.K.Mukherjee, S.S. Moore and R.D. Drinkwater."Phylogenetic analyses of Asian buffalo populations : allozymes, microsatellites and mtDNA."

10.50am Terry Reardon, Jana Skilins and **Steven Cooper** " Genetic relationships amongst Australian and Indo-Papuan *Rhinolophus* (Rhinolophidae, Chiroptera)."

11.10am **R.H.Crozier,** N.Dobric, H.T.Imai, D.Graur, J-M Cornuet and R.W. Taylor." Mitochondrial DNA sequence evidence on the phylogeny of Australian Jack-jumper ants of the *Myrmecia pilosula* complex."

11.30am **R. Kusmierski**, G. Borgia and R.H. Crozier "Labile evolution of exaggerated display traits in bowerbirds suggests reduced effects of phylogenetic constraint."

11.50am **Jennifer Seddon** and Peter R. Baverstock "Molecular phylogenetics of the Australian Chelidae."

12.10pm **Martin S.Elphinstone,** Jennifer M. Seddon and Peter R.Baverstock "Evolution of the Australian Fairy Wrens."

12.30 - 1.30pm **Lunch**

1.30pm **M.J.D. WHITE ADDRESS**
**Manning Clark Lecture Theatre 1**
**Professor John Mattick**

" The intron-exon structure of eukaryotic genes and the evolution of parallel processing."
**Chairperson : Rob Saint**

2.30 -3.00pm **Afternoon Tea - Melville Hall**

**Concurrent Session**

**SYMPOSIUM ON HIGHER PLANT GENETICS**
**Manning Clark Lecture Theatre 1**
**Chairperson : Peter Anderson**

3.00pm **Greg Lawrence**, Jeff Ellis, Jean Finnegan, Peter Anderson and Michael Ayliffe." Genetic and molecular analysis of rust resistance loci in flax: George Mayo's legacy to plant genetics."

4.00pm **M.A. Ayliffe**, G.J. Lawrence, J.G.Ellis and A.J. Pryor."Map-based cloning approach for the isolation of Flax rust avirulence genes."

4.30pm **Bernie Carroll** " Maize Ds transposons and transgenic tomatoes: a powerful combination for cloning important genes from plants".

5.00pm **P.Dodds**, E.Newbegin aand A.E. Clarke."Molecular genetics of gametophytic self-incompatibility in the Solanaceae."

**EVOLUTIONARY GENETICS**
**Manning Clark Lecture Theatre 2**
**Chairperson : John McKenzie**

4.00pm **Roland Toder**, Gudrun A. Rappold, Katrin Schiebel and Werner Schempp "*ANT 3 and STS* are autosomal in prosimian lemurs: implications for the evolution of the pseudoautosomal region."

4.20pm **Jennifer A. Graves** " Sex chromosome evolution and Haldane's rule."

4.40pm **Ary A. Hoffmann,** David J.Clancy and Michael Turelli "Population dynamics of maternally-inherited *Wolbachia* : two contrasting infections in natural populations of *Drosophila simulans* ."

5.00pm **Catherine J. Nock** and Peter R. Baverstock "The evolutionary origins of Australia's Elapid snakes."

7.30pm **Society Dinner**
**A.N.U. Union Building (Ground Floor)**
**Guest Speaker - Max Whitten.**

**FRIDAY 7th July**

**SYMPOSIUM ON THE REGULATION OF GENE EXPRESSION**
**Manning Clark Lecture Theatre 1**
**Chairperson : Rob Saint.**

9.00am **J.Drouin**, T. Lamonerie, C. Lanctot, J.J. Tremblay and M. Therrien. "Homeobox transcription factors and expression of pituitary hormone gene."

10.00 - 10.30am **Morning Coffee/Tea - Melville Hall**

**REGULATION OF GENE EXPRESSION (CONTD)**
**Manning Clark Lecture Theatre 1**
**Chairperson : Rob Saint**

10.30am Stephen L. Gregory, R. Daniel Kortschak, Bill Kalionis and **Robert B. Saint**."The *dead ringer* gene of *Drosophila* identifies a family of regulatory genes that encode a novel highly conserved DNA-binding domain."

11.00am **Chris M.Grant,** Paul F. Miller and Alan G.Hinnebusch. "Regulation of ribosomal reinitiation in the translational control of *GCN 4* expression in yeast."

11.30am **Richard B. Todd**, Meryl A. Davis and Michael J. Hynes "DNA binding of FacB, a transcriptional activator of acetate utilization genes of *Aspergillus nidulans* ."

11.50am **David Tremethick** "The mechanism by which DNA is assembled into chromatin and its role in regulating transcription"

12.10pm **Anita A. Piper** and Suyinn Chong " Methyl sensitive DNA:Protein interactions in an active X specific methylated region in the *Macropus robustus* HPRT gene."

**Concurrent Session**

**POPULATION AND GENERAL GENETICS**
**Manning Clark Lecture Theatre 2**
**Chairperson : Don Colgan**

10.30am **Carla Sgro** and Ary A. Hoffmann "Parental environments can induce high heritabilities for fecundity in *Drosophila melanogaster*."

10.50am **Peter Hunt** and Warwick Grant " Four mutations imparting a dominant ivermectin resistance phenotype in *Caenorhabditis elegans* ."

11.10am **Tania King** and Graham Wallis "Population structuring in an alpine weta hybrid zone as assessed using mitochondrial and microsatellite markers."

11.30am **Benjamin P. Oldroyd** and Ross H. Crozier."Number of matings in the genus *Apis* (Honeybees)."

11.50am **John A. McKenzie** and Janet L. Yen." Genotype, environment and the asymmetry phenotype. Dieldrin-resistance in the Australian sheep blowfly, *Lucilia cuprina*.."

12.10pm E.Lowe, M.E. Montgomery, L.M. Woodworth, D.A. Briscoe and **R. Frankham** "How large do wildlife populations need to be to retain their evolutionary potential ?"

12.30-1.30pm **Lunch**

**Concurrent Session**

**MOLECULAR GENETICS**
**Manning Clark Lecture Theatre 1**
**Chairperson : Tony Howells**

1.30pm **Peter Atkinson**, Steven Whyard, Craig Coates, Alexandra Pinkerton, Hilary Mende, Ken Savillle, Bill Warren and David O'Brochta "*HAT* transposable elements as gene vectors for insects."

1.50pm **Craig J.Coates**, Antony J. Howells, David O'Brochta and Peter W. Atkinson "The use of plasmid based excision assays to determine transposable element mobility in various insect species."

2.10pm **Tomasz M.Wilanowski**, Phil T. Barnes and John B.Gibson "The molecular structure of ultra-fast rare mobility variants of *sn* - glycerol-3- phosphate dehydrogenase in *Drosophila melanogaster*."

2.30pm Nelida Contreras,Val MacLean, **Rob O'Brien** and Dave Shaw. "The genomic and molecular organisation of centromeres in the genus *Caledia* ."

2.50pm **K.Freebairn**, N.Anthony, G. Colebatch, R.ffrench- Constant, J.A. McKenzie and P. Batterham." Dieldrin resistance in the Australian sheep blowfly, *Lucilia cuprina* ."

3.10pm **Paul Sunnucks** and Dinah Hales " Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera:Aphididae)."

**Concurrent Session**

**PLANT GENETICS**
**Manning Clark Lecture Theatre 2**
**Chairperson : Michael Ayliffe**

1.30pm Zhao Xiaochun, P. Sharp, G.Crosbie, I. Barclay and **R.Appels.** "A single gene specifying noodle quality in particular wheat varieties."

1.50pm **R.I.S. Brettell**, B. Witrzens and F.R. Murray "Gene transfer for modifying grain properties of wheat."

2.10pm **R.B. Gupta**, H.S. Bariana, R. Appels and F. MacRitchie "Genetic dissection of grain quality traits in wheat."

2.30pm E.S. Lagudah, S. Chandramohan, **O.Moullet**, R. Eastwood, R. Appels, K. Andersen and S. Andersen."Molecular genetic analysis of the *Cre* 3 locus that confers resistance to cereal cyst nematode in wheat."

2.50pm **Sharon J. Orford** and Jeremy N. Timmis " Specific genes expressed during cotton fibre development."

3.10pm **David R. Smyth** and John Alvarez "Role of the *PINOID* gene in generating flower primordia in *Arabidopsis* ."

3.30 - 5.00pm **Afternoon Tea and Drinks**

**POSTERS AND TRADE DISPLAYS**
**Melville Hall**

5.30pm **Annual General Meeting**

**Manning Clark Lecture Theatre 2**

## SATURDAY 8th JULY

### SYMPOSIUM ON THE GENETICS OF MARINE ORGANISMS
**Manning Clark Lecture Theatre 1**
**Chairperson : Oliver Mayo**

9.00am **Peter Grewe** "Genetics in fisheries management : past accomplishments and future directions."

10.00 - 10.30am **Morning Coffee/Tea - Melville Hall**

### GENETICS OF MARINE ORGANISMS (contd)
**Manning-Clark Lecture Theatre 1**
**Chairperson : Dave Coates**

10.30am **Shane Lavery** "Marine genetic resources in the tropics : how little we know."

11.00am **Don Colgan** and John Paxton "Genetic diversity and stock identification in the Common Gemfish, *Rexea solandri* . "

11.30am **John Benzie** "Coral reef genetics and the biogeography of tropical oceans."

12noon **M.Johnston** "Population biology of silver trevally, Pseudocaranx dentex, from the east coast of Australia and New Zealand."

**Concurrent Session**

### FUNGAL GENETICS
**Manning Clark Lecture Theatre 2**
**Chairperson : Murali Nayadu**

10.30am **Frederick J. Bowring** and D.E.A. Catcheside "Physical analysis of meiotic crossing over and gene conversion events in *Neurospora crassa* and their implications for molecular models of recombination."

10.50am Pam K. Flynn, Brian F. Cheetham and **Margaret E. Katz**. "The isolation of genes involved in the expression of extracellular proteases."

11.10am **Patricia A. vanKuyk**, Brian F. Cheetham and Margaret E. Katz. "Regulation of the extracellular proteases of *Aspergillus nidulans* ."

11.30am **J.P.Silk**, J.J. Burdon, A.J. Pryor, R.F. Park and D.R. Marshall. "The genetic diversity of stem rust, a fungal pathogen of wheat."

11.50am **M. Nayudu** " Biological control of the take-all fungal root pathogen of wheat by *Pseudomonas* bacteria."

12.10pm **Viji Krishnapillai**, Wai Foong Hong, Meena Dass, Jillian Dunphy and Lisa Ryan "Genome organisation of the bacterial plant pathogen *Pseudomonas solanacearum* : virulence and essential genes."

**Concurrent Session**

## MARSUPIAL GENETICS

**Manning Clark Lecture Theatre 4**
**Chairperson : Bill Sherwin**

10.30am **L.M. McKenzie** and D.W. Cooper "The level of genetic variation within two island populations of the Tammar Wallaby (*Macropus eugenii* )."

10.50am **David A. Loebel** and Peter G. Johnston " Methylation analysis of a marsupial X-linked CpG island by bisulphite genomic sequencing."

11.10am **Cushla J. Metcalfe,** Mark D.B. Eldridge, Leon McQuade and Peter G. Johnston " Mapping the distribution of the telomeric sequence $(TTAGGG)_n$ in three *Petrogale* species by fluorescence *in situ* hybridisation."

11.30am **C.M.Watson**, P.G.Johnston, R.L.Hughes, R.Gemmell, M.Smith and D.W.Cooper " X-chromosome involvement in marsupial sex differentiation."

11.50am **E.Gale**, B. Houlden, P.Hoeben, W.Sherwin and P. Timms "Applications of RAPD-PCR fingerprinting in koalas."

12.10pm Alexandra C.C. Wilson and **Mark D.B. Eldridge** "Preliminary analysis of mtDNA D-loop sequence from Black-Footed Rock wallabies (*Petrogale lateralis*)."

12.30pm **Lunch in National Botanic Gardens**

# POSTERS

**Melville Hall**

**Greg Adcock, David Buckle,** S.Fountain, T.H. Loy and S.L.McElroy "Exploring Australian prehistory with the tools of molecular genetics."

**T. Badgery-Parker** and W. Sherwin "Genetic variation in a captive Sticknest Rat colony."

**T.M. Boyce** "The recombinational landscape of *Caenorhabditis elegans* ."

**M.Byrne,** I. Marquez-Garcia, T. Uren, D.N. Smith and G.F. Moran "Isolation and characterisation of microsatellites in eucalypts."

**Z. Chen** and P. Batterham "The cloning and sequencing of the *Notch* homologue from the Australian Sheep Blowfly, *Lucilia cuprina* ."

**Steve Chenoweth** "Genetic population structure of the Australian Bass *Macquaria novemaculeata* determined by mitochondrial control region variation screened using Temperature Gradient Gel Electrophoresis/ Heteroduplex Analysis"

**B.C.Clarke** and R. Appels "The structure of the *Sec - 1* locus on the short arm of chromosome 1R of Rye (*Secale cereale* )."

Stanley Robert, **Michelle Coulson** and Robert Saint "Characterisation of a *Polycomb* interactor."

**Christopher Driver** "Neurological factors contributing to loss of activity with age in *Drosophila melanogaster* ."

**Stephen D. Goodall,** David Haywood, Lynn Hines, Anthony Howells and Peter Atkinson. "The *Sex-lethal* gene of *Lucilia cuprina*.."

**Yasmine Gray**, Mark Tanaka and John Sved "The hybrid excision -insertion model for P elements : production of recombinants and duplication/deficiencies."

K.A.E. Groom and **M.Nayudu** "How *Pseudomonas* bacteria colonize the roots of wheat plants."

K.A.E. Groom and **M. Nayudu** "Exopolysaccharides play a role in biological control protection of *Pseudomonas* bacteria against the take-all fungal root pathogen of wheat."

**Guanglan Guo** and Alan N. Wilton "Angiotensinogen genotypes in pre-eclampsia/eclampsia (PE/E)."

Jane M. Hughes, Stuart E. Bunn and **David A. Hurwood**. "Genetic Differentiation among Populations of *Caridina zebra* (Decapoda: Atyidae) in a Queensland Rainforest."

**Debbie J. Marsh,** Diana L. Learoyd, Scott D. Andrew and Bruce G. Robinson "Somatic mutations in the RET proto-oncogene in medullary thyroid carcinoma."

**Gawain McColl**, Ary Hoffmann and Stephen McKechnie "Response of heat shock genes to selection for heat resistance in *Drosophila melanogaster* ."

**C. Lynne McIntyre**, Pascale Besse, Guillermo Galvez and Philip A. Jackson "Is parental genetic diversity a useful predictor of sugarcane cross performance ?"

**Stephen W. McKechnie**, Nicola H. Stokes and Neil W. Forrester "Natural allelic variation in a sodium channel gene from *Helicoverpa armigera* detected by temperature gradient gel electrophoresis."

**Martin McLoon,** Brian F. Cheetham and Margaret E. Katz "Genetic variation in *Aspergillus fumigatus* strains isolated from Ostriches."

**M.E. Montgomery**, L.M. Woodworth, R.K.Nurthen D.A. Briscoe and R. Frankham "Population size and genetic variation."

Matthew K. Morell, Rod Peakall, **Lynette R. Preston**, Katrina Moore and Rudi Appels "Analysis of genetic variation within and among varieties of the outcrossing species *Lolium perenne* using RAPD."

**R.M. Murphy**, G.C.Webb, G.B. Peters, A.I. Cassady A.C. Cavanagh and K.M. Summers "Chaperonin 10 : an evolutionarily conserved multigene family ?"

**M.Nayudu**, T. Murphy, P.T.W. Wong and J. Ash "Bioluminescence (using luciferase or *lux* ) a unique and cheap markerfor detection of biological control *Pseudomonas* bacteria from the plant rhizosphere."

**Rachel J. Waugh O'Neill**, R.H. Crozier and J.A.M. Graves "The molecular evolution of SRY: an analysis of reproductive isolation and Haldane's rule in Rock Wallabies (*Petrogale* )."

**Peter Papagiannopoulos**, Alex Andrianopoulos, Julie A. Sharp Meryl A. Davis and Michael J. Hynes "The *hapC* gene of *Aspergillus nidulans*, a homologue of the yeast *HAP 3* gene."

**Tracy Parrish** and Mike Crisp "The systematics and hybridisation of two species of Waratah, *Telopea mongaensis* and *T. oreades*."

**J.M. Roberts-Thomson**, J. Norwich, J.J. Martinson, R.M. Harding, J.B. Clegg and B. Boettcher "Australian aboriginal study: alpha-globin haplotype analysis supports a common and ancient origin with New Guinea Highlanders."

**D.M. Rowell,** D.M. Gleeson, N.N. Tait, D.A. Briscoe and A.V. Higgins "Genetic evolution at three levels in the Onychophora."

**A. Ruvinsky** and I. Rogozin "A consequence of illegitimate recombination of line 1 element in mammals."

**Ian Scott** "Are thickheads monophyletic ?: evidence from cytochrome B."

**K.E. Sloper** and R.Baker "Yeast and human ubiquitin specific-proteases."

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LIST OF REGISTRANTS

ADCOCK Greg, Archaeology and Prehistory, RSPAS, ANU, Canberra, ACT 0200
ALPERS Deryn, School of Biological Science, University of New South Wales, P.O. Box 1, Kensington NSW 2033
ANDERSON Peter,
ANDREWS Dan, Human Genetics Group, JCSMR Australian National University PO Box 334 CANBERRA CITY ACT 2601
APPELS Rudi, Division of Plant Industry, CSIRO, Clunies Ross St, Black Mountain ACT 2601
ARMSTRONG Tristan, Division of Botany and Zoology, ANU Canberra 0200BARKER, J.S.F., Dept of Animal Science, University of New England, Armidale NSW 2351
ATKINSON Peter, Division of Entomology, CSIRO, Clunies Ross St, Black Mountain ACT 2601
ATKINS Karen, Dept Genetics University of Melbourne PARKVILLE VIC 3052
AYLIFFE Michael, Division of Plant Industry, CSIRO, Clunies Ross St, Black Mountain ACT 2601
BADGERY-PARKER Tim, School of Biological Science, University of NSW, 2052
BARTOSZEWSKI Slawek, MPG. RSBS. ANU, GPO Box 475, Canberra ACT 2600
BATTERHAM Philip, Genetics Dept, University of Melbourne, Parkville VIC 3052
BELL Charlie, CSIRO Division of Forestry, P.O. Box 4008, Canberra ACT 2600
BENZIE John, Australian Institute of Marine Science, PMB No 3 Townsville Qld 4810
BESSE Pascale, CSIRO-DCTP, 306 Carnody Rd, St. Lucia QLD 4067
BOWRING Frederick, School of Biological Sciences, Flinders University, Bedford Park, SA 5042
BOYCE Thomas, CSIRO Division of Entomology, Canberra ACT 2601
BRETTEL Richard, Division of Plant Industry, CSIRO, Clunies Ross St, Black Mountain ACT 2601
BUTCHER Penny, CSIRO Div Forestry & Forest Products PO Box 4008 Queen Victoria Terrace CANBERRA ACT 2600
BYRNE Margaret, CSIRO Division of Forestry, P. O. Box 4008, Queen Victoria Tce, Canberra ACT 2600
CAMPBELL Bronwyn,
CAMPBELL Hugh D., CMSF, RSBS, ANU, P.O. Box 475, Canberra ACT 2601
CARROLL Bernie, Dept of Agriculture, University of Qld, Brisbane
CARTHEW Sue, Div Environ. Sci & Range Management Roseworthy Campus - Univ Adelaide ROSEWORTHY SA 5371
CHEN ZhenZhong, Dept of Genetics, University of Melbourne, Parkville, Vic 3052
CHENOWETH Steve, Faculty of Environmental Sciences, Griffith University, Nathan
CLANCY David, School of Genetics and Human Variation, LaTrobe University, Bundoora Vic 3083
CLARKE Bryan, Division of Plant Industry, CSIRO, Clunies Ross St, Black Mountain ACT 2601
CLAUDIANOS Charles, Division of Botany and Zoology, Australian National University, GPO Box 4, Canberra ACT 2601
COATES Craig, Division of Entomology, CSIRO, Clunies Ross St, Black Mountain ACT 2601
COATES David, W.A. Herbarium, Dept of CALM, P.O. Box 104, Como WA 6152
COLGAN Don, Evolutionary Biology, Australian Museum, William St Sydney 2000
COLLEY Peter, Molecular Genetics, John Hunter Hospital, Newcastle 2310
COOK Lyn, Div. of Botany & Zoology, ANU ACT 0200
COOK Sheridan, School of Genetics and Human Variation, LaTrobe University, Bundoora Vic 3083
COOPER Steven, E.B.U., South Australian Museum, North Tce., Adelaide S.A. 5000
CORDINER Tom, Dept Genetics University of Melbourne 56 Church St Nth Fitzroy VIC 3068
COULSON Michelle, Dept of Genetics, University of Adelaide, Adelaide 5005
CROZIER, Ross H., School of Genetics, La Trobe University, Bundoora VIC 3083
DABORN Phillip, Genetics Dept, University of Melbourne, Parkville VIC 3052
DAVIDSON Heather, Dept Genetics University of Melbourne PARKVILLE VIC 3052
DAVIS Meryl, Dept of Genetics, Melbourne University VIC 3052
DELBRIDGE Margaret, Dept of Genetics, LaTrobe University, Bundoora Vic 3083
DODDS Peter, Plant Cell Biology Research Centre, University of Melbourne, Parkville Vic 3052
DONALD Penny,
DONALD Tamzin, Dept of Genetics, University of Adelaide, Adelaide 5005
DONNELLAN Steve, Evolutionary Biology Unit Sth Australian Museum North Terrace ADELAIDE SA 5000
DRIVER Christopher, Deakin University, Rusden Campus, Blackburn Rd Clayton Vic

DROUIN Jacques, Institu de recherches clinique de Montréal (IRCM), Quebec H2W IR7 Canada
DUFFY Brendan, Dept of Genetics, University of Adelaide, Adelaide 5005
EASTEAL Simon, Human Genetics Group JCSMR, ANU, Canberra ACT 2601
Eldridge Mark, School of Life Sciences, Macquarie University, North Ryde NSW 2109
ELLIOTT Nick,
ELPHINSTONE Martin, Faculty of R.S. & M., P.O. Box 157, Lismore NSW 2480
ENGLAND Phillip, School of Life Sciences, Macquarie University, North Ryde NSW 2109
EWEN Kelly, 475-485 Mickleham Rd, Attwood VIC 3049
FAIRHALL Astrid,
FIRESTONE Karen, School of Biological Science, University of NSW 2052
FORBES Emma, Dept Genetics University of Melbourne PARKVILLE VIC 3052
FRANKHAM R., School of Biological Sciences, Macquarie University NSW 2107
FREEBAIRN Kris, Dept of Genetics, University of Melbourne, Parkville 3052
GALE Elizabeth, CMB, School of Life Science, QUT, Brisbane QLD 4001
GASKELL Giles,
GIBSON John, MPG. RSBS. ANU, GPO Box 475, Canberra ACT 2600
GOODALL Stephen, Division of Entomology, CSIRO, Clunies Ross St, Black Mountain ACT 2601
GRAMMATICOPOULOS Georgia,
GRANT Chris, University of NSW, School of Biochemistry Sydney 2052
GRAVES Jenny, Dept of Genetics and Human Variation, La Trobe University, Bundoora, 3083
GRAY Yasmine, School of Biological Science A12: University of Sydney, NSW 2006
GREVILLE Warwick, School of Biological Sciences, University of New South Wales, Kensington, NSW 2033
GREWE Peter, Division of Fisheries Research, CSIRO Hobart Tas.
GROVES Colin, Prehistory and Anthropology, ANU ACT 0200
GUO Guanglan, School of Biochemistry, University of New South Wales, Sydney NSW 2052
GUPTA Ram, Division of Plant Industry, CSIRO, Clunies Ross St, Black Mountain ACT 2601
HEALY Marion, Division of Entomology, CSIRO, Clunies Ross St, Black Mountain ACT 2601
HESLEWOOD Margaret, RSM; Southern Cross University, Lismore NSW 2480
HIGGINS Angela, Division Botany & Zoology, Aust. Nat. University, Canberra ACT 0200
HOFFMANN Ary, School of Genetics and Human Variation, LaTrobe University, Bundoora 3083
HOULDEN Bronwyn, School of Biological Science, University of New South Wales, Sydney NSW 2052
HOWELLS Tony, Division of Biovchemistry and Molecular Biology, ANU ACT 0200
HRYSOUDIS Anthony, Monash University, Wellington Rd Clayton Vic. 3168
HUANG JiongLe,
HUNT Peter, c/- CSIRO "Chiswick", New England Highway, Armidale NSW 2350
HURWOOD David, Faculty of Environmental Sciences, Griffith University, Nathan
HYNES Michael J., Dept of Genetics, University of Melbourne, Parkville VIC 3052
IRVINE Danielle,
JANKE Axel, Zoologisches Institut, Universität München, P.O. Box 202136, D-80021 Munchen Germany
JENKINS Nicole, Dept of Genetics & Human Variation, La Trobe University, Bundoora VIC 3083
JOHNSON Rebecca,
JOHNSTON Michael, Division Botany & Zoology, Aust. Nat. University, Canberra ACT 0200
JONES Lynne Dept of Genentics University of Adelaide SA 5005
KATZ Margaret, Dept of Animal Science, University of New England, Armidale NSW 2351
KELLET Mark 301 Crisp St, Hampton 3188
KING Tania Dept of Zoology, University of Otago, POB 56, Dunedin, New Zealand
KRISHNAPILLAI V., Dept. of Genetics & Devel. Biology, Monash University, Clayton VIC 3168
KUSMIERSKI Robert, Dept of Genetics, La Trobe University, Bundoora Vic. 3083
vanKUYK Patricia, Dept of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, NSW 2351
LANE Cinnamon University of Melbourne Vic.
LAU Nelson, Monash University, Wellington Rd, Clayton Vic 3168
LAWLER Susan, Dept of Environmental Management and Ecology,La Trobe University, Albury-Wodonga Campus, POB 963, Wodonga Vic 3689
LAVERY Shane, QABC, Level 4, Gehrmann Labs., University of Queensland QLD 4072
LAWRENCE Greg, CSIRO Division of Plant Industry, GPO box 1600, Canberra ACT 2601
LAYFIELD Sharon, 157 Bambra Rd, South Caulfield Vic. 3162
LEACH Carolyn, Dept of Genetics, University of Adelaide SA 5005
LOEBEL David, School of Biological Sciences, Maquarie University NSW

LOWE Edwin, School of Biological Sciences, Maquarie University, 2104
LUPTON Michelle,
MARSH Debbie, Kolling Institute, Royal North Shore Hospital, St Leonards 2065
MARTIN Jon, Genetics Dept, University of Melbourne, Parkville VIC 3052
MASOUMI Amir, CSIRO Division of Entomology, POB 1700, Canberra, ACT 2601
MATTICK John, Centre for Molecular and Cellular Biology, University of Queensland
MAY Cedric, Institute of Agricultural Research, Wagga Wagga NSw 2650
MAYO Oliver, CSIRO Division of Animal Production, Locked Bag No.1 - Delivery Centre, Blacktown NSW 2148
McCOLL Gawain, Dept of Genetics, Monash University, Wellington Rd, Clayton VIC 3168
MCKECHNIE Steve, Dept. Genetics & Dev. Biology, Monash University, Clayton VIC 3168
MCKENZIE John, Genetics Dept, University of Melbourne VIC 3052
McKENZIE Louise, School of Biological Sciences, Maquarie University NSW
McINTYRE Lynne, CSIRO Division of Tropical Crops and Pastures, 306 Carmody Rd, St Lucia 4067
MCLEAN Lynette, Dept of Animal Science, University of New England, Armidale NSW 2351
MCLOON Martin, Dept of Biochemistry, Microbiology and Nutrition, University of New England Armidale NSW 2351
McPARTLAN H., via 475 Mickleham Rd, Attwood Vic 3049
McQUADE Leon, School of Biological Sciences, Maquarie University, NSW 2109
METCALFE Cushla, Biology Dept, Maquarie University, NSW 2109
MILKINS Kate, 53 View Hill Cres, Eltham Vic 3095
MITCHELL Claire, Dept Genetics University of Melbourne PARKVILLE VIC 3052
MONTGOMERY Meg, School of Biological Sciences, Maquarie University NSW 2109
MORAN Gavin F., CSIRO Division of Forestry, P.O. Box 4008, Canberra ACT 2600
MORGAN-RICHARDS, Mary, School of Biological Sciences, Victoria University of Wellington, POB 600 New Zealand
MORRELL Mathew, PSC, RSBS, ANU PO Box 475, Canberra ACT 2601
MOULLET Odile, Division of Plant Industry, CSIRO, Clunies Ross St, Black Mountain ACT 2601
MURA Roberta, Dept of Agriculture, University of Queensland 4072
MURPHY Rachael, Dept of Genetics, University of Melbourne, Parkville VIC 3052
MURPHY Rachel, University of Queensland, Dept of Surgery, Clinical Sciences Building, RBH Herston 4029
MURRELL Jan, CSIRO Division of Forestry, POBox 4008, Canberra ACT 2601
NAYUDU Murali, Division Botany & Zoology, Aust. Nat. University, Canberra ACT 0200
NEWSOME Tim, University of Melbourne Vic
NGOC TUAN LE, 68 Eley Rd, Box Hill South, Vic 3128
NICHOLLS Rob, Genetics, Case Reserve University, Cleveland OH, U.S.A.
NOCK Cathy, Centre for Conservation Technology, Faculty of Resource Science and Management, Southern Cross University, PO Box 157, Lismore NSW 2480
OAKESHOTT John, Division of Entomology, CSIRO, Clunies Ross St, Black Mountain
ACT 2601
O'BRIEN Rob, Research School of Biological Sciences, ANU ACT 0200
ODGERS Wendy, Division of Entomology, CSIRO, Clunies Ross St, Blackmountain ACT 2601
OLDROYD Ben, School of Biological Sciences, Macleay, University of Sydney NSW
OLLIS David, Research School of Chemistry, ANU, ACT 0200
ON Strevda, Division of Botany and Zoology, ANU ACT 0200
O'NEILL Rachel, Dept of Genetics, La Trobe University, Bundoora 3083
ORFORD Sharon, Dept of Genetics, University of Adelaide 5005
PAPAGIANNOPOULOS Peter, Dept of Genetics, University of Melbourne, Parkville VIC 3052
PARBERY Peter, Dept Genetics University of Melbourne PARKVILLE VIC 3052
PARRISH Tracy, CSIRO Division of Forestry, P.O. Box 4008, Canberra ACT 2600
PASK Andrew, 26 Heath Ave, Ferntree Gully 3156
PASSIOURA Jason, University of WA
PEAKALL Rod, Division Botany & Zoology, ANU, Canberra ACT 0200
PELLEKAAN Sheila van holst, Genetics, School of Biological Sciences A11, University of Sydney NSW
PERKINS Harvey D., CSIRO Division of Forestry, P.O. Box 4008, Queen Victoria Terrace, Canberra ACT 2600
PETRIS Michael, Dept of Genetics University of Melbourne PARKVILLE VIC 3052
PIPER Anita, Dept of Cell and Molecular Biology, University of Technology, Sydney NSW
PRESTON Lynette, Research School of Biological Sciences, ANU ACT 0200
PRIMMER Craig, Dept of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Biomedical Centre, Box 597, S-751 24 Uppsala Sweden

PRYOR Tony, Division of Plant Industry, CSIRO, Clunies Ross St, Black Mountain ACT 2601
PYKE Fiona, Dept. of Genetics, University of Melbourne, Parkville NSW 3052
RAPHAEL Kathie,
ROBERTS-THOMSON June, Dept. of Biological Sciences, University of Newcastle, Newcastle NSW 2308
ROBIN Charles, Division of Entomology, CSIRO, Clunies Ross St, Black Mountain ACT 2601
ROBINSON Nick, Victorian Institute of Animal Science, 475 Mickleham Rd, Attwood, Vic, 3049
ROBSON Meryl, School of Biological Sciences (A12) Sydney University NSW 2006
RODBOURN Louise,
ROFE Ruth, Dept of Genetics, University of Adelaide, Adelaide SA 5005
ROWELL David, Division Botany & Zoology, ANU, Canberra ACT 0200
RUNCIMAN David, School of Genetics and Human Variation, LaTrobe University, Bundoora Vic 3083
RUSSELL Robyn, Division of Entomology, CSIRO, Clunies Ross St, Black Mountain ACT 2601
RUVINSKY Anatoly, Dept of Animal Science, University of New England, Armidale NSW 2351
SAINT Robert, Dept of Genetics, University of Adelaide, Adelaide. SA 5005
SCHAFER Darren, Dept of Biochemistry, Microbiology and Nutrition, University of New England, Armidale NSW 2351
SCHOFIELD Peter, Garvan Institute of Medical Research, 384 Victoria St Darlinghurst NSW 2010
SCHMIDT M., Dept of Genetics, La Trobe University, Bundoora 3083
SCOTT Ian, Research School of Biological Sciences, ANU ACT 0200
SEDDON Jenny, Faculty of Health Sciences, Southern Cross University, P.O. Box 157, Lismore NSW 2480
SERGEANTSON Sue, Deputy Vice-Chancellor, ANU ACT 0200
SGRO Carla, School of Genetics and Human Variation, La Trobe University, Bundoora 3083
SHARKEY Dierdre, School of Biological Sciences, University of New South Wales, Kensington, NSW 2033
SHARP Julie, University of Melbourne, Dept. of Genetics, Parkville Vic 3052
SHARP Peter J., Plant Breeding Institute, Cobbitty Road, Cobbitty NSW 2570
SHEARMAN Deborah, Macleay Bldg, A 12, University of Sydney NSW 2006
SHELDON Bruce, CSIRO PO Box 184 North Ryde NSW 2113
SHERRARD Sharon, 7 Fiona Court, Keysborough 3173
SHERWIN Bill, School of Biological Sciences, University of New South Wales, Kensington, NSW 2033
SILK John, CSIRO Division of Plant Industry GPO Box 1600 Canberra ACT
SKILINS Jana,
SLADE Rob, Dept of Zoology, University of Queensland Qld 4072
SLOPER Katherine, JCSMR, ANU, PO Box 334 Canberra ACT 2601
SMALL Anna, Dept. of Genetics, University of Melbourne, Parkville VIC 3052
SMITH Alana, Monash University, Clayton Vic 3168
SMITH Ellie, Cytogenetics, Children's Hospital Camperdown Sydney, NSW
SMITH Michiko, Genetics Dept, University of Melbourne, Parkville Vic 3052
SMITHWICK Megan, Dept. of Genetics, La Trobe University, Bundoora Vic 3083
SMYTH David, Dept of Genetics and Dev. Biology, Monash University Clayton Vic 3168
SMYTH Kerry-Ann, Division of Entomology, CSIRO, Clunies Ross St, Black Mountain ACT 2601
SPIELMAN Derek,
SPURDLE Amanda, Dept. of Genetics and Human Variation, La Trobe University, Bundoora VIC 3083
STARR Gary, Dept of ES & RM, University of Adelaide, Roseworthy SA 5371
STEMPLE Chris, Dept. of Genetics, University of Melbourne, Parkville Vic 3052
STEWART-MOORE Cathie, Research School of Biological Sciences, ANU ACT 0200
SUNNUCKS Paul, School of Life Sciences, Macquarie University, North Ryde NSW 2109
SYDES Marita, Division Botany & Zoology, ANU, Canberra ACT 0200
SZOEKE Cassandra, Dept Genetics University of Melbourne PARKVILLE VIC 3052
TANAKA Mark, School of Biological Sciences A.12 University of Sydney SYDNEY NSW 2006
TAYLOR Andrea, School of Biological Science, University of NSW, PO Box 1, Kensington NSW 2033
TAYLOR Chris, 475 - 485 Micklewood Rd Attwood Vic 3049
TILLEY Charles,
TIMMIS Jeremy, Dept. of Genetics, University of Adelaide, Adelaide SA 5005
TODD Richard, Dept of Genetics, University of Melbourne, Parkville VIC 3052
TODER Roland, Dept. of Genetics & Human Variation, La Trobe University, Bundoora VIC 3083
TREMETHICK David, JCSMR, ANU, ACT 0200
TROTT Josie, Dept Animal Science B.19 University of Sydney SYDNEY NSW 2006
TURNER M., Division of Plant Industry, CSIRO, Clunies Ross St, Black Mountain ACT 2601
TURNER Rachel, Dept. of Biochemistry, University of New South Wales, Sydney NSW 2052

VANDEBERG John, Southwest Foundation for Biomedical Research, San Antonio, Texas 78228 USA
van KUYK Patricia, Dept. of Animal Science, University of New England, Armidale NSW 2351
WAKEFIELD Matthew, Dept. of Genetics & Human Variation, La Trobe University, Bundoora VIC 3083
WARD Wayne, Victorian Institute of Animal Science, 475-485 Mickleham Rd, Attwood VIC 3049
WARR Coral, Genetics Dept, University of Melbourne, Parkville Vic 3052
WARREN Bill, Division of Entomology, CSIRO, Clunies Ross St, Black Mountain ACT 2601
WATSON Cathy, Biological Sciences, Macquarie University NSW 2109
WHITTEN Max, CSIRO Entomology, Canberra ACT 2601
WHYARD S., CSIRO Entomology, Canberra ACT 2601
WILANOWSKI Tom, Molecular and Population Genetics, RSBS. ANU, GPO Box 475, Canberra ACT 2600
WILCOX Stephen, Dept. of Genetics, La Trobe University, Bundoora VIC 3083
WILKS Ann, Molecular and Population Genetics, RSBS. ANU, GPO Box 475, Canberra ACT 2600
WILLIAMS Jill, Dept. of Genetics, University of Melbourne, Parkville VIC 3052
WILTON Alan, Biochemistry, University of New South Wales, Sydney NSW 2052
WISE Cheryl, John Curtin School of Medical Research, Australian National University, Canberra ACT 2601
WOODWORTH Lynn, Biological Sciences, Macquarie University NSW 2109
WU Yan-Hong, Molecular and Population Genetics, RSBS. ANU, GPO Box 475, Canberra ACT 2600
YEN Janet , 62 Brushy Park Rd, Wonga Park V IC 3115
ZHANG Li, Department of Medicine, Prince Charles Hospital, Chermside Qld 4032
ZHAO Jing Ting, School of Biological Sciences, Sydney University NSW 2006

# PAPER ABSTRACTS

**In alphabetical order by presenting author**

# EXTRACTING THE PAST: THE ANALYSIS OF DNA VARIATION IN PREHISTORIC ABORIGINAL AUSTRALIANS.

**Greg Adcock[1,2], E. S. Dennis[2], T. H. Loy[3], A. J. Peacock[2] and A. G. Thorne[1].**

**1. Division of Archaeology and Natural History, R.S.P.A.S., Australian National University, A.C.T. 0200, Australia.**

**2. CSIRO Division of Plant Industry, Black Mountain, A.C.T. 2601, Australia.**

**3. Centre for Molecular and Cellular Biology, and Department of Anthropology and Archaeology, University of Queensland, St Lucia 4072, Australia.**

Bone from fifteen ancient Aboriginal individuals has yielded amplifiable DNA. To achieve this we developed a methodology for the analysis of ancient human bone. This methodology includes consultation with local Aboriginal groups, minimising of starting material needed and stringent controls for contamination. DNA was extracted from ancient individuals of several hundred years to over 30,000 years of age from Northern Victoria, Western New South Wales and New Guinea. From these extractions we used the polymerase chain reaction to amplify the highly variable mitochondrial control region (D-loop). We targeted the control region to take advantage of the large data-base available for comparison and the techniques for analysis developed in the many phylogenetic and population studies already done. The data-base includes almost a thousand living humans from all parts of the world, including more than a hundred Aboriginal Australians and when compared with our ancient sequences allows us to ask many questions. Most fundamentally we need to be certain that our data are useful for analysis. Can we be certain that the ancient DNA is not some kind of contaminating DNA sequence? How meaningful are population comparisons from a small data set? Then we can ask how much do the ancient sequences resemble people living today, and how do the relationships fit with our ideas about population movements into and within Australia? The ancient data also has the potential to look at evolutionary processes directly. Are there any mutations that can be "dated", and are any ancient lineages ancestral to modern ones?

## A single gene specifying noodle quality in particular wheat varieties

**Zhao Xiaochun[1], P. Sharp[1], G. Crosbie[2], I. Barclay[2], R.Appels[3].**

**[1] University of Sydney, Plant Breeding Institute-Cobbitty, PMB11, Camden, NSW 2570**

**[2] Western Australian Dept. of Agriculture, 3 Baron-Hay Court, South Perth, WA 6151**

**[3] CSIRO, Division of Plant Industry, PO Box 1600, Canberra, ACT 2601**

The establishment of a direct link between noodle quality and specific attributes of wheat flour (Nagao et al. 1977; Oda et al. 1980; Crosbie 1990) has provided the basis for segregating wheat grain for noodle markets. Early studies showed starch from Australian wheats had characteristics that were desirable for Japanese noodle manufacture. The key feature of the starch granules proved to be related to the volume of the gel formed when starch granules were hydrated and heated; this led to a simple flour swelling volume (FSV) test (Crosbie 1990) that allowed the prediction of noodle quality. The analysis of progeny from a cross between the wheat varieties Kulin (poor noodle quality and relatively low FSV) and Reeves (good noodle quality and relatively high FSV) were carried out in order to assess whether FSV, and thus noodle quality, could be linked to the presence or absence of one of the major proteins in the starch granules. Particular attention was focussed on the presence or absence of the granule bound starch synthase (GBSS) protein encoded by a locus on chromosome 4A since this protein is often missing from Australian wheat varieties. The analysis of 34, F5, families derived from a single cross provided evidence for complete linkage between the absence of the GBSS-4A protein and high FSV. The study has therefore identified a major gene that controls the swelling properties of starch granules. Additional factors can influence noodle quality and the data reported suggests that these can now be analysed by examining the variation in FSV that exists in varieties missing the GBSS-4A protein.

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# *HAT* transposable elements as gene vectors for insects.

Peter Atkinson, Steven Whyard, Craig Coates, Alexandra Pinkerton, Hilary Mende, Ken Saville*, Bill Warren* and David O'Brochta*
CSIRO Division of Entomology, Canberra, Australia &
*Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute, College Park, Md, USA

Excision and transposition assays enable the mobility properties of transposable elements in host and non-host insect species to be determined. We have developed these assays for the *hobo* element of *Drosophila melanogaster*. Excision assays show that *hobo* excises in a similar way to the *Ac* element of *Zea mays* and the *Tam3* element of *Antirrhinum majus* suggesting that these 3 elements are members of a single family of transposable elements - the *hAT* family. *Hobo* is capable of accurate plasmid to plasmid transposition in its host species as well as in the house fly, *Musca domestica*, the Queensland fruitfly, *Bactrocera tryoni* and the Old World cotton bollworm, *Helicoverpa armigera*. These results suggest that *hobo* should also be capable of undergoing plasmid to chromosome transposition in these species. A *hobo* vector in which the gene expressing resistance to the antibiotic G418 was inserted into the *hobo* element was consequently used to genetically transform *B. tyroni* showing that *hobo* elements can be used as gene vectors in non-drosophilid insects.

We have also isolated other members of the *hAT* element family from other insects - *Hermes* from *M.domestica* and *Homer* from *B.tryoni*. One of these, *Hermes*, has been used as a gene vector in *D. melanogaster*.

**A Map-based Cloning Approach for the Isolation of Flax Rust Avirulence Genes.**

**M.A. Ayliffe, G.J. Lawrence, J.G. Ellis and A.J. Pryor.**

**CSIRO Division of Plant Industry, PO Box 1600, Canberra, ACT 2601, Australia and the**
**Cooperative Research Centre for Plant Science, PO Box 475, Canberra, ACT 2601, Australia.**

Plants possess complex molecular defense mechanisms that are effective against a broad range of pathogens. Recognition of phytopathogen attack is mediated by a specific resistance gene in the host plant that recognises a complementary avirulence gene expressed in the pathogen. If the invading pathogen is not expressing an appropriate avirulence gene the plant is incapable of recognising pathogen attack, regardless of its resistance gene status.

The molecular nature of fungal avirulence gene products is poorly understood. However recently avirulence genes have been isolated from two fungal pathogens, *Cladosporium fulvum* and *Magnaporthe grisea*, the causative agents of tomato leaf mold and rice blast respectively. These avirulence genes encode relatively small peptides whose functions have not yet been determined. This report will describe a map based cloning approach for the isolation of avirulence genes from the flax rust fungus (*Melampsora lini*). The interaction between this rust pathogen and its host (*Linum usitatissimum* ) has been extensively characterised genetically and was the model system that lead to the formation of the gene-for-gene hypothesis (Flor, 1956) which has remained as central dogma in plant-pathogen interactions.

Molecular markers linked to flax rust avirulence genes have been identified by a variety of PCR based techniques (AFLP, DDRT, RAPD). Analysis of spontaneous mutants of flax rust with these markers suggests that mutation from avirulence to virulence in the pathogen may frequently occur by DNA deletion. A transformation system for this obligate, biotrophic plant pathogen is being developed. Transient gene expression in rust urediospores has been achieved by particle bombardment with a 35S-GUS gene construct. A selectable marker for transformation is being developed around resistance to the fungicide benomyl by *in vitro* mutagenesis of a rust *B*-tubulin gene.

**The second intron controls expression of the *Gpdh* gene in *D.melanogaster*.**

**S. Bartoszewski and J.B. Gibson**
**Molecular & Population Genetics Group, Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra, ACT 2601**

The *sn*-glycerol-3-phosphate dehydrogenase gene *(Gpdh )* of *D.melanogaster* has an interesting pattern of expression. Three different isozymes are expressed with tissue and developmental specificity - they arise from different polyadenylation pathways and the resulting processing.

To study the regulation of *Gpdh* expression we constructed a fragment 13 kb long, including 5 kb upstream and 3 kb downstream of the *Gpdh* transcribed sequences. Flies transformed with this construct have levels of GPDH similar to that of the genomic copy of the gene.

Subsequently, transformations were made with four constructs lacking some DNA sequences. Two of these constructs produced GPDH levels indistinguishable from the control; one had sequences deleted upstream leaving 900 bp 5' to the transcription start site and a second one lacked 3 kb of DNA downstream from the last described polyadenylation signal.

In contrast, the level of expression for a construct lacking the 1.6 kb second intron was reduced by an order of magnitude. Almost no expression was detectable when all three alternative polyadenylation signals were removed. The above results were obtained both in adult flies and in larvae. We conclude that major regulatory elements are located in the second intron and perhaps also in close proximity to the promoter. The polyadenylation signals, which are responsible for tissue specificity are also essential for the level of the gene's expression.

Phylogenetic analyses of Asian buffalo populations : allozymes, microsatellites and mt DNA. J.S.F. Barker[1], S.G. Tan[2], D.J.S. Hetzel[3], C.H. Lau[2], T.K. Mukherjee[4], S.S. Moore[3] and R.D. Drinkwater[3].

[1]Department of Animal Sciences, University of New England, Armidale; [2]Department of Biology, University Pertanian Malaysia, Serdang, Malaysia; [3]CSIRO Molecular Animal Genetics Centre, Brisbane; [4]University of Malaya, Kuala Lumpur, Malaysia.

Some 3500 breeds and strains of about 40 species of domestic animals (i.e. those used to produce food, fibre, power, skins, manure, etc for human use) exist in the world today. These breeds and strains are referred to as the global animal genetic resources. The genetic variation, both between and within breeds, is described as the diversity within each species. Concern about reduction in genetic diversity has been expressed primarily in terms of loss of breeds and strains. Many have become extinct in recent years, and many more are endangered. However, in the global management of animal genetic resources, the fundamental distinction is not between those breeds that are endangered and those that are not, but between those that are perceived to have little or no current utility and those which do have current utility or seem likely to have in the immediate future. For each of these categories, the necessary actions are then preservation or utilization (including breeding programs for genetic improvement).

Decisions about which breeds should be conserved should be based on objective criteria, taking into account not only current utility, but also the maintenance of maximum genetic diversity in the global gene pool of each species. Maintenance of maximum genetic diversity could be achieved by conserving that sub-set of all breeds in a species that show the most genetic differentiation among them. A complete description of the genetic differences between any two breeds is not possible, but measures of genetic distance provide the best available objective description of their genetic differentiation. In 1993, FAO convened a Working Group to examine the feasibility of a global program for estimating genetic distances among breeds for each species of domestic livestock. This Group recommended that such a program should be developed, and that genetic distances should be based on variation at microsatellite loci, because of the high levels of polymorphism that they exhibit and consequently the high resolution discrimination expected between closely related populations of the same species. Allozyme loci have been commonly used to infer genetic distances among populations, while a number of recent studies have used mt DNA variation. It is important then to compare distances based on these three types of genetic variation - protein coding loci, genomic molecular markers and mitochondrial (mt) DNA, to determine if they are concordant, and if not, what differential forces may be affecting them.

We have assayed 17 buffalo populations from Asia for allozyme variation at 53 loci, a subset of 11 of these populations for microsatellite variation at 21 loci, and for mt DNA D-loop and cytochrome b sequences. Results for phylogenies and genetic distance comparisons will be presented.

**Coral reef genetics and the biogeography of tropical oceans.**

by

**John A. H. Benzie**
**Australian Institute of Marine Science**
**PMB No 3**
**Townsville Qld 4810**

Coral reefs include some of the most diverse ecosystems in the planet, and coral reef organisms were the object of some early studies on the relationship between genetic diversity and environmental stability. Since then, much more has been discovered about the dynamic nature of coral reef systems, but relatively little concerning the genetic structure of coral reef species until recently. Surveys of the population genetic structure of a variety of taxa from several phyla are revealing cryptic taxa, a complexity of breeding systems and consistent patterns of genetic differentiation among species within the Great Barrier Reef and in the Indo-Pacific. Some of the patterns of gene flow are not consistent with present- day ocean circulation or with traditional views of marine biogeography. There is growing debate concerning the extent of connectedness of widespread marine species and the nature of speciation in the marine environment. Much genetic work on coral reef species is being carried out in Australia and the paper will review genetic studies on corals, giant clams, starfish, sponges and foraminifera that are providing new insights into the tropical marine environment and the evolution of coral reef systems.

# Physical analysis of meiotic crossing over and gene conversion events in *Neurospora crassa* and the implications for molecular models of recombination..

**Frederick J. Bowring and D.E.A. Catcheside.**
**Flinders University, Bedford Park, S.A.**

The more popular molecular models of genetic recombination posit that crossing over is one of two possible outcomes from the resolution of a recombination intermediate. As gene conversion results from the initiation of a recombination intermediate these models predict that the association between gene conversion and crossing over is 50%. This is based in part on early work by Hurst *et al.* (**1**) who found that about half of the prototrophic recombinants from a cross of parents with different mutant alleles also had a recombinant association of flanking markers. However, some have questioned this association. Carpenter (**2**) has argued for some time that gene conversion and crossing over have different frequencies due to their different purposes: conversion is an early and frequent event resulting from the search for homologous sequences while crossing over is less frequent, occurs later and stems from the formation of structures necessary for the correct segregation of homologs during meiosis I (chiasmata). Indeed, Fogel *et al.* (**3**) have subsequently shown that the observed 50% association was fortuitous while Foss *et al* (**4**) have reviewed the data from a number of different organisms and find the association to be less than 50% although still quite high (approx. 30%).

We have found numerous sequence polymorphisms (SP's) and restriction site polymorphisms (RSP's) in and close to the Neuropsora *am* gene in our laboratory stocks and have used these to examine the nature of recombination events at this locus. We have examined the length and nature of gene conversion tracts and find that some are discontinuous. The segregation of RSP's closely flanking *am* demonstrate that the association between crossing over and gene conversion is less than 10% at this locus.

These data suggest that if crossing over results from the resolution of a recombination intermediate, then at the *am* locus at least, there is a mechanism which ensures a strong bias in favour of resolution without crossing over.

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**Evolution of a multigene family: esterases caught in the act.**

**T. M. Boyce, R. D. Newcomb, K. A. Smyth, C. Robin, C. Claudianos, P. M. Campbell, K. Medveczky, R. J. Russell, and J. G. Oakeshott.**

**Biotechnology Program**
**Division of Entomology**
**CSIRO**
**GPO Box 1700**
**Canberra, ACT 2601**

The diversification of multigene families requires duplication and divergence of family members and may be accelerated by selection for functional diversity. The carboxyl and cholinesterases in insects are a paradigm of multigene family diversification with important practical applications. Members of the alpha carboxyl esterase family have evolved to confer resistance to organophosphate insecticides in a variety of species.

In *Drosophila melanogaster*, the alpha esterases include a cluster of ten genes and one pseudogene. Homologues to at least four of these have been identified in the sheep blowfly, *Lucilia cuprina*, and the housefly, *Musca domestica*. In *L. cuprina*, resistance to diazinon and most OPs is conferred by an allele of one of these genes, αE7. This allele of a carboxyl esterase now acts as a phosphatase - a dramatic change of enzymatic function caused by a single amino acid substitution.

Resistance to a different organophosphate, malathion, is conferred by an allele at another gene in the family, simply known as malathion carboxylesterase. This allele continues to encode a carboxylesterase, but with substantially enhanced ability to degrade the carboxylester bonds of malathion.

RFLP analysis of the family in *L. cuprina* shows that both resistances occur throughout Australia, and are associated with only one or two haplotypes. Selection for both resistances has apparently occurred such that a single allele at each locus is now widespread and common. Intriguingly, flies homozygous for resistance to diazinon are typically not resistant to malathion and vice versa. Recent evidence suggests that this pattern is a constraint imposed by negative epistasis between genes in the family, and that pressure to circumvent this constraint may be driving the family through further duplication and diversification.

## Gene transfer for modifying grain properties of wheat

R.I.S. Brettell, B. Witrzens, F.R. Murray, R. Appels
CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601.

Genetically transformed wheat plants have been generated using the technique of microparticle bombardment. Gold particles are coated with purified plasmid DNA and shot into immature zygotic embryos with sufficient velocity to penetrate the nucleus of target cells from which plants can be regenerated in aseptic culture. The low frequencies of stable transformation for individual cells make it necessary to employ selectable marker genes coding for resistance to antibiotics or herbicides, and select regenerable cultures for resistance to the selective agent. In recent experiments we have recovered independently transformed wheat lines at a frequency of between 0.1 and 0.3% of target embryos. We have demonstrated the stable inheritance of introduced bacterial marker genes by gel blot hybridisation of DNA extracted from progenies of transgenic wheat plants segregating for resistance to the selective agent. The presence of marker gene sequences frequently does not correlate with detectable expression of the gene in progeny plants, suggesting that the transgenes may be progressively inactivated following their introduction into the wheat genome. These observations are consistent with other studies which suggest that transgene inactivation may be a common phenomenon in transgenic plants (Finnegan and McElroy, 1994).

In collaboration with other researchers at CSIRO and the Plant Science Centre, genes predicted to alter wheat grain properties are being isolated and transferred to hexaploid bread wheat cultivars. The genes include those involved in protein and starch biosynthesis in the developing grain. The occurrence of transgene inactivation in wheat leads us to predict that it will be easier to modify biosynthetic pathways by using anti-sense to reduce the synthesis of an undesirable enzyme or storage protein, than by introducing novel genes to boost the amount of a given gene product.

Finnegan,J., McElroy, D. (1994) Transgene inactivation: plants fight back! Bio/technology **12**: 883-888

**Maize *Ds* transposons and transgenic tomatoes: a powerful combination for cloning important plant genes.**

**B. J. Carroll[1,2], C. M. Thomas[2], V. Klimyuk[2], D. A. Jones [2] and J. D. G. Jones[2]**

**[1]Department of Agriculture, University of Queensland, Brisbane and [2] The Sainsbury Laboratory, John Innes Centre for Plant Science Research, Norwich, UK.**

The maize transposon *Activator (Ac)* and its non-autonomous derivative *Dissociation (Ds)* comprise the first transposon system discovered by McClintock. We have introduced *Ds* elements into transgenic tomato and have shown the system to be a powerful combination for cloning genes from plants. The behaviour of *Ds* elements in tomato is similar to its behaviour in maize (Carroll et al. 1995), but the transgenic system offers several advantages including: i) the genetic attributes of tomato, ii) markers to monitor transposition, iii) low *Ds* copy number for efficient cloning of tagged genes, iv) flexible control of transposition by an unlinked transposase gene, and v) the important option of recombinant techniques for improving the efficiency of the system for cloning genes from plants.

We have used this system to clone an important gene in shoot development. *Ds* insertion in the coding sequence of the gene causes cell division to occur in a disorganized fashion during embryogenesis, and all cell division in the shoot and root meristems ceases immediately after germination of mutant seedlings. The sequence of the tagged gene is homologous to a cDNA sequence in the data base. The homologous cDNA is derived from floral buds of another species but no biological function has been assigned to the corresponding gene. Reversion of the tomato mutant to wild type only occured in the presence of the transposase gene, and was due to excision of the *Ds* element and restoration of the reading frame of the gene. Somatic reversion allowed the shoot meristem to resume growth and develop in a normal fashion. Determining the biochemical function of the gene will be the subject of future research. The efficacy of the transgenic tomato system for tagging genes has also been recently demonstrated by the cloning of a disease resistance gene (Jones et al. 1994).

Carroll B. J. et al. (1995) Germinal transposition of the maize element Dissociation from T-DNA loci in tomato. Genetics 139, 407-420.

Jones D.A. et al. (1994) Isolation of the tomato Cf-9 gene for resistance to Cladosporium fulvum by transposon tagging. Science 266, 789-793.

# Evolution of the endosymbiont *Wolbachia*, Drosophilid hosts, and infection of a novel host, *Drosophila serrata*.

**David J. Clancy and Ary A. Hoffmann**

**School of Genetics and Human Variation, La Trobe University, Bundoora, Victoria 3083, Australia.**

ABSTRACT

*Drosophila serrata* was experimentally infected with the maternally inherited endosymbiont *Wolbachia*, which is responsible for cytoplasmic incompatibility in a large number and range of insect hosts. This phenomenon causes reduced egg hatch rate when infected males mate with females which do not carry a compatible Wolbachia strain. This indirectly results in the spread of Wolbachia and its host cytoplasm type through populations. Egg hatch rate from incompatible crosses, fecundity deficit associated with infection, and rate of production of uninfected offspring by infected females (segregation) are three parameters which can affect rate and extent of spread of Wolbachia in populations. *D. serrata*, a novel host, showed extreme values for incompatibility and segregation. These values are compared with those from three naturally occurring *Drosophila-Wolbachia* associations. Differences between these Drosophila infections probably reflect different durations of association between host and parasite. The results are generally consistent with theoretical predictions of evolution of host and parasite; hosts can initially evolve to reduce incompatibility to an extent, probably by controlling parasite load, but Wolbachia will eventually lose its ability to cause incompatibility. In addition, fecundity deficits are probably prone to reduction by host control of parasite load, and evolution of Wolbachia, rather than the host, will reduce segregation.

**The Use Of Plasmid Based Excision Assays To Determine Transposable Element Mobility In Various Insect Species.**

Craig J. Coates[1,2] , Antony J. Howells[2], David A. O'Brochta [3] & Peter W. Atkinson[1].
1 CSIRO Entomology, GPO Box 1700, Canberra, ACT, 2601.
2 ANU, GPO Box 4, Canberra, ACT, 2601.
3 Maryland Biotechnology Institute, University of Maryland, MD 20742, USA.

We have used excision assays as a means to detect the mobilisation of a transposable element from a plasmid in the embryonic soma of various insect species. These assays have previously been utilised to assay the excision of the P and hobo elements from *Drosophila melanogaster* (1,2). We have extended these assays to test the mobility of the hermit element from Lucilia cuprina and the mariner element from *D. mauritiana*.

DNA sequence analysis of the hermit element indicates that it is a member of the hAT family of transposable elements to which hobo also belongs (3). We present data indicating that the hermit element can be mobilised in *D. melanogaster* in the absence of hobo transposase. The addition of hobo transposase increased the frequency of mobilisation, providing functional evidence that hermit is a member of the hAT family of transposable elements. Similar assays carried out in *L. cuprina* also demonstrated hobo and hermit mobility. This indicates that there may be a source of endogenous transposase or host factors necessary for hAT element mobilisation within the *L. cuprina* genome.

The mariner excision assays were carried out in the embryonic soma of D. melanogaster, *D. mauritiana* and *L. cuprina*. No excision events were observed in the absence of exogenous transposase. The addition of exogenous transposase, either under the control of the mariner promoter or a heat shock promoter, resulted in the recovery of excision events from all three species. The types of excision events recovered from *L. cuprina* were identical to those recovered from the Drosophila species. This indicates that the mariner element is capable of correct mobilisation in the embryonic soma of *L. cuprina* and may be able to form the basis of a germ-line transformation system for this species.

The plasmid based excision assays are a rapid means of testing the mobility of various transposable elements in the embryonic soma of insects. The results from these assays may give an indication of the presence of endogenous sources of transposase or host factors required for mobilisation of the various elements. They may also be useful in determining which transposable element system has the greatest potential to act as a gene vector system in a particular insect species.

1. O' Brochta et al., 1988. PNAS 85: 6052-6056.
2. Atkinson et al., 1993. PNAS 90: 9693-9697
3. Coates et al., 1995. Genetica, in press.

**POPULATION GENETIC STRUCTURE, GENOMIC CHANGE, AND HYBRIDISATION IN THE *STYLIDIUM CARICIFOLIUM* SPECIES COMPLEX.**

**David J. Coates and Vicki L. Hamley**
**Western Australian Herbarium, Department of Conservation and Land Management, PO Box 104, Como, WA 6152**

*S. caricifolium* complex taxa are diagnostically differentiated by a range of chromosome re-arrangements and apart from *S. affine* and *S. caricifolium* are generally well differentiated allozymically. Like other *Stylidium* taxa they exhibit unusually high levels of allelic diversity even in taxa which have extremely restricted distributions and relatively small isolated populations. Within taxa significant levels of allozyme divergence can be found, not only between disjunct population groups which are often confined to geographically restricted and unique plant communities, but also between adjacent populations.

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 indicate that hybridization is extensive and ongoing. These data also show that increasing genomic divergence between the two species occurs in a south-north direction and that hybridisation may have occurred in a north-south direction. 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.

*Stylidium* populations have a number of features which may explain the complex evolutionary patterns and levels of diversification observed in this species complex and the genus generally. Field studies suggest that populations are largely inbreeding but that most of the seed set comes from low level cross pollination because of the operation of post zygotic seed aborting lethal systems. Effective population size may fluctuate dramatically over relatively short periods of time in response to fire and other disturbance while population fragmentation and isolation are characteristic of many taxa.

## GENETIC DIVERSITY AND STOCK IDENTIFICATION IN THE COMMON GEMFISH *Rexea solandri*

Don Colgan and John Paxton

The Australian Museum, 6-8 College St., Sydney 2000

The range of the common gemfish, *Rexea solandri*, includes southern Australian and New Zealand waters. Intensive fishing of *R. solandri* developed in the early 1970's, the species becoming the most important winter catch in the southeastern Australian trawl fishery. Since 1987, the catch has declined dramatically and severe management restrictions have been imposed. Similar catch histories have been seen for New Zealand gemfish. From a fisheries management perspective, the most pressing requirements for genetic information are deciding whether the Australian and New Zealand populations may be regarded as distinct and whether there exists more than one stock in Australia. We have sought such information using protein electrophoresis, RFLP analysis of mtDNA and sequencing of mtDNA and the ribosomal ITS regions.

The results show that Australian fish are separated into two genetically distinct stocks with a boundary at the western end of Bass Strait. There is one nearly-fixed allozymic difference between the stocks and fixed differences for mtDNA haplotypes for all 11 studied endonucleases. In contrast, eastern Australian fish are genetically close to those from New Zealand. Rarer allozymes, haplotypes, or polymorphisms in the 12S rDNA are restricted to one region or the other. None of these markers is by itself, statistically significant, although the frequency differences suggest low levels of gene flow between the regions. One variant haplotype was seen in eastern New Zealand fish and not in those from the west coast, but there were no other indications of stock separation from protein electrophoretic or RFLP data.

Sequencing studies of the ribosomal ITS regions are currently in progress. These are aimed at further testing of the genetic similarity between Australian and New Zealand gemfish, and possible sub-division of the latter stock.

# RAPID AND EXTRAORDINARY CHROMOSOMAL EVOLUTION IN AN AUSTRALIAN ERIOCOCCID.

Lyn Cook

**Division of Botany and Zoology**
**Australian National University ACT 0200**

*Apiomorpha* (Hemiptera: Coccoidea: Eriococcidae) is a genus of scale insect in which both males and females induce galls on *Eucalyptus*. The galls induced by females of *Apiomorpha* are generally species-specific and those of some species are amongst the largest and most spectacular of all insect-induced galls. The taxonomy of *Apiomorpha* is based upon the morphology of adult females and, to a lesser extent, the galls they induce. There are currently 39 morphospecies of *Apiomorpha* recognized.

Coccoids possess holokinetic chromosomes but, contrary to the assumption that holokinetic chromosomes show a great propensity for fragmentation, most coccoid families and genera have retained conservative chromosome number ranges. *Apiomorpha*, however, has been found to exhibit chromosome numbers ranging from 2n = 4 to 2n = 164. This range exceeds that previously reported for the whole of the Hemiptera.

Variation in diploid chromosome number has been found within 16 of the 24 morphospecies of *Apiomorpha* for which more than one population has been examined. In only six of these morphospecies has concomitant variation in morphology been detected. The large range of chromosome numbers found within several other morphospecies suggests that several cryptic (morphologically indistinguishable) species may be present.

*Apiomorpha* provides an opportunity to study the role of chromosomal evolution in speciation processes. If it can be demonstrated that chromosomal differences became fixed prior to morphological or genic changes becoming fixed, then chromosome change may be implicated as playing a primary causative role in the speciation of the chromosomally divergent taxa.

**Genetic relationships amongst Australian and Indo-Papuan *Rhinolophus* (Rhinolophidae, Chiroptera).**

**by Terry Reardon, Jana Skilins and Steven Cooper**

**Evolutionary Biology Unit, S.A. Museum, Adelaide, S.A. 5000**

Two morphologically distinct species of horseshoe bats, *Rhinolophus philippinensis* and *Rhinolophus megaphyllus*, have been described in Australia. *R. philippinensis* is restricted in its distribution to Cape York, but has a wide distribution through South East Asia from the Philippines to New Guinea. *R. megaphyllus* is found along the east coast of Australia from Victoria to Queensland and is also found in Papua New Guinea and the Bismark Archipelago. The two species are sympatric on Cape York, and in this region a third form has been observed which is intermediate in size between the two species and has a distinct echolocation call. To resolve the taxonomic status of this third form (henceforth referred to as the "intermediate") we have carried out genetic studies of Australian and Indo-Papuan rhinolophids using both allozyme electrophoresis and sequencing of the mitochondrial DNA (mtDNA) control region.

The surprising results from both studies suggest that the three Australian taxa are monophyletic and recently diverged, a sharp contrast to the traditional view of their phylogenetic relationships based on morphology. No fixed differences were detected at 45 allozyme loci, between each of the three Australian taxa, while representatives of eight other species of *Rhinolophus* showed fixed differences ranging from 0 to 50%. The intermediate was found to contain mtDNA haplotypes that were almost identical to those found within *R.philippinensis*, but distinctly different from those within *R. megaphyllus*. These results suggest that the intermediate is most likely to be either a hybrid resulting from a cross between a female *R. philippinensis* and male *R. megaphyllus* or a morphological variant of *R. philippinensis*. MtDNA sequence data also provide preliminary evidence for genetic subdivision between Queensland and Victorian forms of *R. megaphyllus*.

**Mitochondrial-DNA sequence evidence on the phylogeny of Australian Jack-jumper ants of the *Myrmecia pilosula* complex.**

R. H. Crozier[1], N. Dobric[1], H. T. Imai[2], D . Graur[1,3], J-M Cornuet[1], R. W. Taylor[4].

[1] Department of Genetics and Human Variation, La Trobe University, Bundoora, V 3083, AUSTRALIA [2] National Institute of Genetics, Yata 1,111, Mishima, Shizuoka-ken, JAPAN 411 [3] Permanent address: Department of Zoology, Tel Aviv University, Tel Aviv, 69978 ISRAEL [4] Division of Entomology, CSIRO, PO Box 1700, Canberra, ACT 2601, AUSTRALIA

Australian ants of the *Myrmecia pilosula* species complex include some individuals (in *M. croslandi*) with the lowest possible metazoan chromosome number of 2n = 2. Others in this cluster of sibling species have much higher numbers, the known maximum being 2n = 32. Two species (*M. pilosula* and *M. 'banksi'*) are believed on cytogenetic and morphological grounds to have hybridized over a long period. To investigate the phylogeny and age of this group relative to the congeneric outgroup species *M. gulosa* we sequenced part of the cytochrome *b* gene and the intergenic sequence between it and a primer anchored on the nearby $tRNA^{Ser}_{UCN}$ gene, and analysed the coding region using bootstrapped parsimony and NJ trees using the numbers of synonymous and non-synonymous codons per site. The intergenic space demonstrated a profusion of repeated sequences and only very closely related sequences (as judged by that for cytochrome *b*) showed detectable similarity at this almost 100% A+T region. In agreement with prodictions from karyotype studies, the phylogenetic analyses showed that *M. croslandi* is the sister group to the other siblings; the time of separation of *M. croslandi* from the rest of the *pilosula* group is unexpectedly ancient. Other relationships were poorly resolved, but the results suggest that *M. banski* and *M. pilosula* cluster together, as expected on cytogenetic grounds, and the tentative suggestion of close affinity of the *M. pilosula* samples and two 'PB' samples supports derivation of PB from female *M. pilosula* and male *M. 'banksi'*.

# Cloning and Characterization of Marsupial Spermatogenesis Genes.

**Margaret L. Delbridge and J.A.M. Graves.**
**Dept. of Genetics and Human Variation, La Trobe University, Bundoora, Victoria, 3083.**

In humans, deletion mapping and molecular analysis have identified regions of the Y chromosome associated with male infertility. The genes in these regions are presumed to be involved in spermatogenesis.

One group of genes which are candidate spermatogenesis genes, the YRRM gene family, has been localized to a 1-2 Mb region on Yq (Ma et al., 1993). Two members of this gene family have been cloned and show strong homology to a family of nuclear RNA-binding proteins, hnRNP, which are associated with RNA processing, possibly by influencing RNA splice site choice. Unlike the hnRNP's which are widely expressed, YRRM expression is specific to developing germ cells in the testis.

The TSPY sequences may also be involved in spermatogenesis. These sequences have a testis-specific expression and have been localized to Yp in humans (Arnemann et al, 1987). Comparisons of genomic sequences from several Y derived cosmids revealed that 30-60 TSPY sequences are tandemly repeated as part of the 20kb DYZ5 repeat units. Several cDNA have also been analysed and differ in single base substitutions, size and exon composition. The different transcripts are thought to be derived from divergent duplicate genes or products of alternative splicing.

However, the function of both YRRM and TSPY gene families is unknown, and the presence of multiple copies of these genes makes investigation of their role in spermatogenesis difficult to ascertain. Comparisons of these genes in humans and other mammals with their homologues in the distantly related marsupial species is a stringent test of function, as only functionally important genes are likely to be conserved over such large evolutionary distances. In particular, it seems likely that functionally important male-specific genes located on the Y in humans will also be conserved on the Y chromosome in other mammals.

Using human cDNA clones for these two genes as probes, we have shown that both genes appear at a single autosomal locus in marsupials. We have cloned a marsupial cDNA homologue of the YRRM family which has up to 90% homology to a region of a member of the hnRNP family, hnRNPG, and considerable homology (50-60%) to YRRM genes. This clone displays both autosomal and male-specific hybridization patterns in marsupials and has testis specific expression. We believe these data may have interesting implications for Y chromosome evolution. Other marsupial YRRM cDNA clones have different restriction digest patterns, which may be the result of alternative splicing events, or products of more than one genetic locus. We hope that analysis of these marsupial genes will enable us to extrapolate to the analysis of the function, patterns of expression and alternative splicing of these genes in humans.

Arnemann, J. et al.(1987), *Genomics* **11**: 108-114
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Molecular genetics of gametophytic self-incompatibility in the Solanaceae.

P.Dodds, E. Newbigin, A.E. Clarke.

Self-incompatibility (SI) is a genetically controlled mechanism that prevents inbreeding in many families of flowering plants (de Nettancourt, 1977). In many plants, SI is determined by a single locus (the *S* locus) with multiple alleles. Pollen expressing the same allele as the stigma on which it germinates is unable to cause fertilisation. At least two distinct systems operating in different plant families can be distinguished on the basis of their genetics; sporophytic SI (SSI) in which the SI phenotype of the pollen is determined by the diploid genotype of its parent, and gametophytic SI (GSI), in which the pollen phenotype is determined by its own haploid genotype. GSI has been extensively studied in the family Solanaceae, which includes tobacco, tomato, petunia and potato. The products of the S-locus in the pistil have been identified as glycoproteins with ribonuclease activity and have been termed S-RNases. The ribonuclease activity of the S-RNases is essential for SI and the arrest of incompatible pollen tube growth is associated with degradation of pollen tube rRNA. There is evidence for the entry of S-RNases into pollen tubes and a current model for the action of GSI proposes that the S-RNases act as selective cytotoxic agents, inhibiting the growth of incompatible pollen tubes by degrading their RNA.

The nature of the recognition process between pollen and pistil is not understood. We have recently shown that S-RNases are also expressed in developing pollen of *Nicotiana alata* (a self-incompatible tobacco species), thus raising the possibility that the pollen component of GSI may be identical to the pistil component. Alternatively there may be another gene located at the S-locus which is responsible for determining the pollen SI phenotype. Early genetic experiments indicated that the pollen and style components of the S-locus could be independently mutated. Current approaches to identifying the pollen component of GSI in the Solanaceae will be discussed.

# B CHROMOSOME SEQUENCE ORGANISATION IN AN AUSTRALIAN NATIVE PLANT SPECIES.

Tamzin Donald, Carolyn Leach, Tricia Franks and Jeremy Timmis.

*Department of Genetics, University of Adelaide, Adelaide, S.A. 5005.*

B chromosomes are a form of supernumerary DNA which are found in all major taxonomic groups of organisms, particularly plants. By and large, B chromosomes appear to be mildly deleterious to the individuals which carry them. Although the B chromosomes significantly increase the genome size (by up to 40% in a rye plant with 8 B chromosomes), no active genes have yet been unequivocally localised on these chromosomes by genetic or molecular means. Nevertheless, the presence of B chromosomes has been shown to be associated with potentially significant changes in chromosome behaviour, such as pairing and recombination, and it is therefore thought that they play a significant biological role. The mechanisms which are responsible for these effects remain to be isolated.

Fluorescent *in situ* hybridisation (FISH) with biotinylated rDNA revealed the presence of an rRNA gene cluster on one pair of A chromosomes and also on the B chromosome of *Brachycome dichromosomatica* an Australian native ephemeral plant of the arid regions of South Eastern Australia. This species contains only two pairs of A chromosomes and up to three B chromosomes. No molecular differences between the A and B rDNA clusters were detected by Southern analysis and, because of this and the commonly observed association of B chromosomes with the nucleolus, it is thought that members of both A and B clusters of rRNA genes may be expressed.

A second tandemly repeated sequence was located, also by FISH, at the centromere of the B chromosome. This cluster contains a 176 bp repeat unit which is present in approximately $1.8 \times 10^5$ copies on each B chromosome[1] but only at very low copy number in the A chromosome complement. The presence of this sequence at the centromere may have some influence on the abnormal separation (nondisjunction) of B chromosomes which is observed at pollen grain mitosis. Such abnormal behaviour is thought to account for the accumulation and maintenance of B chromosomes in this and many other species.

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## HOMEOBOX TRANSCRIPTION FACTORS AND EXPRESSION OF PITUITARY HORMONE GENE

**J. Drouin, T. Lamonerie, C. Lanctöt, J.J. Tremblay and M. Therrien.**
**Institut de recherches cliniques de Montréal (IRCM), Québec H2W IR7 Canada**

The pituitary gland provides a simple and well characterized system to study cell differentiation and its relation to pattern formation. As the pituitary derives from the most anterior part of the neural ridge, the control of the pituitary organogenesis is likely to be integrated with mechanisms for head development. Members of the POU-homeo family of transcription factors have been involved in late events of pituitary cell differentiation; indeed, the pituitary - specific Pit1 factor is required for maintainance growth hormone, prolactin and TSH- producing pituitary cells as well as for cell-specific transcription in those cells. Anterior pituitary cells expressing the pro-opiomelanocortin (POMC) gene are the earliest ones to differentiate, much before the Pit1-dependant lineages. As the results of our analysis of the mechanisms for cell-specific transcription of the POMC gene, we have identified homeobox containing transcription factors that are involved in POMC transcription. One of these is a novel homeobox transcription factor Ptx1 (pituitary homeobox 1). Few homeobox genes have been implicated in head formation in contrast to the well documented role of Hox genes in patterning of the trunk: the implication of Pxt1 and other homeobox genes in pituitary transcription and possibly in patterning of head structures, provides new tools to understand this complex developmental process. The role of homeobox genes as transcription factors as well as for development of the head will be discussed and contrasted with the properties of Hox genes involved in pattern formation in the trunk.

**Molecular evidence for the timing of human evolution**

**Simon Easteal**
**Human Genetics Group, John Curtin School of medical Research, The Australian National University**

The evolutionary rate of non-coding DNA, with a few exceptions, appears to be uniform among mammalian lineages - there is a mammalian DNA clock. This conclusion is reached by comparing rates of substitution in different lineages relative to each other, using a more distantly related species as a reference. It does not depend on interpretation of the fossil record, but it does have substantial implications for the interpretation of the fossil record and the divergence times of a wide range of mammalian taxa. It suggests a Triassic divergence of placental and marsupial mammals, early Cretaceous divergence of rodents and primates, and an Oligocene separation of rats and mice. These dates are all much earlier than those currently accepted. They are not, however, inconsistent with the fossil record and are supported to some degree by biogeographical evidence. Among primates, the DNA clock suggests that many divergence times were later than currently believed. Molecular data have already resulted in extensive reinterpretation of many important primate fossils, and it would appear that the process reinterpretation needs to be extended. Specifically, the DNA clock implies: 1. A catarrhine - platyrrhine split approximately 30 Ma ago; this is consistent with the earliest platyrrhine fossil, *Branisella* at 26 Ma ago, but it implies that *Aegyptopithecus*, rather than being an ancestral catarrhine, was either ancestral or a sister group to all simians; 2. A hominoid - cercopithecoid split approximately 20 - 18 Ma ago; this is consistent the first appearance of bilophodont molars, which distinguish cercopithecoids, but it implies that *Proconsul*, rather than being an ancestral hominoid, was either ancestral or a sister group to all catarrhines; 3. An orangutan - African ape split 9 - 8 Ma ago, which implies that *Sivapithecus* was ancestral or a sister group to all great apes; 4. A 4 - 3.6 Ma human - chimpanzee divergence. Previous molecular-based estimates of this split range from 2.7 to 9.5 Ma ago, but the consensus is that divergence was approximately 5 - 7 Ma ago, predating the bipedal *Australopithecus aferensis* (as well as the newly discovered *A. ramidus*). A 4 - 3.6 Ma divergence implies a bipedal ancestry of chimpanzees.

**Preliminary Analysis of mtDNA D-Loop Sequence from Black-Footed Rock-Wallabies (*Petrogale lateralis*).**

**Alexandra C. C. Wilson & Mark D.B. Eldridge**

***School of Biological Sciences, Macquarie University, North Ryde, N.S.W., 2109***

Mitochondrial DNA D-loop was sequenced in order to assess the extent of interrelationship and genetic differentiation within the six taxa of the *Petrogale lateralis* complex. Information was obtained from twenty-two animals. Phylogenetic analysis of sequence data, approximately 200 base pairs, was performed using PAUP version 3.1 (Swofford, 1993). These preliminary findings identify *P. l. purpureicollis* as a highly distinct taxon and *P. l.* "MacDonnell Ranges" and "West Kimberley" races as discrete taxa within *P. lateralis*. The remaining three taxa were indistinguishable. These results when compared with morphological, electrophoretic and mtDNA RFLP analysis suggest that; *P. l. purpureicollis* represents a separate species; *P. l.* "MacDonnell Ranges" and *P. l.* "West Kimberley" represent at least distinct forms of *P. lateralis* and finally that *P. l. pearsoni* and *P. l. hacketti* are the result of a recent radiation.

# EVOLUTION OF THE AUSTRALIAN FAIRY WRENS

Martin S. Elphinstone, Jennifer M. Seddon and Peter R. Baverstock
Centre for Conservation Technology
Faculty of Resource Science and Management
Southern Cross University
Lismore. NSW. 2480.

Two conflicting phylogenies have been proposed for the ten recognised species of Australian fairy wrens (genus *Malurus*). One of these phylogenies is based on morphology, the other on allozyme electrophoresis. The aim of this study is to distinguish between them using an independent technique - DNA sequencing. DNA sequence data from the mitochondrial Control Region (1.4kbp) and intron 2 (700bp) of the nuclear myoglobin gene will be presented and discussed.

## TAXONOMY OF QUOLLS

Karen B. Firestone[1,2], William B. Sherwin[1]

[1]School of Biological Science, UNSW, Sydney 2052
[2]CRC, Taronga Zoo, P.O. Box 20 Mosman, NSW 2088

Microsatellites are powerful tools for examining genetic variation at many different taxonomic levels (i.e. individual, species, and genus levels). Quolls (Marsupialia:Dasyuridae) are among the most severly affected species in terms of decline in population numbers and distribution since European settlement. We are using microsatellite loci to determine genetic variation within and between different species of quolls.

Markers have been designed from a Tasmanian eastern quoll individual and have been applied successfully to three species of quolls: *D. viverrinus* (eastern quoll), *D. maculatus* (tiger quoll or spotted-tailed quoll), and *D. geoffroii* (western quoll or chuditch). *D. hallucatus* (northern quoll) will be used as an outgroup.

Genetic variation within each species is also being examined: tiger quolls (mainland vs. Tasmanian; and mainland vs. mainland); eastern quolls (mainland vs. Tasmanian; and Tasmanian vs. Tasmanian); and western quolls (mesic vs. xeric) are being examined for genetic variation at these loci.

Levels of genetic variation within populations will be determined. Genetic variation between historic populations (from museum samples) and extant populations of tiger quolls from the mainland as well as historic and extant populations of eastern quolls from Tasmania are being examined.

We hope to continue genetic analysis using these markers in conjunction with a related ecological study of tiger quolls in the Barrington Tops region of NSW as an aid in determining pedigrees of individuals.

**HOW LARGE DO WILDLIFE POPULATIONS NEED TO BE TO RETAIN THEIR EVOLUTIONARY POTENTIAL?**

**E. Lowe, ME. Montgomery, LM. Woodworth, D.A. Briscoe and R. Frankham**
**School of Biological Sciences, Macquarie University, NSW 2109**

A fundamental question in conservation biology is "How large do populations need to be to retain their evolutionary potential?". Franklin (1980) suggested that an effective size ($N_e$) of 500 was sufficient, based on the equilibrium between loss of quantitative genetic variation and its replenishment by mutation. Lande and Barrowclough (1987) reached a similar conclusion, based on a model of stabilising selection, drift and mutation. However, Lande (1995) suggested that a $N_e$ of 5,000 was necessary as most mutations are deleterious.

We have made the first empirical estimate of this quantity. Populations of *Drosophila melanogaster* were maintained for 50 generations at effective sizes of 25 (8 replicates), 50 (6), 100 (4), 250 (3), and 500 (2). Cage population of each population plus inbred lines, the base population and a new wild population were founded with 750 flies and forced to evolve in response to increasing concentrations of a heavy metal pollutant ($CuSO_4$). Extinction concentrations were regressed on $(1 - 1/2N_e)^{50}$. The $N_e$ required to maintain evolutionary potential was estimated as the $N_e$ corresponding to the intersection of the extinction concentration for the wild population with the regression lines through the remaining points. The estimate was in the order of hundreds, in line with Franklins' original prediction.

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# Dieldrin resistance in the Australian sheep blowfly, *Lucilia cuprina*.

**K. Freebairn[a], N. Anthony[b], G. Colebatch[a,c], R. ffrench-Constant[b], J.A. McKenzie[a] and P. Batterham[a].**

**a. Department of Genetics, University of Melbourne, Parkville 3052, Victoria. b. Department of Entomology, University of Wisconsin, Madison. c. CSIRO Division of Entomology, Canberra 2601, A.C.T.**

Mutagenesis and selection has been used to investigate the number of genetic options available to *L. cuprina* in responding to the strong selection imposed by insecticides. For both dieldrin (a cyclodiene) and diazinon (an organophosphate) the mutants isolated were indistinguishable genetically and toxicologically from those which arose in the field (McKenzie et al.,1992; Smyth et al.,1992).

In this study the molecular mechanism of dieldrin resistance was examined. Genomic DNA was extracted from two susceptible strains and six resistant strains (one field strain and five laboratory strains selected after mutagenesis). Primers based on the cyclodiene resistance gene (*Rdl*) in *Drosophila melanogaster* were used to PCR amplify a 128 base pair region from each strain. The PCR product included the nucleotide sequence that encoded the amino acid change responsible for cyclodiene resistance in *D. melanogaster* ( alanine$^{302}$ to serine). These products were cloned and sequenced. The region amplified in *L. cuprina* strains showed high homology to that of *D. melanogaster*, with the resistant strains also displaying the same alanine to serine substitution. The importance of these results with respect to microevolution, insecticide resistance and the use of *Drosophila* as a genetic model will be discussed.

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## Applications of RAPD-PCR fingerprinting in koalas

**E Gale[1], B Houlden[2], P Hoeben[1], W Sherwin[2] and P Timms[1].**
**[1]Centre for Molecular Biotechnology, School of Life Science, Queensland University of Technology, Brisbane, Qld., 4001; [2]School of Biological Sciences, University of New South Wales, Sydney, NSW, 2052.**

Background: Genetic analysis of both free-range and captive populations is an important tool for their effective management. However, it has remained largely untouched in the koala (*Phascolarctos cinereus*) despite a reputation as one of Australia's most popular native marsupials. Over the past 100 years, koala populations in south-eastern Australia have endured large fluctuations. This was caused by local extinctions, an extensive program of relocations, and restocking from populations which have arisen from small numbers of founders. Consequently, these populations have endured bottlenecks which may have resulted in a severe reduction in genetic variation. North-eastern populations do not appear to have suffered the same bottlenecks and theoretically should have a larger gene pool. More recently, the range of the koala has been seriously reduced as a result of the increased urban development of essential koala habitats. Subsequent overcrowding often results in considerable reorganisation of population social structure, accompanied by a decrease in genetic diversity within these populations, which may ultimately lead to reduced fitness in the koala. In captive populations, determining parentage of individuals is necessary to avoid excessive inbreeding and to maximise the available gene pool. We have previously developed the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for DNA fingerprinting of koalas. The aim of this study was to explore further the applications of this technique with respect to free-range and captive populations of koalas.
Methodology: We analysed 10-20 koalas from seven free-range populations (Mutdapilly and Gold Coast, Queensland; Nowendoc, New South Wales; French Island, Phillip Island and South Gippsland, Victoria; Kangaroo Island, South Australia), and 11-19 animals from two captive populations (Lone Pine Koala Sanctuary, Queensland; Featherdale Wildlife Park, New South Wales). Thirty 10-mer primers were screened, five were selected which generated twenty-six reproducible polymorphic markers (loci) with a size range between 0.1-1.3kb, and for >90% of these markers no deviation from expected Mendelian inheritance was observed.
Results & Discussion: Analysis of the seven free-range populations for polymorphisms at 14 polymorphic RAPD loci indicates that the south-eastern koalas, as a whole, are significantly less heterogeneous than the north-eastern populations. This was reflected in the high genetic distances observed between Queensland and Victorian populations (0.300). Interestingly, the genetic distance observed between Victorian and South Australian koalas was also high (0.471). Hierarchical analysis using Wright's F-statistics suggests that there are no defined state boundaries which differentiate Australian koala populations. Paternity determination by exclusion analysis of 11 and 19 animals from Featherdale and Lone Pine respectively was successful using 26 polymorphic RAPD loci. This study has demonstrated the effective application of RAPD-PCR technology to genetic analysis of both free-range and captive koala populations, and indicates that north-eastern koala populations may exhibit greater genetic diversity, in comparison with southern animals.

This project was supported by the Australian Koala Foundation, the Australian Research Council and Lone Pine Koala Sanctuary.

# Regulation of ribosomal reinitiation in the translational control of *GCN4* expression in yeast

Chris M. Grant[1], Paul F. Miller and Alan G. Hinnebusch

National Institutes of Health, Laboratory of Molecular Genetics, NICHD, Bethesda, MD 20892, U.S.A.
[1]Present address: School of Biochemistry and Molecular Genetics, University of New South Wales, Sydney 2052.

Expression of the GCN4 protein of yeast is regulated by four small open reading frames (uORFs) in the *GCN4* mRNA leader (reviewed in 1). These sequences mediate increased translation of *GCN4* under conditions of amino acid deprivation. uORF4 (counting from the 5' end) is a strong translational barrier that is sufficient to prevent *GCN4* expression in the absence of the other uORFs. In contrast, uORF1 is a weak translational barrier that is required to overcome the inhibitory effect of uORF4 under amino acid starvation conditions. According to our model, ribosomes translate uORF1 and resume scanning the *GCN4* mRNA leader. Amino acid limitation activates the eIF-2$\alpha$ kinase GCN2 that reduces the levels of ternary complex (eIF-2, GTP and $tRNA_i^{Met}$) in the cell. As a consequence, many ribosomes fail to rebind the ternary complex until after scanning past uORF4, and reinitiate at *GCN4* instead. Our experiments address the sequence requirements at uORF1 that are required for reinitiation events downstream.

We used random mutagenesis to determine the sequence context at the uORF1 termination region required for efficient reinitiation at *GCN4* (2). Heterogeneous sequences in the ten nucleotides following the uORF1 stop codon were found to allow efficient reinitiation at *GCN4,* with the common feature of being AT-rich. In addition, the triplet preceding the uORF1 stop codon had a strong effect on *GCN4* expression, with AT-rich codons being the most permissive for reinitiation. We propose that the nucleotide context surrounding the stop codon affects the rate of the termination process and hence the probability of ribosomal release from the mRNA versus continued scanning and reinitiation at *GCN4*. In addition to the uORF1 termination context, sequences in the long leader segment preceding uORF1 are required for efficient reinitiation at *GCN4*. Placing uORF1 at the position in the leader normally occupied by uORF4 abolished the ability of uORF1 to promote reinitiation at *GCN4*. This property of uORF1 was recovered by restoring the leader segment normally upstream of uORF1 in this construct. In addition, deleting the leader segment upstream of uORF1 in a wild type construct, or moving this leader segment further upstream of the uORF1 start codon by means of spacer insertions also impaired uORF1 positive regulatory function. We suggest that sequences in the leader segment upstream of uORF1 promote reinitiation either by facilitating the rebinding of initiation factors needed for reinitiation at *GCN4* or by antagonizing ribosome release from the mRNA after uORF1 translation.

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## SEX CHROMOSOME EVOLUTION AND HALDANE'S RULE.

**Jennifer A. Marshall Graves**
School of Genetics and Human Variation, La Trobe University, Bundoora, Vic 3083.

Haldane[1] first pointed out that where interspecies hybrids show asymmetry (i.e. sex differences) in viability, or especially fertility, it is always the heterogametic sex which is affected. In mammals and Diptera with XX female:XY male sex chromosome systems, the male is affected, while in birds and Lepidoptera with a ZW female:ZZ male system, it is always the female. It is difficult to find a unifying system which accommodates these different systems, and which explains why fertility specifically is affected. Here I propose that the asymmetry in fertility can be accounted for by the interactions between genes which diverged from allele pairs on the X and Y (or Z and W) chromosomes as the sex chromosomes evolved.

In mammals the X and Y chromosomes share homology and are clearly derived from an ancestral autosomal pair[2]. The differential part of the Y chromosome is exempt from recombination and evolves rapidly by drift. The Y is much degraded, and most alleles trapped within regions isolated from recombination have become inactive pseudogenes, or are deleted altogether. Only genes with a selectable function are likely to survive on the Y chromosome, and the only selectable function open to a gene on the Y is a role in male differentiation, since no Y-borne gene can be essential for survival, or required for a general function in both sexes. Indeed, almost all of the 12 or so genes on the human or mouse Y (even including the testis determining factor, SRY)have a homologue on the X, and serve a function in sex determination or spermatogenesis[3]. The set of genes retained on the Y are not necessarily the same in all mammals (indeed, there are differences in the gene content of the mouse and human Y), but they all determine male-specific functions.

Because of the similarity in structure and sequence of the X and Y-shared genes and their products, these male-specific functions are likely to involve interaction of the products of the erstwhile alleles. Thus, at least some Y-borne genes will act by interfering with the female-producing action of their X-linked partners and diverting development of gonad or germcells along the male pathway. There may be one or several such gene pairs, or hundreds.

Y-linked genes evolve rapidly, and variants are frequently fixed in populations by drift and/or hitchiking. Compensatory changes will be selected for in interacting X linked partners. When two species diverge, the X-Y shared gene pairs diverge independently, and when these species are hybridized a few million years later, heterospecific interactions between the diverged X and Y alleles may no longer be effective. Males arising from hybridization between two species must have received an X from one species and the Y from the other, and if the heterspecific interaction fails, the pathway of male gonad/ germcell development is blocked so the animal is sterile. There may be one or several, or hundreds of such incompatible interactions between diverged X and Y-borne former alleles, blocking the pathways of male gonad or germcell differentiation at one point or another.

This model may be generalized to other species with an XX female:XY male sex chromosome system. Even though there is unlikely to be any homology between the X and Y chromosomes of mammals and Dipterans, the same principles may apply to the interactions of Y-borne fertility factors with evolutionarily related X linked genes. Similarly, the same principle may apply in reverse to species with ZW female:ZZ male. If a W allele no longer inhibits its erstwhile Z allele, the latter is expressed as the default (male) condition, and products essential for female reproduction are absent, leading to female sterility. Similar arguments may explain Y-autosome interactions in which a Y- (or W-) borne retroposon inhibits the action of the autosomal gene from which it evolved.

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# Genetics in fisheries management : past accomplishments and future directions.

Peter Grewe.

Division of Fisheries Research, CSIRO, Tasmania

# ABSTRACT

## The earliest fossil representatives of the human line

**Colin P. Groves**
Department of Archaeology & Anthropology,
Australian National University

A new fossil taxon was described in September, 1994, by White, Suwa and Asfaw, as being the earliest known representative of the human lineage. It was dubbed *Australopithecus ramidus*, but more recently was referred by the describers to a new genus, *Ardipithecus*. As the remains are 4.4 million years old, the new taxon would, if its ascription to the Hominini (=human/australopithecine lineage) were confirmed, set a new minimum date for separation of the human and chimpanzee lineages.

Twenty-three characters were extracted from the type description for a cladistic analysis. These characters are presumed to be uncorrelated because they occur independently an array of other hominine taxa. Those which refer to the dentition were examined in the Natural History Museum (London) collection of chimpanzees, in order to confirm whether the states assigned to them in that species by White *et al.* were correct and non-polymorphic.

Using PAUP Version 3.0 (D.L.Swofford, 1989), heuristic search and bootstrap (100 replicates), a cladistic analysis was run on the following taxa: *Ardipithecus*, *Pan troglodytes* (chimpanzee), *Gorilla gorilla*, *Australopithecus afarensis*, *Australopithecus africanus*, *Paranthropus robustus* and *Homo sapiens*, using orang plus gibbon as outgroup. Two shortest trees (length 41m Consistency Index 0.854) were found, differing only in the relative placements of the three last taxa. The consensus sequence is -

G(Pan(Ard(A.afar(A.afric(H.Sap., Par)))))

The *Ardipithecus* + other Hominini clade is supported by 95 bootstrap replicates; the clade containing the other Hominini is supported by 99 replicates. *Ardipithecus* is thus confirmed as the earliest known (and extremely primitive) member of the Hominini, whose separate existence is therefore indicated back to 4.4. million B.P.

## Genetic dissection of grain quality traits in wheat

**RB Gupta1, HS Bariana2, R Appels1 and F MacRitchie2**
**CSIRO Division of Plant Industry, 1GPO Box 1600, Canberra, ACT 2601 and**
**2PO Box 7, North Ryde, NSW 2113**

Wheat dough quality traits such as extensibility, mixing time, resistance and breakdown are the key dough processing parameters for defining end-use suitability of wheat samples. In order to define the genetics of these traits, a large number of F2 derived progeny from wheat crosses involving parents with extreme dough phenotypes was developed. The segregation patterns based on 260 F4 progeny from Mexico 8156 x Israel M68 crosses indicate that these dough parameters are controlled by multigenes. Low dough mixing time appeared to be dominant over high mixing time. A strong correlation between dough mixing time and dough resistance was found. Analysis of glutenin subunit profiles by SDS-PAGE confirmed well-known linkage between Glu-D1 subunits 5+10 and high mixing time and resistance. Despite a tight linkage between mixing time and resistance, a few segregants with the desirable combination of low mixing time and moderate resistance could be identified. Dough mixing time and dough breakdown segregated almost independently. Further work to identify molecular markers segregating independently between various dough attributes is in progress. A PCR based bulked F2 DNA segregant analysis will be performed following the confirmation of the dough phenotypes of the progeny at F5 generation.

**Population dynamics of maternally-inherited *Wolbachia*: two contrasting infections in natural populations of *Drosophila simulans***

Ary A. Hoffmann[1], David J. Clancy[1], Michael Turelli[2]

[1]School of Genetics and Human Variation, La Trobe University, Bundoora

[2]Population Biology, University of California, Davis, California

Microbes of the genus *Wolbachia* are present in 10-15% of insects. They are transmitted by their hosts via the maternal parent and are responsible for cytoplasmic incompatibility among insect populations when infected males mate with uninfected females. This phenomenon results in *Wolbachia* spreading through natural populations as we have demonstrated for the R infection of *Drosophila simulans*. By monitoring the infection status of thousands of flies with a PCR assay, we show that the frequency of the R infection and its rate of spread in populations can be predicted by its maternal transmission efficiency and field incompatibility. This infection has also resulted in the rapid spread of a mitochondrial DNA variant in *D. simulans* populations. In contrast, we show that another *Wolbachia* infection of *D. simulans*, designated "A", does not cause incompatibility. Even though the A infection is present at a lower intracellular density than R, the infection exhibits perfect maternal transmission. The new infection may behave as a neutral variant in natural populations and theoretical considerations suggest that it may represent the evolutionary outcome of interactions among *Wolbachia* infections and their host.

**Hypervariable microsatellite loci reveal low levels of genetic variation in bottlenecked koala populations from south eastern Australia.**

**Bronwyn A. Houlden, Phillip England and William B. Sherwin, University of NSW, Sydney 2052.**

Genotyping of koalas at simple sequence repeat microsatellite loci has revealed a significant difference in the levels of allelic diversity and heterozygosity between populations throughout Australia. High levels of allelic diversity (mean of 6.0-8.0) and observed heterozygosity (mean 0.69-0.84) were found in four wild populations of koalas from Queensland and New South Wales. In contrast, lower levels of genetic variation (mean allelic diversity 1.7-4.3; observed mean heterozygosity 0.25-0.48) were detected in three mainland Victorian populations, in two populations from islands in Westernport Bay, and from Kangaroo Is. in S. Australia. Following near extinction in the 1920s, southeastern Australian koalas have been involved in an extensive program of relocations. The relocated animals were from islands in Westernport bay, themselves founded by very few individuals in the late 1800s and early 1900s. This relocation strategy has had a homogenizing effect on variation throughout Victoria, and its long-term genetic consequences are discussed.

**Four mutations imparting a dominant ivermectin resistance phenotype in *Caenorhabditis elegans*.**

**Peter Hunt and Warwick Grant CSIRO Animal Health Armidale N.S.W. 2350.**

Although ivermectin resistance mutations are common in the nematode *Caenorhabditis elegans*, (approx. 1 in 240 haploid genomes mutagenised by 0.05M ems) dominant mutations are very rare (less than 1 in $10^6$ haploid genomes mutagenised by 0.05M ems). Recessive mutations have been allocated to 29 loci on all six *C. elegans* chromosomes and the dominant mutations described in this study map to two loci.

The genetic characterisation of the dominant ivermectin resistance genes will be described. These mutations show many pleiotropic phenotypes and two, Dyf (Amphid Dye Filling Defective) and Avr (Avermectin Resistance), are discussed in detail. These phenotypes exhibit varying degrees of dominance and allelic variation. Evidence for intergenic noncomplementation involving these mutations is also presented. A number of the Avr loci have been cloned and although the majority have novel sequences, two of the cloned genes are microtubule-associated proteins. Intergenic noncomplementation and genetic dominance are characteristics previously ascribed to mutations in molecular motor genes and microtubule structural genes. It is proposed that a number of the Avr mutations, including the dominant mutants, may represent defects in cytoskeletal associated components of the chemosensory cilia of *C. elegans*.

**Structure, Function and Evolution of the Mammalian Mitochondrial Genome**

**Axel Janke, Neil J. Gemmell*, Arndt von Haeseler, and Svante Pääbo**

**Zoologisches Institut, Universität München, P.O. Box 202136, D-80021 Munchen Germany**

***Department of Genetics and Human Variation, La Trobe University, Bundoora 3083, Australia. Present address: Department of Genetics, University of Cambridge, Cambridge CB2 3EH, England**

The complete sequences of the mitochondrial genomes of a marsupial (*Didelphis virginiana*) and a monotreme (*Ornithorhynchus anatinus*) have been determined. Protein-coding sequences were aligned and used to reconstruct the phylogeny of eutherians. It could be shown that the rodents represent an early eutherian branch, followed by primates, artiodactyls, carnivores and whales. The data furthermore indicate also that the lineages leading to mouse and rat have diverged as much as 35 million years ago.

Using the protein-coding sequences of frog and chicken as outgroups, the data suggest that marsupials and monotremes are sister groups. Thus the eutherians may represent the most ancient divergence among mammals.

Although the mitochondrial genomes of the marsupial and the monotreme show the same over-all genomic organization as in eutherians, frog and fish, they have some unique features. First, 5 tRNA genes around the origin of light strand replication are rearranged in marsupialia. Second, in marsupial mitochondria the gene coding for the tRNA for aspartic acid carries the anticodon GCC instead of the expected GTC. cDNA sequencing revealed that the second position of the anticodon is posttranscriptionally editel as the existence of a transcript from one of the strands, indicate that it may have an unknown function.

# Population biology of silver trevally, *Pseudocaranx dentex* from the east coast of Australia and New Zealand

Michael Johnston
Division of Botany and Zoology,
Australian National University
ACT 0200 Australia

Silver trevally, *Pseudocaranx dentex* is a temperate water marine species distributed throughout southern Australian and New Zealand waters. The main Australian fishery operates between Port Macquarie and the NSW/Victorian border. Knowledge of the stock structure of trevally within the main Australian fishery, and between the Australian and New Zealand fisheries is fundamental to the rational management of this species. Protein electrophoresis of samples collected both within, and between Australian and New Zealand waters was used to investigate the genetic structure of silver trevally. Additionally, some aspects of the population biology of Australian trevally were examined and compared to those for their New Zealand conspecifics to provide additional information on the stock structure.

Trevally sampled within Australian waters exhibit statistically significant but minor genetic heterogeneity with no clear geographic pattern between some, but not all Australian locations indicating that although gene flow is high ($Nm$ = 1-36) some population structuring remains. Significant intralocality heterogeneity was also detected in two Green Cape populations sampled one month apart. The results support the conclusion that despite minor population structuring, Australian populations of silver trevally can be considered a single stock. Comparison of allele composition and frequency between Australian and New Zealand samples showed not only significant heterogeneity at all three resolvable polymorphic loci, but also the presence of private alleles at both the GPI and EST-4 loci. Furthermore, significant differences in growth rates, asymptotic length and both the size and age at sexual maturity were observed between samples. The results indicate silver trevally from Australian and New Zealand waters can be managed as distinct stocks.

# *Cyclin E* transcriptional regulation during *Drosophila* development

**Lynn Jones, Helena Richardson and Rob Saint.**
**Dept. of Genetics, University of Adelaide, South Australia, 5005.**

Cell proliferation during *Drosophila* development occurs in a complex spatio-temporal pattern that is coordinated with differentiation. This regulation is important for correct animal development. Controlled cell proliferation can be achieved by the developmentally regulated expression of cell cycle components such as *Drosophila cyclin E (DmcycE)*. Cyclin E, a G1 cyclin, acts as a regulatory subunit of the cyclin dependent serine/threonine kinase (cdk2). The cyclin E/cdk2 complex functions during the G1 to S phase transition, promoting entry into S phase. During *Drosophila* development, *DmcycE* transcription occurs in all proliferating tissue and in most cases shows a dynamic expression pattern mimicking the pattern of S phases (Richardson et al, 1993). Null *DmcycE* mutants show a cell cycle arrest prior to the S phase at cycle 17 during embryogenesis (Knoblich et al, 1994) demonstrating that DmcycE is essential for S phase. In contrast, ectopic *DmcycE* transcription can drive cells prematurely into S phase (Knoblich et al, 1994; Richardson et al, 1995), indicating that DmcycE is sufficient for S phase entry and that regulated transcription of *DmcycE* is important for appropriate cell proliferation during development.

We are interested in how developmental signals control cell proliferation during development. Specifically how zygotic *DmcycE* transcription is regulated during *Drosophila* embryogenesis and larval development. To address this question, promoter deletions have been generated by the imprecise excision of a P element located 14kb 5' from the start of zygotic *DmcycE* transcription. Subsequent mapping identified one mutation that deleted most of the putative zygotic *DmcycE* promoter, leaving approximately 1kb of genomic DNA 5' from the start of transcription. Phenotypic analysis of this lethal mutation has revealed that *DmcycE* expression appears to be similar to wild type during embryogenesis. This result strongly suggested that the 1 kb region immediately 5' from the start of *DmcycE* transcription has some of the regulatory sequences necessary for appropriate *DmcycE* expression during development. This result was confirmed by placing the 1kb region upstream of a *LacZ* reporter gene and generating transgenic flies. *LacZ* expression was observed in flies containing the transgene in epidermal tissues, in the proliferating central nervous system during embryogenesis, and in the larval brain. In addition, reporter constructs covering other regions of the *DmcycE* promoter have been generated. Analysis of these constructs have identified further sequences that drive *DmcycE* expression in the proliferating embryonic peripheral nervous system. Future work is aimed at refining these regions to identify the specific sequences that are directing *DmcycE* transcription. This work will provide insight into how developmental signals control *DmcycE* transcription and therefore cell proliferation during *Drosophila* development.

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# THE ISOLATION OF GENES INVOLVED IN THE EXPRESSION OF EXTRACELLULAR PROTEASES.

**Pam K. Flynn, Brian F. Cheetham & Margaret E. Katz**
**Department of Biochemistry, Microbiology & Nutrition,**
**University of New England, NSW 2351.**

A number of systems of wide domain regulation exist in *A. nidulans* which control the synthesis of enzymes involved in nitrogen, carbon, sulphur & phosphorous nutrition. Extracellular proteases are normally secreted in this organism in response to nitrogen, carbon or sulphur nutrient limitation.

We have isolated a strain, designated *xprE1*, which is defective in the secretion of proteases under nitrogen & carbon starvation growth conditions but not under sulphur starvation growth conditions. The mutation has been mapped to chromosome VI, approximately 17 map units from the gene encoding sulphate permease, *sB*, & is recessive to its wild-type allele in diploid studies.

We have screened two cosmid libraries in order to clone *xprE*. An *A. nidulans* chromosome-specific cosmid library was screened by co-transformation using a plasmid, *pMOO6*, which carries the *argB* gene as a selectible marker. The other library carried the *trpC* gene as a selectible marker allowing direct selection to be undertaken. Even though transformation frequencies were high we have been unable to clone *xprE*.

During a co-transformation experiment we created a new mutation which has been shown to be associated with *pMOO6* plasmid insertion. This new mutation, *xprH1*, suppresses *xprE1* under all growth conditions & exhibits raised levels of extracellular proteases particularly under carbon-limiting growth conditions. It has been mapped to chromosome II & is recessive to its wild-type allele.

Plasmid rescue has been undertaken in order to obtain flanking genomic DNA regions at the points of plasmid insertion. Non-plasmid sequences from a rescued plasmid have been identified & sequence analysis is underway.

**Population structuring in an alpine weta hybrid zone as assessed using mitochondrial and microsatellite markers**

**Tania King and Graham Wallis**

**Department of Zoology and Centre for Gene Research**
**University of Otago**
**PO Box 56**
**DUNEDIN**
**New Zealand**

Observations of a clinal colour polymorphism in a central Otago population of an alpine 'tree' weta (*Hemideina maori*, Orthoptera: Stenopelmatidae) led to the discovery of a hybrid zone between the two colour forms. Extensive molecular genetic analyses have been undertaken to locate markers that correlate with colour which can then be used to describe characteristics of the zone. RFLP analyses of amplified mitochondrial genes (COII - COIII) have uncovered distinct haplotypes but so far, nuclear markers remain elusive. A number of microsatellite loci have been characterised however, and are uncovering high levels of polymorphism across the range of both colour forms. These data are used to describe patterns of population structuring and gene flow among schist outcrops (tors) on which weta are exclusively found. Structuring of these nuclear loci are then related to patterns of introgression for mitochondrial markers.

**GENOME ORGANISATION OF THE BACTERIAL PLANT PATHOGEN *Pseudomonas solanacearum*: VIRULENCE AND ESSENTIAL GENES**

**VIJI KRISHNAPILLAI, WAI FOONG HONG, MEENA DASS, JILLIAN DUNPHY AND LISA RYAN**

**DEPARTMENT OF GENETICS AND DEVELOPMENTAL BIOLOGY, MONASH UNIVERSITY, CLAYTON 3168, VICTORIA**

We have constructed a partial macro-restriction map of strain PS1000 of *P. solanacearum* by pulsed field gel electrophoresis and Southern analysis. This map is now being used to physically map a range of virulence and essential genes. The virulence genes include those encoding a large cluster of hypersensitive response and pathogenicity genes (*hrp*), a large cluster for extracellular polysaccharide synthesis (*eps*), avirulence (*avrA*) and a global virulence regulatory gene *phcA*.

The following conclusions are made:

1) Analysis of the locations of these genes suggests there is clustering of these genes which has been confirmed by detailed partial restriction analysis using *Dra*I, *Xba*I and *Ase*I of a 347 kb *Spe*I chromosomal fragment (F) identified on the basis of its carriage of a ribosomal DNA cluster (*rrn*1).

2) An important finding is the chromosomal location of *hrp* in strain PS1000, a sequence which was originally located on a megaplasmid in another strain (GMI1000) (Boucher *et al* 1991, Mol. Gen. Genet. **205**:270). This and other data suggest that gene shuffling of virulence genes between different replicons is a genomic feature and may contribute to the evolution and spread of such genes.

3) Another important observation is the identification of homologues of virulence genes from the human pathogen *Pseudomonas aeruginosa*, encoding a novel RNA polymerase sigma factor ($\sigma^{24}$ = *algU*) and *mucA*, both essential for alginate biosynthesis which is important in bacterial pathogenesis in cystic fibrosis. These contiguous genes map to *Spe*I fragment doublets C/D of 431 kb.

4) We have also physically mapped the location of the origin of chromosomal DNA replication (*oriC*) to *Spe*I fragment H on which is also located biosynthetic genes. Other essential genes which have also been mapped include those for a cluster of five transfer RNAs (tRNA), 4.5S RNA, *rpoN* ($\sigma^{54}$ of RNA polymerase) essential for virulence gene expression in *P. aeruginosa* and *denA denB* (nitrite reductase and cytochrome C-551, respectively).

This type of mapping data is expected to provide a basis for a more detailed analysis of the genome of a range of strains, particularly to identify the basis of virulence gene shuffling which is hypothesised to be mediated by repetitive DNA (transposable?).

# Labile evolution of exaggerated display traits in bowerbirds suggests reduced effects of phylogenetic constraint

R. Kusmierski*, G. Borgia† & R.H. Crozier*

*Department of Genetics and Human Variation,
La Trobe University, Bundoora, Victoria 3083, Australia

†Department of Zoology,
University of Maryland, College Park, MD 20742, USA

Bowerbirds (Ptilonorhynchidae) have among the most exaggerated sets of display traits known including bowers, decorated display courts and bright plumage that differ greatly in form and degree of elaboration among species . Here we present phylogenetic evidence, obtained from mitochondrial cytochrome b sequence data, of frequent reversals and convergences in behavioral and morphological male display traits. Lost traits are replaced with complex, qualitatively distinct, yet highly integrated alternatives. The lability of these traits and the complex, coordinated nature of replacements indicates a non-arbitrary pattern of trait evolution, relatively free from phylogenetic constraint, resulting in the observed differences in bower architecture.

# Regulation of the extracellular proteases of *Aspergillus nidulans*.

**Patricia A. vanKuyk, Brian F. Cheetham & Margaret E. Katz**
**Department of Biochemistry, Microbiology, & Nutrition.**
**University of New England, Armidale NSW Australia 2351**

The proteases of *Aspergillus* species have become an area of great interest, due to the economic importance of hydrolytic enzymes produced by filamentous fungi and the use of *Aspergillus* to produce heterologous proteins. Early studies of *A. nidulans* confirmed the existence of enzymes from the neutral and alkaline classes of endo-proteases (1). Unfortunately the methods used were unsuitable for the detection of proteases of the acid and thiol classes, therefore it was unknown if *A. nidulans* was able to produce proteases of these types.

With the intent of studying the regulation of the extracellular proteases of *A. nidulans* we have cloned two protease structural genes. Both an alkaline protease gene (*prtA*), and an acid protease gene (*prtB*), have been cloned through the use of PCR-generated heterologous probes and a lambda library of *A. nidulans* genomic DNA. The deduced amino acid sequences of *prtA* and *prtB* both show a high degree of amino acid identity to other *Aspergillus* proteases of their respective classes.

Enzyme assays, polyacrylamide gel electrophoresis, and RNA blots have been employed to study the regulation of the protease structural genes. *A. nidulans* secretes extracellular proteases in response to carbon, nitrogen, or sulphur nutrient-limiting conditions, and pH regulation may also play a role in this system. We have shown that *prtA* transcript levels are high in mycelia transferred to medium lacking a nitrogen, carbon, or sulphur source and undetectable in medium containing glucose, ammonium and sulphate indicating that the control of alkaline protease production is at the level of transcription or mRNA stability (2). Northern blot analysis showed that the 1.8 kb transcript is expressed in response to nitrogen deprivation regardless of environmental pH, at least for the range of pH values (3.5-9.5) which were tested. However, preliminary studies suggest that the acid protease(s) may not be expressed under neutral and alkaline conditions.

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**Marine Genetic Resources in the Tropics: How little we know.**

**Shane Lavery**

**QDPI Agricultural Biotechnology Centre, and**
**Department of Zoology, University of Queensland.**

The management of biological resources for either sustainable use or conservation requires that the appropriate units of management be identified. In the marine environment, where direct observation of occurrence, behaviour and dispersal are so difficult, the analysis of genetic diversity can play an important role in delineating management units, be they at the species or population levels.

Relatively few marine genetic studies have been undertaken in northern Australian waters. This paper will review some of those studies from the past and present, highlighting that surprisingly little is known about genetic diversity in much of our tropical and subtropical marine fauna.

Some important themes will include: (1) the continuing need for genetic species identification of both adult and juvenile forms, (2) the great variety of patterns of genetic connectivity found in northern Australian marine organisms, (3) the strong, but unpredictable, relationship between dispersal potential and extent of gene flow, and (4) the need to use the most powerful techniques available in defining management units.

**Genetic and molecular analysis of rust resistance loci in flax: George Mayo's legacy to plant genetics.**

**Greg Lawrence, Jeff Ellis, Jean Finnegan, Peter Anderson and Michael Ayliffe**

**CSIRO Division of Plant Industry, PO Box 1600, Canberra ACT 2601 and The Cooperative Research Centre for Plant Science, PO Box 475, Canberra ACT 2601.**

In flax, 31 rust resistance genes are located at five loci, designated *K, L, M, N* and *P,* that contain 2, 13, 7, 3, and 6 genes respectively. Work by Harold Flor (North Dakota) and George Mayo, Ken Shepherd and Rafiqul Islam (Adelaide) found that test-crosses involving pairs of rust-resistance genes at the *M* locus generated rare recombinants expressing both parental resistance genes as well as recombinants expressing neither parental resistance specificity. In contrast, with the *L* group of rust-resistance genes, test-crosses involving 11 different genes in 19 pair-wise combinations yielded either no recombinants at all or only recombinants with neither parental specificity. These observations, interpreted on the basis of the modified cis-trans test proposed by Shepherd and Mayo, suggest that the *L* group of genes are alternative forms of the same gene (alleles) whereas the *M* locus is likely to contain an array of separate, closely-linked genes.

Recently the *L6* gene for rust resistance in flax was cloned after it was tagged with the maize transposable element *Ac*. DNA probes from the *L6* gene were found to hybridize to a family of DNA fragments that mapped to the *M* locus, indicating sequence similarities between genes at the *L* and *M* loci. The molecular data indicate that the *L* locus is simple and contains a single gene with multiple alleles while the *M* locus is complex and contains a tandem array of genes of similar sequence: thus the molecular data are in full agreement with the locus structure inferred from the earlier genetic analyses.

Analysis of the *L6* gene sequence and of a cloned cDNA predict two products, a 1294 amino acid polypeptide and a truncated product of 705 amino acids. These products result from two different mRNAs that are produced because one of the introns is apparently not spliced from all pre-mRNA transcripts. Both protein products have a potential signal peptide and a nucleotide (ATP or GTP) binding site (NBS) consisting of P-loop and kinase-2 motifs. The carboxy-terminal half of the larger *L6* product is rich in leucine residues (18%) and contains a direct repeat of about 150 amino acids with 74% identity. The putative truncated product, which lacks the major part of the leucine-rich region including the two repeats, contains an extra 29 amino acids at its C-terminus that are not in the longer product. The *L6* gene product shows significant similarity to the products of two other disease resistance genes that also contain NBS and leucine-rich regions, namely the Tobacco Mosaic Virus resistance gene *N* of tobacco and the bacterial resistance gene *RPS2* of *Arabidopsis thaliana*. This similarity suggests that these genes may function by a common mechanism, possibly acting in ATP/GTP dependent transduction of signals encoded by the corresponding pathogen avirulence genes.

# Methylation Analysis of a Marsupial X-Linked CpG Island By Bisulphite Genomic Sequencing

**David A. Loebel and Peter G. Johnston**

*School of Biological Sciences,*
*Macquarie University NSW, 2109.*

There is considerable evidence that DNA methylation is important in the maintenance of X-inactivation in eutherians. CpG islands on the inactive X are heavily methylated, whereas those on the active X are largely unmethylated (1). X-inactivation in marsupials appears to be less complete than in eutherians and activity states of X-linked loci differ between species. For example there is detectable activity of the paternal G6PD allele in all adult tissues of the Virginia opossum (*Didelphis virginiana*), but in wallaroos (*Macropus robustus*) no activity is detected in any adult tissue except for cultured fibroblasts (2).

There is only one published account of DNA methylation in a marsupial X-linked CpG island. The 5' CpG island of G6PD of *D. virginiana* was examined using methylation sensitive restriction enzymes and Southern blotting (3). No methylation differences were found. However, since many CpG sites cannot be assayed by Southern blotting, and given that *D. virginiana* shows incomplete paternal inactivation of G6PD, it is conceivable that methylation of some CpG dinucleotides in CpG islands may play some role in marsupial X inactivation.

Recently, a more sensitive method of detecting all methylated cytosines in a stretch of genomic DNA was devised (4, 5). This method is based on conversion of cytosine but not 5-methylcytosine to uracil by sodium bisulphite, followed by strand specific amplification of the converted DNA by PCR. We have applied this method of methylation analysis to studying methylation of the CpG island at the 5' end of the G6PD gene of *M. robustus*. Analysis of 45 CpG dinucleotides in tissues, sperm and cultured fibroblasts indicates that both the active and inactive alleles appear to be largely unmethylated. There appear to be no functional methylation differences between the two G6PD alleles in the CpG island. However, within the body of the gene, the active X chromosome is heavily methylated while the inactive is mostly unmethylated. This methylation pattern is conserved between Australian and American marsupials and is widespread (3,6), implying possible functional significance.

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## Intron-exon structure of eukaryotic genes and the evolution of parallel processing

**John S. Mattick**
**Centre for Molecular and Cellular Biology**
**The University of Queensland**

Our understanding of the relationship between genetics and biological function is rooted in the one gene-one protein hypothesis, and in the classical studies of the *lac* operon and the "genetic code", i.e the triplet code specifying amino acids in protein coding sequences. The concept of DNA as a relatively stable, inheritable source of template information for proteins, transduced through a temporary and discrete RNA readout, has become an article of faith and implicitly, but very powerfully, influenced our ideas on the structure of genetic systems. Accordingly , cells and organisms are thought of as being built from a myriad of structural and catalytic proteins, whose expression is generally controlled by other proteins which bind to DNA. This is a biochemical rather than an informatic perspective, which, apart from local analysis of promoter function, gives little thought to the problem of how gene activity might be integrated and regulated in four dimensions.

That something might be wrong with this simple view of the structure of genetic systems was first suggested by the discovery in 1977 of intervening sequences, or introns, which account for over 90% of all transcripts in animals. Because they did not code for protein, they were immediately assumed to be non-functional, and then rationalised as the legacy of early molecular evolution that had persisted in slower growing more complex organisms because of a long-term advantage in the shuffling of protein domains, but had been pruned from bacteria and unicellular eukaryotes under pressure to streamline their genomes. However there is now good circumstantial evidence that nuclear introns derived from self-catalytic group II introns that first entered the eukaryotic lineage via the mitochondrion. The likely reason for the absence of introns in prokaryotic protein-coding genes is the intimate coupling of transcription and translation, whereas the separation of these processes in eukaryotes allowed a greater opportunity for invasion of such genes by self-splicing parasitic sequences, a process which was made easier by the subsequent evolution of the spliceosome. As the sequence requirements for splicing were reduced, these elements became free to drift and explore new genetic space.

Introns have become enormously successful as a class of sequences, and now dominate the transcriptional output of the higher organisms. They also have many of the hallmarks of information - high sequence complexity, non-random nucleotide distribution, and intriguing patterns of conservation between species. It is completely plausible that introns have evolved function, and that there are in fact two levels of information produced by gene expression in the higher organisms - mRNA and iRNA - allowing the simultaneous expression of both structural (*i.e.* protein-coding) and networking information, and multiplex contacts between different genes via RNA signals that are implicit in the primary transcript. This would potentially include RNA-DNA, RNA-RNA, and RNA-protein interactions, and there are known or implied examples of all three. This hypothesis also predicts that some genes will have evolved to express only iRNA, and there is clear evidence for this in such cases as the *XIST* and *H19* transcripts of mammals, and in the *bithorax* region of *Drosophila*. Hence there are three types of genes - those that encode only protein, those that encode only iRNA, and those that encode both. This hypothesis would potentially explain a number of puzzling genetic and molecular genetic observations. Such a dual mRNA + iRNA system constitutes a form of parallel processing or neural network, which would have vastly expanded the options for regulating more complex genetic datasets and programs, and which may have been an essential prerequisite for the evolution of multicellular organisms.

## Genotype, environment and the asymmetry phenotype. Dieldrin-resistance in the Australian sheep blowfly, *Lucilia cuprina*

John A. McKenzie and Janet L. Yen

Department of Genetics, University of Melbourne,
Parkville, Victoria 3052

Dieldrin-resistant (*Rdl/Rdl* and *Rdl/+*) and susceptible (+/+) phenotypes of *L. cuprina* were scored for departures from bilateral symmetry for bristle characters after development at different temperatures, larval densities or concentrations of dieldrin. The asymmetry phenotype of resistant flies was dominant and independent of developmental temperature and larval density. The asymmetry of susceptibles increased for temperatures and larval densities above and below standard rearing conditions. A positive correlation was observed between asymmetry score and dieldrin-concentration for all genotypes. The susceptible phenotype did not attain the asymmetry score of resistants in any environment. Resistant phenotypes showed an anti-symmetric pattern in each environment; fluctuating asymmetry was observed for susceptibles.

The relevance of the results of genetic and general or specific environmental stresses to estimates of developmental perturbation will be discussed as will the difficulty of interpreting departures from symmetry at the population level if the genotype is not defined.

## THE LEVEL OF GENETIC VARIATION WITHIN TWO ISLAND POPULATIONS OF THE TAMMAR WALLABY (Macropus eugenii)

**L.M. McKenzie and D.W. Cooper**
**School of Biological Sciences, Macquarie University, NSW, 2109**

Levels of genetic variation were investigated for two island populations of tammar wallabies (*Macropus eugenii*). One population was from Garden Island in Western Australia and the other was from Kangaroo Island in South Australia. The genetic distance between the two is large: none the less they interbreed freely in captivity. The levels of heterozygosity, percent polymorphism and mean average percent difference were calculated based on RFLP data. Despite the significantly smaller size of Garden Island (1054 ha) in comparison to Kangaroo Island (450,000 ha) no difference was detected in any of these tests for genetic variation. Furthermore, the level of heterozygosity observed in these tammar wallaby populations does not differ significantly from that observed in sheep and humans.

**Mapping the distribution of the telomeric sequence $(TTAGGG)_n$ in three *Petrogale* species by fluorescence *in situ* hybridization.**

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

School of Biological Sciences
Macquarie University, NSW 2109

The development of fluorescence *in situ* hybridization (FISH) has provided a powerful technique for the detection of specific sequences on metaphase chromosomes. Using this technique it has recently been demonstrated the sequence $(TTAGGG)_n$ is most likely to be the functional vertebrate telomere. Hybridization to non-telomeric sites has been reported in a large number of vertebrates. It has been speculated that non-telomeric sites of the $(TTAGGG)_n$ sequence are related to chromosomal rearrangements during karyotypic evolution. The *Petrogale* group (rock wallabies) are an ideal group for this area of research as they are karyotypically diverse (20 distinct taxa) and the pattern of karyotypic evolution has been well studied. The distribution of telomeric sequences was mapped using FISH to metaphase chromosomes of three *Petrogale* species. While some results were consistent with sites of known chromosomal rearrangements, others are not.

**Comparison of diversity in the nuclear and chloroplast genomes of *Eucalyptus nitens*.**

**M. Byrne, T.L. Parrish and G.F. Moran**
**CSIRO Division of Forestry, P.O. Box 4008, Queen Victoria Terrace, Canbera, ACT 2600**

*Eucalyptus nitens* is an eastern Australian species with a large range and a highly disjunct distribution. Genetic diversity in the chloroplast genome was assessed for 10 individuals from each of eight populations using RFLPs with heterologous probes. The level of diversity in the chloroplast genome was high and the pattern of diversity suggests the presence of two lineages within the species. Diversity in the nuclear genome was assessed using 40 anonymous RFLP loci. Information about the RFLP probes was available from the genetic linkage map of the species and probes that detected a single locus were selected to cover the whole of the genome. Several loci that were monomorphic in the mapping pedigree were also used to avoid any bias towards polymorphic loci. Variation was determined for 20 individuals from each of 8 populations covering the natural range of the species. The level of diversity within the nuclear genome was also high with up to 12 alleles detected at loci. Levels of diversity and distribution of diversity within and between populations and regions will be compared to that detected with isozyme loci. The pattern of diversity in the nuclear genome will be compared with that of the chloroplast genome.

**Variation in the rate of chromosome evolution within the New Zealand weta (Orthoptera: Stenopelmatidae)**

Mary Morgan-Richards

**School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, NEW ZEALAND**
**e-mail: weta5@matai.vuw.ac.nz**

It is generally accepted that the rate of karyotype evolution is not constant between, and even within lineages and research into the reasons for this are producing some interesting results. There are many who favour an explanation based on variation in fixation rates of novel chromosome rearrangements. However, where rate change within a lineage has been studied in detail mutation rates, fixation rates and selection have all been evoked to explain chromosome shuffles, karyotype revolutions and karyotypic megaevolution. I present data suggesting that a rapid rate of karyotype change has arisen independently in two species of New Zealand weta. The rate of chromosome change does not correlate with rates of gene flow or reproductive ecology and involves many different types of chromosome rearrangements.

**Molecular Genetic analysis of the *Cre3* locus that confers resistance to cereal cyst nematode in wheat.**

E.S. Lagudah[1], S. Chandramohan[1], O. Moullet[1], R. Eastwood[2], R. Appels[1], K. Andersen[2] and S. Andersen[2].

1. CSIRO, Div. of Plant Industry, GPO Box1600, Canberra, ACT 2601
2. Victorian Institute of Dryland Agriculture, Private Bag 260, Horsham, VIC 3400
3. Kallerupvej 57, 2640 Hedehusene, Denmark

The *Cre3* gene locus derived from *Triticum tauschii* confers a high level of resistance in wheat to the Australian pathotype of *Heterodera avenae* (causal agent of cereal cyst nematode, CCN). *Cre3* is physically mapped to the distal long arm of chromosome 2D. The relationship between *Cre3* and other CCN resistance genes in wheat (*Cre1* ) *T. tauschii* (*Cre4* ) and barley (*Ha2*, *Ha3* ) was deduced from host-pathotype interactions and chromosomal location. The results revealed *Cre1*, *Cre4*, *Ha2* and *Ha3* constituted one homoeologous group as distinct from the *Cre3* locus. A clone, csE20, isolated from bulked F2 *Cre3*/*cre3* segregant analysis on low copy DNA which detects an RFLP that cosegregates with CCN resistance was used to screen a λ genomic library made from a *T. tauschii* line carrying the *Cre3* gene. Analysis of a csE20 positive λ clone showed the presence of a gene sequence encoding nucleotide binding sites (NBS), ATP/GTP and kinase2, and a leucine rich region(LRR). The NBS/LRR subclone was shown to represent a gene family of which one member cosegregated with resistance to CCN at the *Cre3* locus. Further analysis of the NBS/LRR sequence encoded by the *Cre3* locus revealed similarities to other resistance gene products reported for bacterial (*Rps2* ), viral (*N* ) and fungal ($L^6$) pathogens

**Biological control of the take-all fungal root pathogen of wheat by *Pseudomonas* bacteria.**

**M.Nayudu, K.A.E. Groom, K. Turnbull, J. Fernance, T. Murphy, P.T.W.Wong[1], and J. Ash**

**Division of Botany & Zoology, Faculty of Science, Australian National University, Canberra, ACT 0200; [1]Biological & Chemical Research Institute, New South Wales Department of Agriculture, Rydalmere, NSW**

The most frequent type of Plant-microbe interaction in soil is probably transient in nature and involves loose association. The anti-fungal nature of some associative bacteria involved in such interactions has enabled them to be used in Biological control protection against certain fungal diseases. The take-all fungal disease of wheat is the most significant root disease which conservatively leads to 10% loss of the annual Australian wheat crop (Murray and Brown, 1987). A non-fluorescent Australian *Pseudomonas* species (strain AN5) is able to effectively protect against the take-all fungal pathogen, *Gaeumannomyces graminis* var *tritici*, in agar plate bioassays and pot experiments. It also significantly increase wheat yield in biological control protection against take-all in field trials at dryland sites, where most wheat is grown in Australia. Molecular genetic analysis of strain AN5 has shown antibiosis is the most significant factor in biological control protection. Antibiotic production is specified by genes in two regions of the AN5 genome. Bacterial exopolysaccharides (EPS) have been shown to play an essential role in this plant-microbe interaction. We have for the first time characterised the unique mode of infection by the *Pseudomonas* bacteria into the roots of wheat by scanning electron microscopy, using spot inoculation. This shows the bacteria induce degradation of the epidermal layer of the root and some bacteria actually live inside the root surface.

This *Pseudomonas* bacteria has been shown to have wide host range in root colonization by marking with bioluminescence genes (luciferase or *lux* ), as a prelude to field release. Biological control bacteria are an example of how soil bacteria have evolved in response to wheat monocultures.

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## Imprinted genes, phenotypes and mechanisms of imprinting

**Robert D. Nicholls, Department of genetics, Case Western Reserve University and Center for Human Genetics, University Hospitals of Cleveland, Cleveland, 10900 Euclid Ave., OH 44106, USA. (e-mail: rxn19@po.cwru.edu)**

Genomic imprinting in mammals refers to a phenomenon by which a specific subset of genes are differentially marked during gametogenesis, resulting in differential expression during embryogenesis and in the adult, depending upon the parental origin. Several lines of evidence in the mouse, including (1) pronuclear transplantation studies, (2) the abnormal phenotypes associated with uniparental disomy for specific chromosome regions produced by breeding of balanced or Robertsonian translocations, and (3) transgene studies, first demonstrated that paternal and maternal inheritance was unequal in mammals. In recent times, endogenous genes have been identified in the mouse and human, including at this time ten such genes in each species; current estimates predict about 100-200 imprinted genes. Conceptually, imprinting requires at least four successive steps during the life cycle: (1) *erasure* of the existing imprint in the gamete; (2) *establishment* of the new imprint in the gamete; (3) *maintaintenance* of the epigenetic mark through successive mitoses; (4) *manifestation* of the imprint during development by regulation of gene expression, leading to parent-of -origin specific gene expression.

At this time, most work in the mouse has concentrated on the oppositely imprinted genes *H19* and *Igf2*, located within 70 kb of each other in distal mouse chromosome 7, and on the *Igf2r* gene located in proximal chromosome 17 in the mouse. A model for reciprocal imprinting of the former two genes involving transcription factor competition for a pair of enhancers downstream of *H19* has been proposed by Tilghman and co-workers. An apparent imprinting "box" has been identified in the *Igf2r* second intron, which is postulated by Barlow to be the epigenetic mark for this gene. Consistent with the proposed role for DNA methylation in at least maintainance of imprinted gene expression, each of these genes is deregulated in mice deficient for DNA methyltransferase, the enzyme responsible for setting methylation patterns in the mammalian genome.

An important role for imprinting has been recognized in several human inhertited diseases, including Prader-Willi (PWS), Angelman(AS) and Beckwith-Weidemann (BWS) syndromes, as well as many pediatric and adult tumors. Multiple molecular mechanisms give rise to BWS and to PWS or AS, associated with genetic abnormalities in human chromosome 11p15 and 15q11-q13 respectively. Recent studies have given significant insights into the molecular mechanisms associated with imprinting in chromosome 15q11-q13, and this model will be highlighted. We and others have identified at least five imprinted genes in this region (eg., *SNRPN, IPW, ZNF127)* and associated functional imprinting with DNA methylation imprints and asynchronous DNA replication. Several novel types of gene arrangement have been identified for these genes. More recently, we have identified overlapping deletions in familial PWS and AS patients with a mutation in the imprinting process, which defines an autosomal imprinting center (IC) that controls imprinting of each imprinted gene within 15q11q13. I will present a molecular model for function of the IC, and speculate on the evolutionary origins of imprinting.

In closing, understanding the molecular basis of imprinting may lead to therapeutic approaches for those genetic disorders and cancers which imprinting is fundamental.

# The Evolutionary Origins of Australia's Elapid Snakes

Catherine J Nock and Peter R. Baverstock
Centre for Conservation Technology
Faculty of Resource Science & Management
Southern Cross University
PO Box 157, Lismore, NSW 2480

The Elapidae includes the most poisonous snakes in the world - the Mambas of Africa, the Cobras of India, and the Taipans, Tigers and King Browns and Red-bellies of Australia. Given the paucity of the fossil record of the Elapidae in Australia and their high degree of endemism, there has been considerable debate over the origin of this group.

The traditional view, based on morphology, is that the Elapids and the sea snakes (the Hydrophiidae) are two separate groups. Another controversial hypothesis, based on microcomplement fixation, is that one branch of the sea snakes - the Laticaudinae or sea kraits - gave rise to Australia's terrestrial Elapids which in turn gave rise to a further group of sea snakes (the Hydrophiinae).

A 416 bp fragment of the mitochondrial 12S ribosomal RNA is being sequenced for representatives of the African and Asian Elapids, all the major Australian Elapid lineages and the Hydrophiidae in order to resolve the conflicting hypotheses and elucidate the origins of Australia's Elapids.

Preliminary results will be presented and discussed.

# The Genomic and Molecular Organisation of Centromeres in the Genus *Caledia*.

Contreras, N., Maclean, V., O'Brien, R. and Shaw, D.D.
Eukaryote CHromosome Organisation,
Research School of Biological Sciences,
Australian National University

Despite its fundamental role in the evolution of complex genomes, the molecular structure of the centromere from any higher eukaryote remains unknown. Among lower eukaryotes, the centromeres from several yeast species have been comprehensively analysed and defined at the structure/function level but equivalent DNA sequences have not been detected in higher eukaryotes. The centromeric regions of many higher eukaryotes are embedded in long arrays of highly repeated DNA that have impeded the isolation and characterisation of functional centromeres, although in *H.sapiens*, recent evidence suggests centromeric activity may involve a subset of the 171bp $\alpha$- satellite sequences.

The grasshopper genus *Caledia* is composed of several taxa in which the centromeres on all chromosomes (2n=24) show major intra- and inter-taxon evolutionary change. The centromere can occupy a wide range of positions along all twelve chromosomes, even within the same taxon.

In an attempt to understand the molecular structure of the centromere and its interaction with centromeric proteins to form the kinetochore, we have shown by immunofluorescence that CREST antisera cross-react with the centromeres of *Caledia* and the same antisera also bind to 4 putative centromeric proteins after separation by SDS-PAGE. We have also isolated and sequenced an 894bp DNA fragment that hybridises to the spindle attachment region during mitosis and meiosis. This sequence is highly conserved within each genome with an average of 150 copies per chromosome. The sequence also binds to a 32kDa protein which appears to be a ribonucleoprotein (RNP) that forms part of the nucleolus during interphase and then migrates to the centromeric regions during mitosis, as detected by NOR-specific $AgNO_3$ staining. The same 32kDa protein also shows positive silver staining on Western blots using the same protocol. The RNA component of the RNP is 380bp in length and protein binding affinity to the 894bp centromeric sequence is lost after RNase A digestion. This raises the novel possibility that centromeric DNA may bind with the RNA component of a small ribonucleoprotein during the formation of the kinetochore.

## NUMBER OF MATINGS IN THE GENUS *APIS* (HONEYBEES)

**Benjamin P. Oldroyd(1), Ross H. Crozier(2)**

**1. School of Biological Sciences, University of Sydney, N.S.W. 2006**
**2. School of Genetics and Human Variation, La Trobe University, Bundoora, Victoria 3083**

It has been postulated that multiple mating by social insect queens is adaptive because: 1) Genetically diverse colonies may be able to tolerate a wider range of environmental conditions, perhaps by increased polyethism or the potential for caste differentiation, or by increased tolerance to pathogens. 2) Multiple mating eliminates the possibility of a queen mating with a single drone carrying the same sex allele as herself. 3) Multiple mating can reduce conflict between workers and queens over the preferred sex ratio.

Understanding the evolution of multiple mating requires good estimates of the number of matings in a broad range of species. We have been using microsatellite markers to detect paternity and maternity of workers so that the number of males that mated with queens can be determined. We will present data from *Apis mellifera*, *A. dorsata* and *A. florea*. The three other *Apis* species remain to be examined. The results show that in the species examined: 1) There was only one queen per colony. 2) With a rare exception, all drones were laid by queens, none by workers. 3) Queens mated with more than 6 to >30 drones depending on the species. These results substantially increase previous estimates of the number of matings in the genus. We hope to correlate these findings with the ecological range of each species, and thereby test the predictions of hypothesis (1) above.

**Dienelatone Hydrolase: An α/β Hydrolase Fold enzyme**

**David Ollis**

The Research School of Chemistry , The Australian National University, Canberra, ACT, 0200.

Dienelactone hydroalse (DLH) is a small hydrolytic enzyme that does lactone hydrolysis. The structure of DLH has been determined and refined at high resolution. DLH consists of eight β strands surrounded by α helices with an active site found in a crevice near the center of the molecule. The key catalytic residdues of DLH are a triad of rsidues which a linked by hydrogen bonds in a manner reminescent of the catalytic triads found in theserine and cysteine proteases. Although DLH has an overall structure which differs markedly from the trypsin like serine proteases and the papaine like cysteine proteases, it has recently been found to be structurally very similar to a number of other hydrolytic enzymes that have ben called the α/β hydrolase fold enzymes. The similarities of these enzymes will be discussed along with the functional significance of the α/β hydrolase fold.

# SPECIFIC GENES EXPRESSED DURING COTTON FIBRE DEVELOPMENT

**Sharon J. Orford and Jeremy N. Timmis**

**Department of Genetics, University of Adelaide, South Australia 5005**

The commercial cotton fibre in Australia is a single specialised cell of the outer epidermis of the ovule of *Gossypium hirsutum*. Fibre differentiation begins at about the time of anthesis and is characterised by synchronous growth of many epidermal cells without cell division. The fibre length and other qualities determine the quality of the resulting spun thread.

We have studied genes which are expressed during differentiation of these cells. Five groups of cDNA clones have been isolated which appear to be expressed only in developing fibre cells. One of these cDNA types has been isolated and characterised elsewhere (John et al., PNAS., 89, 5769-5773). Sequence analysis of the most abundant cDNA group indicates that it encodes a protein which has previously been characterised in other plant systems where it is not confined to fibres or any fibre-like tissues. Sequence data comparisons of the three remaining cDNA classes indicate that they encode previously uncharacterised proteins. Northern analyses show that several of the mRNAs represented are expressed early during fibre development. Genomic clones corresponding to these temporarily expressed, fibre-specific mRNAs have been isolated in order to characterise their promoter regions.

These cDNAs identify four new fibre-specifically transcribed genes. The possibility of manipulation of the structure of such genes, the tissue-specific expression of heterologous genes and the modification of temporal expression of key genes in fibre development, offers exciting prospects for the alteration of fibre characteristics in transgenic plants.

# MITOCHONDRIAL D-LOOP DIVERSITY IN AUSTRALIAN ABORIGINES

**Sheila van Holst Pellekaan, Marianne Frommer and Barry Boettcher.**
**Genetics,School of Biological Sciences,**
**University of Sydney.**

ABSTRACT

Mitochondrial DNA sequences provide useful data regarding recent human population patterns. Mitochondrial D-loop sequences derived from Australian Aboriginal blood samples given by people from the Darling River region of NSW and from Yuendumu in central Australia demonstrate considerable diversity from the 'Cambridge reference sequence', with the greatest number of variable sites noted in descendants of the Ngiyampaa and northern Paakintyi people from the Darling River area. The region between nucleotide 16179 and 16194 shows more diversity in these samples than in those from southern Paakintyi descendants or the Warlpiri people from Yuendumu. The instability of this and other specific regions/sites compared to apparently conserved segments raises questions of different constraints operating within the hypervariable region. Some specific mitochondrial genotypes are present in both southern Paakintyi and Warlpiri people, geographically more distant from each other than southern Paakintyi are from northern Paakintyi and Ngiyampaa.

The mitochondrial genotypes identified for this sample show diversity which, when grouped according to family history, suggests conformation to the general distribution of language groups in the Darling River region. Conflict following European settlement after 1788 decimated the populations of the area and caused a local population bottleneck which needs to be considered in evaluating the data. It is likely that fewer individuals survived in this region than in Yuendumu. The genotypes presented are not necessarily representative of the pre-European settlement population.

# Methyl Sensitive DNA:Protein Interactions in an Active X Specific Methylated Region in the *Macropus robustus* HPRT gene.

**Anita A. Piper and Suyinn Chong**
**Department of Cell and Molecular Biology, University of Technology, Sydney NSW 2065**

Mammalian X chromosome inactivation serves to equalise sex differences in the dosage of X-linked gene products and is probably the result of interactions between various mechanisms controlling chromatin structure and replication timing. Numerous studies have investigated the cytosine methylation status of X linked genes, since methylation provides a logical explanation for cell memory and somatic inheritance of the inactivated X. A correlation between transcriptional silence and promoter hypermethylation has been demonstrated for the inactive X-linked allele of numerous eutherian constitutive genes. However since this hypermethylation occurs largely after X inactivation has begun its role may be as a secondary reinforcing mechanism. Moreover since no such correlation between promoter hypermethylation and inactivity has yet been demonstrated for metatherian X linked genes or eutherian X linked tissue specific genes this role may be restricted to eutherian constitutive genes. In contrast, there are intragenic sites in both eutherian and metatherian genes that appear to be methylated specifically on the active X and which could be involved in X inactivation, for example by marking replication origins, delineating chromatin boundaries or regulating binding of inactivating proteins.

To investigate these possibilities we performed *in vitro* DNase I footprinting experiments on a region in intron 3 of the kangaroo HPRT gene that we have previously demonstrated to be methylated specifically on the active X only[1]. This region is relatively enriched in CpGs and has the appearance of a small CG island. Three areas of protein binding to unmethylated DNA were found; Footprint 1 contains both a HpaII and a HhaI site previously demonstrated to be methylated on the active X but not on the inactive X, as well as a further CpG dinucleotide whose *in vivo* methylation state is as yet unknown. Footprint 2 also includes a potentially differentially methylated CpG whose methylation state is unknown, but footprint 3 has no CpG dinucleotides. DNA-protein interactions within footprint I were investigated further using gel shift assays, which showed several high specificity interactions using nuclear extracts prepared from both kangaroo tissues and HeLa cells. The binding of one or more of these is abolished by methylation of the HpaII site but not the adjacent HhaI site contained within the footprint. Binding of the methylation sensitive factor appears to promote the binding of a second factor to nearby bases to produce a multicomponent binding complex. We hypothesise that represser factors binding to the unmethylated inactive X linked footprint I site interact with the promoter, perhaps in concert with factors binding to the other footprints, to silence transcription. Methylation of this binding site on the active X would prevent binding therefore maintaining the gene active. These findings are analogous to those recently reported for the imprinted autosomal Igf2r gene for which an intragenic active-allele-specific methylated region has been identified which is suggested to act as a silencer of the gene on the inactive allele in the unmethylated state[2]. Future experiments will focus on defining the various binding sites more accurately and examining the effects of this region on the transcription of reporter genes, to test whether it can act as a silencer for the X linked marsupial HPRT gene.

1. Piper, A.A., Bennett, A.M., Noyce, L., Swanton, M.K. & Cooper, D.W. (1993). *Somat. Cell & Molec. Genet.*, 19, 141-159.

2. Stoger, R., Kubicka, P., Liu, C.-G., Kafri, T., Razin, A., Cedar, H. and Barlow, D.P. (1993). *Cell* 73, 61-71.

# A wide-range survey of cross-species microsatellite amplification in birds

Craig R Primmer[1] Anders Pape Müller[2] and Hans Ellegren [1]

1 Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Biomedical Centre, Box 597, S-751 24 Uppsala, Sweden.
2Zoological Institute, Department of Population Biology, Copenhagen University, Universitetsparken 15, DK-2100, Copenhagen Ø, Denmark.

The possibility to perform cross-species microsatellite amplification in birds was surveyed by analysing sets of prmers developed from the swallow (*Hirundo rustica*) and the pied flycatcher (*Ficedula hypoleuca*) genomes on a panel of 58 different bird species. There was a significant and negative relationship between microsatellite performance and the evolutionary distance between the original species and the tested species (as measured by DNA-DNA hybridisation). As a rough indicator of expected cross-species microsatellite performance we estimate that 50% of markers will reveal polymorphism in avian species with a DNA-DNA hybridisation DTmH value of 5 compared to the original species. The established relationship between performance and evolutionary distance agrees very well with data obtained from some mammalian microsatellite cross-species studies.
An analysis of 10 species from within the family *Hirundinidae* with the swallow primers revealed extensive polymorphism in all species studied (average probabilities of identical genotypes ranging from $6x10^{-4}$ to $6x10^{-7}$). There were distinct allele frequency differences between the Hirundinidae species and we envisage that microsatellite cross-species amplification will be a useful tool in phylogeny construction and species identification.

# GENE DUPLICATION AND DIVERGENCE IN THE α ESTERASE CLUSTER OF *DROSOPHILA MELANOGASTER*

**C. Robin[1,2], R.J.Russell[2], J.G. Oakeshott[2].**
**1.Australian National University and**
**2.Division of Entomology, CSIRO, Canberra, Australia**

We present analyses of the gene duplication events and the associated pattern of divergence of eleven genes in the α esterase cluster of *Drosophila melanogaster*.

At the sequence level, the esterases of the α cluster have diverged substantially ( 37-66% aa. identity ). There is no evidence for recent gene conversion events although the presence or absence of introns suggests that either several ancient recombination events or a number of independent intron losses have occurred. We also present data suggesting an esterase pseudogene located within an intron of another esterase gene was once active.

We have used a structural model based on acetylcholine esterase to interpret differences between the α esterases and other esterases sequenced to date. The α esterases can be distinguished by a deletion and two insertions, in loops at the lip of the active site gorge, and by absence of a conserved disulphide bridge. Esterases from several species within the genus *Culex* share these features and the lack of signal peptides, suggesting that these may be distant homologs to the α esterases of *Drosophila melanogaster*.

We are now extending the analysis of gene duplication and divergence by comparing the α cluster of *Drosophila melanogaster* with that of other *Drosophilid* species, in particular *Drosophila buzzatii*.

# The *dead ringer* gene of *Drosophila* identifies a family of regulatory genes that encode a novel highly conserved DNA-binding domain.

**Stephen L. Gregory, R. Daniel Kortschak, Bill Kalionis and Robert B. Saint.**

**Department of Genetics, University of Adelaide, Adelaide, South Australia, 5005.**

Our analysis of the *dead ringer* (*dri*) gene of *Drosophila melanogaster* has led to the identification of a new gene family, termed the ARID family, that encodes proteins containing a novel, highly conserved DNA binding domain. This domain bears no homology to known DNA binding domains. The ARID family is an important family of regulatory genes as it includes yeast *SWI1*, that encodes a member of the yeast SWI complex involved in widespread transcriptional regulation, a gene encoding a human retinoblastoma binding protein and other mouse and human regulatory genes. The conserved domain in the mouse B cell-specific DNA binding protein, Bright, exhibits 84% identity to DRI over a 90 amino acid region, making this one of the most conserved DNA binding domains identified. Curiously, gel retardation and optimal binding site screens revealed that the *in vitro* sequence specificities of DRI are strikingly similar to those of many homeodomain proteins, suggesting a cooperative or competitive relationship with homeodomain proteins *in vivo*.

Maternal *dri* mRNA and the protein derived from it are found distributed throughout the embryo during the syncytial stages. During gastrulation, *dri* is expressed in mesodermal tissue and, following germ band retraction, in a restricted set of tissues including salivary gland ducts, pharyngeal muscles, parts of the gut and some CNS cells. Two enhancer trap P-element insertions in *dead ringer* give an embryonic lethal phenotype with disruption of the hindgut as the most obvious defect. Thus we propose that *dead ringer* encodes a novel DNA-binding protein that is developmentally regulated and is required for successful embryogenesis, specifically for correct hindgut formation.

# The ligand-gated ion channel receptor superfamily: Heritable mutations in the glycine receptor help to define structural domains of the receptor

Peter R Schofield

Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst 2010

Fast synaptic neurotransmission is mediated by receptors of the ligand-gated ion channel receptor superfamily. Excitatory neurotransmitters, such as acetylcholine, or inhibitory neurotransmitters, such as γ-aminobutyric acid and glycine, bind to their cognate receptors and activate an integral ion channel that allows the passage of either sodium or chloride ions, respectively.

Each receptor comprises five subunits which form a pseudosymmetric pentameric complex with a central ion channel. Each subunit of the ligand-gated ion channel receptor comprises a large extracellular domain of about 225 amino acids that forms the ligand binding site, four hydrophobic domains of which the second is believed to form the ion channel lumen and an intracellular domain located between transmembrane domains three and four. Additionally, each receptor subunit shares approximately 70-80% primary sequence identity with subunit subtypes of the same receptor class (eg. α1 vs α2 subunits of the glycine receptor), approximately 35% primary sequence identity with other subunits of the same receptor class (eg. α1 vs β subunits of the glycine receptor) and between 20-35% primary sequence identity with subunits of other receptors.

The glycine receptor is the primary inhibitory receptor in the spinal cord and brain stem. Pharmacological blockade, by the antagonist strychnine, or genetic defects in either the α or β subunits lead to characteristic symptoms of muscular rigidity. The autosomal dominant neurological disorder startle disease or hyperekplexia, is caused by missense mutations in the α1 subunit resulting in Arg at position 271 being altered to either Leu or Gln. Characterisation of this mutation shows that it defines a critical site involved in the transduction of ligand binding into the opening of the chloride channel. The murine mutation *spasmodic* is due to a missense mutation in which Ser replaces Ala at position 52 of the α1 subunit. This mutation reduces the efficacy of glycine in gating the channel.

Using the complementary approach, of site-directed mutagenesis and functional expression studies, we have mapped additional residues of the receptor which are involved in either agonist and/or antagonist binding, defining two additional domains of the receptor. Amino acids Phe159, Gly160 and Tyr161 define one domain while Lys200, Tyr202 and Thr204 define the second. These two domains are located at positions in which ligand binding residues have been identified in other ligand-gated ion channel receptors indicating that all members of this class of receptors bind ligands by a similar mechanism.

The understanding of the mechanism of operation of the glycine receptor has been greatly aided by the characterisation of naturally occuring genetic mutations and by site-directed mutagenesis. Since the ligand-gated ion channel receptors comprise a multigene family, knowledge of the structure and function of each individual receptor has aided our understanding of the mechanisms of fast synaptic neurotransmission mediated by this family of receptors..

## Molecular Phylogenetics of the Australian Chelidae

Jennifer Seddon and Peter R Baverstock
Faculty of Resource Science and Management, Southern Cross University, Lismore NSW 2480

The Chelidae (Testudines: Pleurodira), a family of freshwater turtles, are found only in Australasia and South America and are presumed to be of southern origins. DNA sequence data was obtained to examine the controversial phylogenetic relationships of the Chelidae.

The 12S ribosomal RNA gene was amplified by PCR and 411 bp sequenced using an automated sequencing technique for 22 taxa, including 16 Australian and South American Chelidae and, as outgroups, representatives of the five taxa of the Pelomedusidae (Testudines: Pleurodira) and *Carrettochelys* (Cryptodira: Carettochelyidae).

Analysis using parsimony and neighbor joining on weighted and unweighted data sets supports a geographical division of the Chelidae. The data lend support to the placement of the Australian Chelidae as a monophyletic group and within this for a separation of the long and short-necked taxa. It is suggested, therefore, that the Australian Chelidae have diverged in isolation. The failure to resolve the phylogeny of a monophyletic group of Australian short-necked Chelidae containing *Emydura, Elseya dentata, Elseya latisternum, Rheodytes* and *Elusor* suggests that this group may provide an example of rapid cladogenesis.

In addition, the rate of sequence evolution of 12S rRNA in the Chelidae and in another Gondwanan vertebral group, the marsupials, is compared.

**Parental environments can induce high heritabilities for fecundity in *Drosophila melanogaster***

**Carla Sgrò and Ary A. Hoffmann**

**School of Genetics and Human Variation, La Trobe University, Bundoora 3083**

It is well known that the environment can influence estimates of genetic variation. However, previous work has not considered if parental environments affects the expression of genetic variation in the progeny generation, outside the context of maternal effects. A parent-offspring regression was used to compare the effect of two culture temperatures on the heritability for fecundity, development time and wing length. When parents were cultured and tested at 14°C, heritabilities for fecundity were high (>60%) regardless of the temperature at which progeny were cultured and tested. In contrast, heritabilities were low and generally not significant when parents were cultured and tested at 28°C. Genetic variation for wing length was not influenced by parental environments, while development time heritabilities showed complex patterns.

**The genetic diversity of stem rust, a fungal pathogen of wheat**

**J.P. Silk, J.J. Burdon, A.J. Pryor, R.F. Park*, and D.R. Marshall***
**CSIRO Division of Plant Industry, Canberra,A.C.T.**
***The University of Sydney Plant Breeding Institute, Cobbitty N.S.W.**

The stem rust pathogen, *Puccinia graminis* f.sp. *tritici*, under Australian environmental conditions has proven to be destructive and economically damaging. Effective crop protection through the incorporation of resistance genes has been confounded by the ability of the pathogen to undergo genetic change. This enables new pathotypes to attack commercial varieties and challenges breeders to find new sources of resistance and incorporate different gene combinations. Genetic changes have been attributed to the mechanisms of migration, spontaneous mutation, sexual recombination and somatic hybridisation. The study aims to examine the origin and maintenance of genetic diversity of this pathogen.

As the pathogen is an obligate biotroph, and the culturing of large amounts of material is difficult, a PCR-based molecular marker system has been applied. From a selected population of over forty pathotypes, representing the four main Australian stem rust phenotypic lineages and forty five years of pathogen evolution, the progenitors of the four lineages were examined with 120 RAPD primers and by PCR analysis of the ribosomal intergenic spacer region. Of the 84 RAPD primers that amplified a pattern, 64 demonstrated polymorphism between lineages. Polymorphism was also seen in the amplification of the ribosomal IGS-1 region. Thirty seven RAPD primers have been chosen to study the remaining pathotypes, with indications that variation between lineages increases with temporal separation, and very little variation within a lineage. In a somatic hybridisation study using two genetically different parent lines, ninety potential somatic hybrids have been collected for molecular study.

# Role of the *PINOID* gene in generating flower primordia in *Arabidopsis*

David R Smyth and John Alvarez

**Department of Genetics & Developmental Biology, Monash University, Clayton, Vic 3168**

In *Arabidopsis thaliana*, the apical meristem first generates a series of primordia on its flanks which develop into rosette leaves. Following floral induction, flower primordia are produced instead. These are elevated as the stem elongates, and they continue to arise indefinitely on the flanks of the extending inflorescence apex.

A series of allelic mutations, named *pinoid*, has been isolated in which the inflorescence meristem loses the ability to specify floral primordia. A few flowers may arise initially, but their production soon ceases and no other defined structure develops in their place. The inflorescence meristem continues to extend, producing a naked, pin-like growth.

The effect of *pinoid* mutation on the apical meristem is limited to primordia arising on its flanks after it has become an inflorescence apex. The earlier production of leaf primordia is not affected. Also, even if the flanking primordia are converted from flower to inflorescence primordia (as in *cauliflower apetala1* mutants), *pinoid* mutations still disrupt their formation. However the flower meristem is not affected in the same way as the inflorescence meristem. The production of floral organ primordia is not abolished in *pinoid* mutant flowers, even if the flower meristems are caused to proliferate indefinitely (as in *agamous* mutant plants).

We propose that the wild-type *PINOID* gene product helps trigger the initiation of primordia on the flanks of the inflorescence apex. When this function is compromised in *pinoid* mutants, the trigger is disrupted and the growth of primordia cannot be initiated.

**The Origins of the Lemba "Black Jews" of Southern Africa: Evidence from Y Chromosome Studies**

Amanda B. Spurdle* and Trefor Jenkins

**Department of Human Genetics, School of Pathology, The South African Institute for Medical Research and University of the Witwatersrand, Johannesburg, South Africa**

***Present Address: School of Genetics and Human Variation, La Trobe University, Bundoora, Victoria**

The ability of Y chromosome polymorphisms to provide a record of male-specific gene flow and human evolution has long been recognized, and numerous studies using different Y chromosome markers have indicated the value of this approach. The Lemba population of Southern Africa is a group of Bantu-speakers that claims to be one of the lost tribes of Israel. Oral tradition states that their ancestors were craftsmen from a huge town across the sea, and they came to southern Africa to trade their goods. Stranded by the news of war in their home town, they settled permanently in the area, and took Bantu wives. Historically, the Lemba were distinct from their Bantu-speaking neighbours by their livelihood, physical appearance, dress, customs and rituals, and even today the cultural differences between the Lemba and other Bantu-speakers are recognized.

Population allele frequency data for four different Y-specific polymorphic loci were analyzed in an attempt to establish the genetic origins of the Lemba. The results suggest that at least 50% of the Lemba Y chromosomes are Caucasoid in origin, about 40% are Negroid, while the ancestry of the remainder cannot be resolved. Furthermore, the Caucasoid genes found in the Lemba are definitely not European in origin, but appear rather to be Semitic. Data on the history of Semitic peoples and their association with Africa has been combined with the genetic evidence to construct theories about the ancestry of the Lemba.

**Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae)**

**Paul Sunnucks and Dinah F. Hales**
**School of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia.**
**E-mail psunnuck@rna.bio.mq.edu.au. Telephone 02) 850 8203, FAX 02) 850 8245.**

PCR products corresponding to part of the cytochrome oxidase subunits I and II region of mitochondrial DNA (mtDNA COI-II) were deduced (from consistent 'ambiguous' positions on sequencing gels) to consist of multiple haplotypes in three aphid species of the genus *Sitobion*. We investigated the molecular basis of these observations.

The consistent conclusion from sequences of cloned PCR products and Southern blots is that many separate transposed sequences exist in the three *Sitobion* species examined. These findings indicate a frequency of mtDNA transposition unprecedented in invertebrates, and parallelled only by the high level reported from humans. Multiple sequences were also inferred from sequences from some closely-related aphid genera but not more distant ones.

Analysis of nucleotide substitution patterns revealed that some differences between mtDNA and transposed sequences were consistent with having occurred under selective constraints in mtDNA evolution, but other substitutions resembled pseudogene evolution. The COI-II sequences form a robust phylogenetic tree, with mtDNA sequences in each species being the most derived (modern). These data all indicate that transpositions have occurred repeatedly over time in these species. Individual transposed sequences have retained most of the characteristics of ancestral mtDNA types, whilst incurring pseudogene-like mutations. Our data suggest that transposed sequences are on aphid chromosomes rather than other possible locations.

We believe that *Sitobion* aphids (and other species exhibiting mtDNA transposition) may be important vehicles for studying the molecular evolution of mtDNA regions and nuclear pseudogenes, including direct comparison of rates of evolution of these.

# DNA binding of FacB, a transcriptional activator of acetate utilization genes of *Aspergillus nidulans*.

Richard B. Todd, Meryl A. Davis and Michael J. Hynes.

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

The *facB* gene of *Aspergillus nidulans* encodes a transcriptional 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 gel mobility shift assays to demonstrate that FacB is a DNA binding protein that binds to specific sequences from FacB-regulated promoters. Footprinting assays have been used to define two dissimilar sequences to which FacB binds in the *amdS* promoter. An analysis of binding to mutant FacB binding sites has been performed.

*In vitro* mutagenesis has been used to alter specific cysteine 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.

**_ANT3_ and _STS_ are autosomal in prosimian lemurs: implications for the evolution of the pseudoautosomal region**

Roland Toder,[1] Gudrun A. Rappold,[3] Katrin Schiebel,[3] and Werner Schempp[2]

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

2 Institute of Human Genetics and Anthropology, University of Freiburg, Breisacher Straße 33, D-79106 Freiburg, Germany

3 Institute of Human Genetics, University of Heidelberg, Im Neuenheimer Feld 328, D-69120 Heidelberg, Germany

Molecular studies on X and Y chromosomes in humans, non-human primates, and mice have identified the pseudoautosomal region, a genetically unique segment of homologous sequences which recombine and fall within the meiotic pairing region. On the human X chromosome, two genes, steroid sulphatase (*STS*) and ADP/ATP translocase (*ANT3*) map to the most distal band on Xp22.3 at a physical distance of 6.2 Mbp. While the functional human *STS* gene is located about 5 Mbp proximal to the pseudoautosomal region of the X and a non-functional pseudogene on the long arm of the Y, *STS* is pseudoautosomal in mice. This observation was interpreted as the result of a pericentric inversion in the Y chromosome of primates, with the mouse X and Y chromosomes reflecting the ancestral situation in eutherian mammals.

Comparative *in situ* hybridisation in various primate species has revealed a pseudoautosomal location for the human *ANT3* and an X-specific location for the *STS* gene throughout the higher primate species up to the New World monkeys. However, neither *ANT3* nor *STS* map to the X chromosome in lemurs, the next evolutionary relatives. Instead, we demonstrate co-hybridisation of *ANT3* and *STS* sequences on an autosome of two prosimian species of the genus *Lemur* and *Eulemur*. These results suggest either an autosome to X/Y translocation after the simians radiated from the prosimians, resulting in a pseudoautosomal location of genes like *ANT3* and *STS*. In simian primates, *STS* then became X-specific by a pericentric inversion in the Y chromosome followed by mutational inactivation of the Y allele. Or it could mean that a region containing these genes was added to the X and Y of an ancestral eutherian, and secondarily lost from the sex chromosomes of a prosimian ancestor, thus lacking in the prosimian branch.

## The Mechanism by which DNA is Assembled into Chromatin and its Role in Regulating Transcription

DAVID TREMETHICK

**The John Curtin School of Medical Research, The Australian National University.**

In the nucleus of all eukaryotic cells, DNA is complexed with about an equivalent mass of histones and non-histones proteins to form chromatin, which is a periodic structure made up of repeating, regularly-spaced subunits, the nucleosomes. Recent genetic studies have clearly demonstrated that the organization of a gene into chromatin can control the ability of transcription factors to associate with a promoter. To investigate the role of chromatin structure in controlling the transcription of gene, an *in vitro* chromatin assembly system has been developed utilizing extracts prepared from *Xenopus laevis* oocytes. It was found that the assembly of a gene into chromatin does indeed prevent the binding of transcription factors to DNA. This suggests that chromatin may play a role in determining which subset genes are transcribed in a cell. This finding also suggests that for a specific gene to be transcribed, nucleosomes must be excluded, displaced or its position on the DNA altered. In order to understand how the chromatin structure of a gene is altered to allow transcription, it is first necessary to understand how DNA is assembled into chromatin. Fractionation of the oocyte extract, and the eventual purification of all chromatin assembly components, will reveal the mechanism by which chromatin is assembled. Current fractionation work has shown that the assembly of DNA into chromatin is a sequential, time dependent process occurring in at least four steps. Two steps are required for the formation of the nucleosome, and a further two steps are needed to space these nucleosomes in order to generate a regular array. Most interestingly, in contrast to fully assembled chromatin, these chromatin intermediates facilitates the binding of transcription factors to DNA.

# GENES AND GENE FAMILIES THAT AFFECT PLASMA LIPOPROTEIN PHENOTYPES AND SUSCEPTIBILITY TO ATHEROSCLEROSIS

**J.L. VandeBerg, J.E. Hixson, C.M. Kammerer, D.L. Rainwater and J.W. MacCluer. Southwest Foundation for Biomedical Research, San Antonio, Texas, 78228, U.S.A.**

The single greatest risk factor for atherosclerosis is the ratio of plasma cholesterol carried by low density lipoproteins (LDLs) to that carried by high density lipoproteins (HDLs). Other characteristics of plasma lipoprotein phenotype also contribute to risk of atherosclerosis. Genetic determinants of lipoprotein phenotypes include at least ten apolipoprotein genes some of which belong to gene families, three lipoprotein receptor genes, and eight genes for enzymes that affect lipoprotein metabolism. Research by our group with human subjects and with baboons (a model that closely resembles humans) is defining the contributions of polymorphisms at these gene loci to variations in lipoprotein phenotype in the population at large. Some conclusions are that 1) genotype at the locus encoding apolipoprotein E (apoE) is responsible for 6% of the variance in atherosclerosis in young adults, 2) genotype for an apolipoprotein B (apoB) insertion/deletion polymorphism affects LDL levels and atherosclerosis in American blacks and Hispanics, 3) genotype at the locus encoding the LDL receptor affects LDL and apoB levels on basal and atherogenic diets and affects LDL response to an atherogenic diet, 4) genotype at the locus encoding lecithin; cholesterol acyltransferase (LCAT) affects plasma LCAT activities, HDL levels, and distribution of cholesterol among HDL size classes, 5) genotype at the locus encoding apolipoprotein A-IV (apoA-IV) affects HDL levels, and 6) plasma concentrations of Lp(a), a highly atherogenic lipoprotein, is determined primarily by genotype at the locus encoding apolipoprotein (a).

# GATING OF LIGHT-SENSITIVE ION CHANNELS IN *DROSOPHILA MELANOGASTER*

Coral G. Warr and Leonard E. Kelly
Department of Genetics, University of Melbourne, Parkville VIC 3052 AUSTRALIA

The *Drosophila* visual system has been well characterised at both the genetic and molecular level, and is an excellent model system for the study of the gating of non-voltage-gated ion channels. Two genes encoding light-sensitive cation channels (known as *transient-receptor-potential* (*trp*) and *transient-receptor-potential-like* (*trpl* )) have been isolated using a combination of genetic and molecular techniques.

The *trpl* gene was originally isolated as a calmodulin-binding protein, which suggested a mechanism by which intracellular $Ca^{2+}$ levels might gate this channel. Accordingly, *in vitro* binding studies of the putative calmodulin-binding sites in *trpl* have been carried out. These studies have identified two different calmodulin-binding sites in the carboxy-terminal region of the *trpl* protein. One of these sites shows little homology to the consensus calmodulin-binding motif, and binding of calmodulin to this site is independent of calcium, an unusual result which may act to remove a basal conductance and is likely to be involved in $Ca^{2+}$-dependent channel gating. The other site is highly homologous to the consensus calmodulin-binding motif, and binding of calmodulin to this site is calcium-dependent. Phosphorylation of this site *in vitro* has been shown to alter the site's affinity for calmodulin, thus phosphorylation may affect the modulation of the channel by calmodulin. The interpretation of models for ion channel gating in light of these results will be discussed.

Recent investigations have indicated that *trpl* may be part of a new family of cation channel genes, as a novel protein that cross-reacts with anti-*trpl* antibodies has been identified. This protein species is expressed outside the visual system, and is highly abundant in thoraces and abdomens.

# X-CHROMOSOME INVOLVEMENT IN MARSUPIAL SEX DIFFERENTIATION

**C.M. Watson[1], P.G. Johnston[1], R.L. Hughes[2], R. Gemmell[2], M. Smith[3] and D.W. Cooper[1].**

[1] **School of Biological Sciences, Macquarie University, NSW, 2109**

[2] **Department of Anatomy, University of Queensland, QLD, 4072**

[3] **Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA, 5000**

Intersexual animals are those which have some aspects of one sex, some of the other but a complete set of neither. Their occurrence in marsupials raises questions about the nature of sex differentiation, especially the pouch, mammary glands and the scrotum. We now summarise information on 26 intersexual marsupials. Six types have been described. Their combined frequency is probably about 1/500-1/1000 animals.

They are:

(1) males with a pouch, mammary glands, no scrotum and undescended testes;

(2) females with an empty scrotum, but lacking mammary glands and pouch;

(3) females with a hemi-pouch and mammary glands on one side and an empty scrotum on the other;

(4) a male with a hemi-pouch and mammaries on one side and a hemi-scrotum and a testis on the other;

(5) animals lacking pouch, mammaries and a scrotum;

(6) an animal having only a hemi-scrotum on one side.

About half these animals are karyotypically abnormal. The possession of a pouch and mammaries, but no scrotum, is associated with two X chromosomes. The complementary type, possession of a scrotum but no pouch or mammaries is associated with one X. The Y does not seem to influence this dichotomy, although it clearly is testis determining in marsupials. The data are compatible with a dosage hypothesis. They are also compatible with an imprinting hypothesis, with the paternally derived X being necessary for mammary gland development. The occurrence of two intersexual bandicoots in the same litter suggests that a single gene could be involved. The presence of two homeobox genes on the mouse X chromosome raises the intriguing possibility that a homeobox gene may be responsible for the mammary/scrotum switch in marsupials. In interpreting the data it is important to recall (a) that the scrotum in marsupials and eutherians are not homologous organs and (b) that X-inactivation in marsupials is paternal.

# The molecular structures of ultra-fast rare mobility variants of *sn*-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster*

Tomasz M. Wilanowski[1], Phil T. Barnes[2] and John B. Gibson[1]

[1] **Molecular and Population Genetics Group,**
**Research School of Biological Sciences,**
**Australian National University,**
**G.P.O. Box 475, Canberra, ACT 2601, Australia**

[2] **Department of Zoology, Connecticut College,**
**P.O. Box 5406, New London, CT 06320, U.S.A.**

*sn*-glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8) has an important role in energy metabolism and lipid biosynthesis in insects. In *D. melanogaster* two common variants of GPDH - slow (S) and fast (F), with respect to their electrophoretic mobility - have been found in most populations sampled. A rare variant, electrophoretically faster than GPDH-F, has been designated ultra-fast (UF). Alleles with this phenotype have been reported from locations in Europe, Asia, Africa, North America and Australia. In the course of a detailed molecular study, the $Gpdh^{UF}$ alleles from different populations have been found to be phenotypically and structurally heterogeneous. The possible origins of these alleles and their significance in natural populations will be discussed.

**The Evolution of Genomic Imprinting in Mammals.**

**Stephen A. Wilcox, Megan J. Smithwick, and Jennifer A. Graves.**
**School of Genetics and Human Variation, La Trobe University, Melbourne, Australia.**

Genomic imprinting is observed in a variety of animals, but among the higher vertebrates it has been documented only in mammals. The first observation that imprinting occurred in mammals was the demonstration that the paternal X chromosome was preferentially inactivated in female marsupials. Inactivation in eutherian mammals is random in the embryo, but paternal in the extraembryonic tissues, suggesting that imprinted X-inactivation may be ancestral in mammals. In monotremes evidence for X-inactivation is sparse.

More recently, parental imprinting has been demonstrated in regions of mouse and human autosomes. In man and mouse there are at least two conserved autosomal imprinted regions. These imprinted regions contain several genes that have either the maternal or the paternal copy inactivated. One on human chromosome 11 and mouse chromosome 7 includes the genes IGF2, H19, and the other on human chromosome 15 and mouse chromosome 7 includes SNRPN/SNURF, and ZNF127. The evolutionary purpose served by genomic imprinting is still unclear, but one interesting idea is that of a "parental tug of war" between maternal and paternal growth factors expressed in embryo and extraembryonic membranes.

We are taking an evolutionary approach to studying the mechanism, and the advantages of genomic imprinting by cloning and comparing imprinted genes between humans, mice, and their most distantly mammalian relatives, the marsupials and monotremes. Comparisons of sequence location and expression of these genes in eutherians, marsupials, and monotremes should permit us to assess the roles of gene position, sequence elements, DNA synthesis timing, and methylation in the control of imprinting, as well as the relation to the mode of reproduction in the three groups of mammals.

We have cloned the IGF2, SNRPN, and ZNF127 homologues from marsupial cDNA, lambda genomic, and cosmid libraries. Sequence comparisons of these genes reveal homology to the human and mouse sequences. These clones have been localised, by radioactive and flourescence *in situ* hybridisation, to the chromosomes a marsupial, the tammar wallaby.

**Recent coalescence of human mitochondrial genomes results from natural selection and not the movement of people out of Africa.**

Cheryl Wise, Michaela Sraml and Simon Easteal
**Human Genetics Group, John Curtin School of Medical Research, Australian National University**

The approximately 150,000 year coalescence time of human mitochondrial genomes is usually explained as reflecting the age of *Homo sapiens*, with a geographically restricted origin somewhere in Africa followed by a range expansion throughout Africa, Asia and Europe, which resulted in the displacement of the pre-existing *H. erectus*. This historical explanation is consistent with some paleontological interpretations, but not with others that imply evolutionary continuity between *H. erectus* and *H. sapiens* throughout Africa, Asia and Europe. It implies an effect on both mitochondrial and nuclear genome diversity. An alternative, which would have had no direct effect on nuclear genome diversity, is that a mitochondrial genome was spread through human populations by directional natural selection beginning approximately 150,000 years, with all current variation being generated subsequently.

We have tested this hypothesis by comparing nuclear and mitochondrial genome diversity in humans and other related species. Mitochondrial control region sequences and microsatellite allelic distributions are presented for a sample of unrelated chimpanzees. Comparison of these data with the equivalent data from humans reveals that humans have a substantially lower ratio of mitochondrial to nuclear genome diversity than chimpanzees. The relative extent of mitochondrial and nuclear genome diversity in chimpanzees is similar to that seen in other simians. This discrepancy is inconsistent with a neutral model of human mitochondrial genome evolution and can be explained by a recent "selective sweep" of a mitochondrial genome through regionally dispersed human populations, which were established two million years ago, and which have persisted to the present.

We have further tested the selective neutrality of the mitochondrial genome by estimating the ratio of replacement (nonsynonymous) to silent (synonymous) nucleotide substitutions within and between humans and chimpanzees for the mitochondrially encoded NADH dehydrogenase subunit 2 (ND2) gene. The results show evidence for departure from neutral evolution, as do similar studies in Drosophila and mice, and suggest that evolutionary inferences based on patterns of mitochondrial DNA variation should consider selection as a force.

# Defective *hobo* insertion 5' to the distal start site of the alcohol dehydrogenase gene in *Drosophila melanogaster* affects only adult ADH activity

**Yan-Hong WU and John B. Gibson**

***Molecular & Population Genetics Group, Research School of Biological Sciences, The Australian National University P.O.Box 475, Canberra, A.C.T.0200***

The alcohol dehydrogenase gene (*Adh*) in *Drosophila melanogaster* has two promoters. The proximal promoter initiates transcription principally in the larval stages, while the distal promoter is utilised first in late 3rd instar larvae but then in adults. A naturally occurring variant, $Adh^{HA51}$, was shown to have very low ADH activity in adults but nearly normal levels in larvae. The level of mRNA in adults was reduced but not in larvae. Comprehensive molecular analyses of the *HA51* variant have shown that the coding region of the gene does not contain any mutations that might affect activity. These analyses revealed a 2.4Kb defective *hobo* element inserted between the distal TATA box and the transcription start site.

The 2.4Kb defective *hobo* insertion is unstable in somatic cells. When the *hobo* element was deleted from somatic cells, 6bp remained at the insertion site. Strains were isolated in which the number of copies of the *hobo* element was apparently reduced. However in these lines adult ADH activity remained low. The relationship between ADH activity and somatic deletion of *hobo* will be discussed.

# POSTER ABSTRACTS

**In alphabetical order by presenting author**

# EXPLORING AUSTRALIAN PREHISTORY WITH THE TOOLS OF MOLECULAR GENETICS.

Greg Adcock[1], David Buckle[1], S. Fountain[1], T. H. Loy[2] and S. McElroy[3].

1.Division of Archaeology and Natural History, R.S.P.A.S., Australian National University, A.C.T. 0200, Australia.

2. Centre for Molecular and Cellular Biology, and Department of Anthropology and Archaeology, University of Queensland, St Lucia 4072, Australia.

3. U.S.D.A. Forest service PSW Research Centre, Albany, CA 94710, USA

Molecular genetics has the potential to revolutionise the study of human prehistory. To answer "what can DNA recovered from material found in archaeological site tell us?", the limits of molecular techniques need delineation. Prehistorians require the maximum amount of information from a site or an artefact often from minimal material. The "information" from ancient DNA comes in the form of genetic variation detected using the polymerase chain reaction and sequencing. We have worked mainly with Australian material in our efforts to define useful limits for the application of ancient DNA. We have developed techniques to extract and amplify DNA routinely and efficiently because many archaeological samples are available only as minute traces, or must be conserved. We have shown that DNA amplified from human bone can estimate the relationships and origins of individuals and even sex them. We have also used eukaryotic ribosomal (28S) primers to determine the species of origin of blood residues adhering to stone tools. Identification of the origins of residues will help us to better understand tool use and cultural practice. Work is under way on reliably amplifying various single-copy genes to access a wealth of genetic markers that can distinguish populations and detect genetic diseases.

## Genetic Variation in a Captive Stick-Nest Rat Colony

T Badgery-Parker and W Sherwin. School of Biological Science, University of New South Wales.

The Stick-nest rat (*Leporillus conditor*) ranged across much of southern Australia, but is now extinct on the mainland. Animals from the remaining natural population on the Franklin Islands, Nuyts Archipelago, SA have been used to establish a captive breeding program for reintroduction of animals to other offshore islands. It is important to maintain genetic variation in such a program to avoid inbreeding depression and to maximize the ability to adapt to new environments on release. We are using the program SPARKS to calculate the amount of inbreeding and the loss of variation in the captive colony, and the amount of original variation transferred to the new populations. We are also predicting the future genetic and demographic characteristics of the populations, using the stochastic program VORTEX. The results of these models will assist in the management of the colony. We hope to use DNA markers to directly monitor the genetic variation in the captive and new populations, and to test the computer models. For this, we are developing microsatellite DNA markers. Using mouse PCR primers, we have amplified four microsatellite loci and made primers specific for *Leporillus*. We have checked the inheritance of these markers in animals of known pedigree and we are now surveying the captive colony for variation at these loci. We have also screened a Stick-nest rat genomic DNA library for microsatellites, and intend to develop additional markers from this library.

# The recombinational landscape of *Caenorhabditis elegans*.

**T. M. Boyce**

**Biotechnology Program**
**Division of Entomology**
**CSIRO**
**GPO Box 1700**
**Canberra, ACT 2601**

The frequency and distribution of recombinational events in genomes can have profound effects on genomic and organismal evolution. Frequency of recombination has recently been shown to be significantly correlated with levels of genetic polymorphism in natural populations of *D. melanogaster* (Begun and Aquadro, 1992; Kindahl and Aquadro, in press). Recombination is also expected to play a role in determining the location and abundance of repetitive sequences (Charlesworth, 1994).

The role of recombination in these processes can only be effectively evaluated in organisms for which there is a relatively detailed knowledge of how recombination rate varies by physical position in the genome. To date, only the recombinational landscape of *D. melanogaster* has been described (Kindahl and Aquadro, in press) at least in part because appropriate data is not available.

One of the few organisms for which such data is available now is the nematode *Caenorhabditis elegans*. Measures of recombination per unit physical distance (coefficients of exchange) vary widely both within and among chromosomes. A general pattern for all chromosomes but the X has recombination lowest in the middle of the contigs, in the so-called gene clusters. This pattern is remarkably similar to that observed in *D. melanogaster*. Several spikes of high recombination across short physical distances occur, typically at least one on each chromosome. In some cases these spikes may simply reflect overestimates of recombination, but some spikes occur among well characterised and well mapped loci.

Regression of the abundance of repetitive elements against coefficients of exchange confirm predictions by Charlesworth (1994) that the largest mean array size of some repetitive elements will occur in regions of low recombination.

**Isolation and characterization of microsatellites in eucalypts**

**M. Byrne, I. Marquez-Garcia, T. Uren, D.N. Smith and G.F. Moran**
**CSIRO Division of Forestry, P.O. Box 4008, Queen Victoria Terrace, Canberra, ACT 2600**

Microsatellite sequences have been shown to be a highly variable type of genetic marker, useful for identification, mapping and diversity studies. Clones containing microsatellite sequences in *Eucalyptus nitens* were identified by screening a Sau3A genomic library with a CA and GA repeat sequence probes. Positive clones were sequenced and primers designed for the unique sequence flanking the microsatellite. Different magnesium chloride concentration and annealing temperatures were required for different microsatellite markers for resolution of the bands.

Conditions have been resolved for nine microsatellite loci, however two of these exhibit null alleles making them less useful. Inheritance and segregation of alleles was tested in a three generation pedigree. The bands were inherited correctly and segregation according to Mendelian principles for five of the loci tested. The level of variation detected at the microsatellite loci was determined by screening twenty unrelated individuals from five natural populations of *E nitens*. The ability to detect these loci in other eucalypt species was determined by testing four individuals from six species representing the subgenera symphyomyrtus, monocalyptus and corymbia. Most of the loci were detectable in the species from the symphyomyrtus and the monocalyptus subgenera but not from the corymbia subgenus.

# The Cloning and Sequencing of the *Notch* Homologue from the Australian Sheep Blowfly, *Lucilia cuprina.*

Z. Chen and P. Batterham

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

Previous work has shown that the *Scalloped wings*(*Scl*) gene of *L. cuprina* is homologous to the *Notch* (*N*) gene of *Drosophila melanogaster*. *Scl* and *N* mutants share phenotypic similarity for a number of traits including dominant wing notching and recessive embryonic lethality due to the abnormal expansion of the nervous system. These mutants also show a significantly increased level of developmental asymmetry. That is, if a characteristic such as the number of bristles is measured on the left and right sides of the organism there is a significant difference between the sides. Asymmetry has been also found to be associated with insecticide resistance in *L. cuprina*. Individuals homozygous for the diazinon resistance gene show elevated levels of asymmetry. In natural populations, a *Modifier* gene has arisen which returns the level of asymmetry in resistant individuals to normal. This *Modifier* maps to the region of the *Scl* locus on chromosome 3 and in genetic experiments behaves as an allele of *Scl*. This observation is supported by the earlier finding that *Scl* and *N* mutants are asymmetric and that the *Notch* gene controls genes of the *achaete-scute* complex which in turn control bristle production.

Here the isolation and sequence analysis of cDNA clones spanning the *Scl* gene from a non-modifier strain will be described. The full length *Scl* cDNA is 8503 bp in length and encodes a protein of 2653 amino acids. In the coding region 65.1% of nucleotides and 73.7% of amino acids are identical to those in *Notch*. The hypothesis that the *Modifier* is an allele of the *Scl* gene is currently being tested by the sequencing of the *Scl* gene from a modifier strain.

**Genetic population structure of the Australian Bass *Macquaria novemaculeata* determined by mitochondrial control region variation screened using Temperature Gradient Gel Electrophoresis/ Heteroduplex Analysis**

Steve Chenoweth
Faculty of Environmental Sciences
Griffith University, Nathan

Mitochondrial DNA (control region) variation was used to investigate geographic genetic population structure of a native, catadromous (freshwater living- saltwater spawning) fish, the Australian Bass (*Macquaria novemaculeata*). Fish were sampled non-destructively with muscle biopsies from four river systems spanning some 600km along the South Queensland and Northern New South Wales coast. Mitochondrial DNA variation was detected using a PCR-amplified 450bp fragment of left hand control region. This fragment was screened for variation using optimised Temperatutre Gradient Gel Electrophoresis and Heteroduplex Analysis (TGGE/HA) and subsequent cycle sequencing.

Eight distinct haplotypes were found from 72 individuals. Population subdivision estimates indicated marked subdivision between catchments (Fst = 0.1630). Parsimony analysis on 300bp of control region sequence indicated that the most geographically widespread and frequent haplotype formed the central node of the parsimony network.

It was hypothesised that this most common mtDNA haplotype was an ancestral type from which others were subsequently derived, as other haplotypes were not as widely distributed. Frequencies of mtDNA haplotypes in M. novemaculeata appear to be influenced by genetic drift and low levels of gene flow either by passive dispersal of larvae or by direct movement of adults, most probably associated with flooding events. The catadromous life history pattern of M. novemaculeata is likely to be an influential factor in its geographic genetic population structure.

# The structure of the *Sec*-1 locus on the short arm of chromosome 1R of rye (Secale cereale).

B.C. Clarke and R. Appels.
C.S.I.R.O. Division of Plant Industry, P.O. Box 1600, ACT.

Introduction .

*Secale cereale* (rye) has been used extensively as a source of useful genes for the improvement of wheat [Baum, 1991 #63], and the 1R chromosome in particular is a widely utilised source of disease resistance. It is present in wheat lines as either a substitution, mostly for chromosome 1B, or a translocation consisting of the short arm of 1R and the long arm of either 1B, 1A or 1D. The short arm of chromosome 1 (1RS) from 'Petkus' rye carries genes conferring resistance to leaf rust *Lr26* (*Puccinia recondita*); stem rust *Sr31* (*Puccinia graminis*); and stripe rust *Yr9* (*Puccinia striiformis*); as well as powdery mildew *Pm8* (*Erysiphe graminis*). Unfortunately the benefits associated with the 1R chromosome are accompanied with low flour quality which forms a dough that gives a poor stand in fermentation, adverse dough mixing properties and reduced loaf volume. These problems have been attributed to the "sticky dough" characteristic which is believed to be caused by the product of the ω-secalin genes, a family of small monomeric proteins of molecular range ($M_r$) 50-55 kd encoded by the *Sec-1* locus.

The isolation of new λ clones from the *Sec-1* locus and extensive DNA sequencing have shown that the locus consists of approximately 20, 9 kb units tandemly repeated. Analysis of the 5' upstream region have revealed the presence of putative scaffold attachment sites as well as standard promoter sequences. The studies have also demonstrated the presence of a 1.8 kb region that is variable among the different λ clones and this finding has important implications for the evolution of the locus.

## Characterisation of a *Polycomblike* interactor.

**Stanley Robert, Michelle Coulson and Robert Saint.**
**Department of Genetics, University of Adelaide, Adelaide, SA 5005.**

Members of the *Drosophila* Polycomb Group (Pc-G) of genes are required for the maintenance of correct spatial expression of the homeotic genes of the Antennapaedia and Bithorax Complexes. Mutations in Pc-G genes result in ectopic homeotic gene expression, and clonal analysis has demonstrated that the correct pattern of homeotic gene expression must be maintained throughout most of *Drosophila* development for normal formation of adult segmental structures[1].

It is not known how the Pc-G genes function to maintain repression. A possible mechanism is through the formation of a multimeric protein complex that modifies chromatin structure. While there is some evidence for a multimeric complex, no Pc-G member has been demonstrated to have DNA binding ability. However, Pc-G members are observed to bind at multiple sites on polytene chromosomes. We have been studying one Pc-G member, *Polycomblike* (*Pcl*), and have recently isolated an interactor of the *Pcl* gene product by screening an expression library with PCL protein. The *Pcl* interactor is expressed ubiquitously in embryos, and has multiple polytene binding sites, overlapping with those of PCL and other Pc-G proteins[2]. Recently, a unique zinc-finger-like motif, termed the PHD finger[3], was identified in PCL and various other proteins by database searches. Preliminary results suggest that the PHD finger domain of PCL mediates binding to the interactor protein.

The discovery of a protein that binds to PCL and to multiple sites on polytene chromosomes supports the idea of a multimeric repressive complex assembled at specific chromosomal sites.

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# Neurological Factors Contributing to Loss of Activity with Age in *Drosophila melanogaster*

Christopher Driver, Deakin University, Rusden Campus

A possible role is proposed for the age associated changes in behaviour in Drosophila as a useful model for similar changes in humans.

Locomotory activity was measured by several techniques, including the number of times a group of flies breaks an IR beam. The measurements are taken shortly after recent disturbance and are principally alarm stimulated activity. This activity deteriorates substantially with age, males deteriorating faster than females. Females are initially more active than males.

Non-neurological factors conceivably may contribute to these changes. Evidence is presented suggesting that the physical and neuromuscular capacity for activity does not limit activity in old age. However, alterations in folic acid metabolism contribute to this loss with age and additional folic acid supplementation in the diet was used for further studies to eliminate this effect.

The effects of mutants *ebony* and *stoned C*, and the drugs ephedrine, nicotine and tacrine have led to a preliminary model of the neurological of CNS control of activity and the way it changes with age. It is proposed that an activity initiating circuit is cholinergic ( nicotinic ) and is inhibited by two other circuits, one which is dependant on noradrenaline. Only the activating circuit changes with age. The differences between the sexes in initial activity can be explained by dfferences in the noradrenergic activity or responsiveness.

A mutant, *akaal*, has been produced by CaSpeR mutagenesis, which is initially less active but which deteriorates more slowly with age. The effect is most marked in females. This mutant will be used to further clarify the sex based differences in deterioration.

CONCLUSION: The changes with age in behaviour observed in Drosophila which mirror age pathologies in humans, appear to be mirrored by cellular changes which are also similar. The ready accessability of this model to examine make it potentially a very useful model for human brain ageing.

**A study of horse breed evolution using DNA profiles.**

Kelly R. Ewen[1,2], Michael Westerman[2] and Margaret E. Matthews[1].

[1]Victorian Institute of Animal Science
475-485 Mickleham Road, Attwood, 3049, Victoria, Australia.
[2]School of Genetics and Human Variation
LaTrobe University, Bundoora, 3083, Victoria, Australia.

Modern horses are believed to have originated from three ancient horse types, light, heavy and pony. Breeds have evolved with selection of desirable traits and usually with ntroduction of other qualities by cross-breeding with other horse types. Breed societies have documented the origin and influences on most breeds. It has been difficult to study the evolution of the horse breeds at a genetic level up until now because of this cross-breeding. Variable microsatellite markers consisting of short tandem repeats occurring throughout the genome of both plants and animals have been found to be useful in studying populations. This study employed these markers to study the evolution of modern horse breeds. Breeds with known Arabian influence clustered together. Within this group, horse breeds with known close relationships such as the Standardbred and Thoroughbred breeds, demonstrated this by grouping using this method. Microsatellite markers therefore, were found to reflect the known history of horse breed development.

# The *Sex-lethal* gene of *Lucilia cuprina*

Stephen D. Goodall, David Haywood, Lynn Hines*, Anthony J. Howells, Peter W. Atkinson
**Division of Entomology, CSIRO, Black Mountain, ACT, 2601.**
***Division of Wildlife and Ecology, CSIRO, Black Mountain, ACT.**

In *Drosophila melanogaster* sexual development is determined by the ratio of X chromosomes to autosomes. A key gene involved in this pathway, which leads from chromosome ratio to sexual differentiation, is *Sex-lethal* (*Sxl* ) (reviewed in Parkhurst and Meneely 1994). This gene plays an important role in dosage compensation, germ-line sexual differentiation, and somatic sexual differentiation. *Sxl* is initially transcribed only in female embryos, but is later transcribed in both sexes. Active SXL product is required for the correct splicing of *Sxl* transcripts in females, male transcripts contain an extra exon (exon 3) which contains two termination codons, prematurely terminating translation. This leads to a truncated non-functional product in males.

The primary signal of sexual determination differs in *Lucilia cuprina* . It depends on the presence or absence of a Y chromosome (Bedo and Foster.1985). Due to the key nature of the *Sxl* gene in *D. melanogaster*, it was thought a homologue might be found in *L. cuprina*. Using degenerate PCR primers, a region of the *L. cuprina Sxl* RNA binding domain was amplified and used to probe a pupal *L. cuprina* library. Three clones were recovered. These clones have been sequenced, and show high homology to different transcript types of *D. melanogaster Sxl*. An early embryonic library was probed, hoping to find early *Sxl* transcript types as seen in *D. melanogaster*. Although positively hybridising clones were recovered, these were similar to those seen for the later stages of development. Northern analysis indicates no difference in transcript sizes visible between the sexes. Reverse transcriptase PCR shows no difference between the sexes for the exon2-5 region between males and females in *L. cuprina*.

These findings show that the *Sxl* product is highly conserved between *L. cuprina* and *D. melanogaster*, although no male type transcripts from *D. melanogaster* have been identified in *L. cuprina*. The role *Sxl* plays in the development of *L. cuprina* will be the subject of future studies.

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Parkhurst, S. M. & Meneely, P. M. (1994). Sex determination and dosage compensation: lessons from flies and worms. Science 264: 924-932

# THE HYBRID EXCISION-INSERTION MODEL FOR P ELEMENTS: PRODUCTION OF RECOMBINANTS AND DUPLICATIONS/DELETIONS

Yasmine Gray, Mark Tanaka and John Sved; School of Biological Sciences, A12; University of Sydney; Sydney, NSW 2006

P elements are known to induce male recombination in *Drosophila melanogaster*. In order to study the mechanisms by which this recombination is induced, we have used a stable P element transposase source, P[Δ2-3], to activate a non-autonomous P element, P[CaSpeR], and its derivatives. Using this system, we have established that a single P[CaSpeR] element in the presence of P[Δ2-3] can induce male recombination frequencies of approximately 1%. When two P[CaSpeR] elements are located at the same location on homologous chromosomes in the presence of P[Δ2-3] the recombination frequency is about 20%.

We have identified derivatives of the P[CaSpeR] element in which either the left or right end is deficient. When a single end-deficient element is combined with P[Δ2-3], or when two same-end-deficient elements located at the same location on homologous chromosomes are in the presence of P[Δ2-3], recombination frequencies drop to below 0.2%. However, when opposite-end-deficient element located at the same site on homologous chromosomes are combined with P[Δ2-3] male recombination is observed at levels of 30% and greater. Therefore, left-end-deficient and right-end deficient elements are complementary.

We propose a model for P element-induced male recombination based on our findings in which opposite P element ends on separate chromosomes combine as a 'hybrid' P element and undergo a pseudo-excision, freeing the two P element ends. However, unlike normal P element excision, the elements' sequences are still covalently linked to their respective chromosome arms by their deficient ends. This hybrid element then undergoes a pseudo-insertion event, in which several outcomes are possible. This model specifically predicts recombination events as well as duplication, deletion and inversion events. We have found examples of each of these chromosomal rearrangements as well as the predicted 8bp target site duplication characteristic of P element insertion events.

The model can be extrapolated to explain male recombination in the case of a single complete P[CaSpeR] element, as well as two homologous P[CaSpeR] elements, in the presence of P[Δ2-3]. In addition, since the empirical findings are in agreement with the model of hybrid excision-insertion, this aberrant transposition may account for many of the various phenomena associated with P element activity, including hybrid dysgenesis. This model is also strikingly similar to recent work on other transposable elements, namely Ac/Ds and Tam3 and could represent a general transposition mechanism among transposable elements.

# Cloning and Characterizing the Marsupial *XPCT* Gene: A Marker for the Human X Inactivation Centre.

**Matthew J. Wakefield and J. A. M. Graves**
**School of Genetics and Human Variation, La Trobe University, Kingsbury Drive, Bundoora, Vic, 3083.**
**Ph 03 479 2770 email genmjw@genome.latrobe.edu.au**

X Chromosome inactivation in eutherians has long been established to be controlled by a *cis* acting site known as the X inactivation centre (*xic*). A candidate gene for the controlling element, *Xist*, has been cloned from Human and Mouse. This gene has unusual properties as it is transcribed only from the inactive X chromosome, encodes for a large RNA molecule with no ORF or protein product, and the RNA is localized within the X chromosome to the inactive X.[1,2] We are interested in the evolution of the X inactivation centre, in particular its relationship with differences between Eutherian and Marsupial X inactivation. Marsupial X inactivation differs from Eutherian X inactivation in that the Paternal X is always inactivated and the pattern of X inactivation is tissue specific.[3] We hypothesise that these differences may reflect differences in, or the absence of, a Marsupial *Xist* homologue. We have therefore set about cloning marsupial homologues and analogues of the *Xist* gene. Despite using several screening approaches including PCR and hybridization of cloned and oligonucleotide probes to genomic and cDNA libraries we have been unable to detect any homologues to date. In order to complement this approach we have begun cloning and mapping genes that flank *Xist* in eutherian species. The first of these genes, *SCL16A2*, has been cloned from Tammar Wallaby and localized to the X chromosome by southern blotting with a Wallaroo X only hybrid panel. This gene, also known as *DSX128E* and *XPCT*, is located at the distal boundary of the Human X inactivation centre region (determined by chromosome breakpoints) and is less than 600Kb from *Xist*.[4,5] This gene will provide a valuable marker for F.I.S.H. to determine the position of genes from the Human X inactivation centre relative to genes known to undergo tissue specific inactivation, and also to the hypothesised Marsupial X inactivation centre[6]

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# Angiotensinogen Genotypes in Pre-eclampsia/Eclampsia (PE/E)

**Guanglan Guo and Alan N. Wilton**
**School of Biochemistry and Molecular Genetics**
**The University of New South Wales, Sydney NSW 2052**

Pre-eclampsia/eclampsia (PE/E) is a common disease of human pregnancy with 1-5% incidence in the most parts of the world. It is characterised clinically by high blood pressure, proteinuria and oedema. Symptoms develop after 20th week of pregnancy and resolve following pregnancy termination. The aetiology of PE/E is not clear. Epidemiological studies show that the cause of PE/E is largely genetic. Women whose sister and/or mother have been affected are at much greater risk of the disease than women with no family history of the PE/E. One popular model is that a defective interaction between endothelium and placenta is the cause of PE/E. In normal pregnancy the foetal trophoblast cells invade the maternal spiral arteries near the placenta which leads to enlargement and increase in blood supply. This does not occur sufficiently in PE. As a result, the placenta become ischaemic in the second half of pregnancy. Placental ischaemia causes damage to the maternal vascular endothelium resulting endothelial dysfunction. The pathological changes in PE/E involve liver, kidney, heart, brain and the coagulation system. It is a multi system and multi factorial syndrome.

Recently, two studies suggested that the susceptibility gene for PE/E is associated with a molecular variant at the angiotensinogen (AGT) locus. AGT is precursor protein to AGT II, which plays a primary role in the regulation of blood pressure by renin-angiotensin system. Hypertension is a late, secondary event in PE/E pathological sequence. Therefore, AGT is unlikely to be the gene responsible for PE/E. To test this we have typed 16 PE/E families for microsatellite markers from the AGT region and we are typing common variation in AGT for linkage analysis of PE/E as well as examining frequencies of affected and control samples.

## Genetic Differentiation among Populations of *Caridina zebra* (Decapoda: Atyidae) in a Queensland Rainforest.

Jane M. Hughes, Stuart E. Bunn & David A. Hurwood.
Co-operative Research Centre for Tropical Rainforest Ecology and Management
Faculty of Environmental Sciences
Griffith University, Nathan.

The role of dispersal in the genetic structuring of populations is widely accepted. The greater the extent of dispersal between populations, the more homogeneous they will be. Restricted dispersal results in effective isolation and leads to genetic differentiation between populations. The aims of this study were firstly to determine the extent of genetic differentiation among populations of the freshwater shrimp Caridina zebra within the Tully River drainage using allozyme electrophoresis, and secondly to examine genetic differentiation among populations from the Tully and Herbert River drainages that are geographically close but separated by considerable stream distance. The results indicated that most of the sites sampled in the Tully River showed a high degree of differentiation suggesting that there is little gene flow among populations. Sites that shared a low altitude confluence displayed levels of differentiation an order of magnitude higher than those with a high altitude confluence. There was a. relatively low amount of differentiation between certain sites from the Tully and Herbert River that were geographically close. One hypothesis suggested for these data is that the Tully River captured the headwater stream of the Herbert River.

**SOMATIC MUTATIONS IN THE RET PROTO-ONCOGENE IN MEDULLARY THYROID CARCINOMA**

**Debbie J. Marsh[1,3], Diana L. Learoyd[1,2,3], Scott D. Andrew[1] and Bruce G. Robinson[1,2,3].**

**[1]Molecular Genetics Unit, Kolling Institute of Medical Research, [2]Department of Endocrinology, Royal North Shore Hospital, St Leonards 2065, Sydney, NSW, Australia and [3]University of Sydney, Sydney, NSW, Australia.**

Medullary thyroid carcinoma (MTC) represents 5% of all human thyroid cancer. It occurs in both familial and sporadic forms at the frequencies of 25% and 75% respectively. In the familial form, MTC occurs as the main symptom in three syndromes: familial medullary thyroid carcinoma (FMTC) where it occurs alone, multiple endocrine neoplasia type 2A (MEN 2A) where it occurs in conjunction with phaeochromocytomas and sometimes hyperparathyroidism, and multiple endocrine neoplasia type 2B (MEN 2B) where in addition to the symptoms of MEN 2A, mucosal neuromas and a Marfanoid habitus are present. Germline mutations have been identified in exons 10, 11, 13 and 16 of the RET proto-oncogene segregating with the disease phenotype in MEN 2A, MEN 2B and FMTC. Somatic mutations in the RET proto-oncogene have also been identified in sporadic MTC. We have examined paraffin-embedded and frozen tissue from clinically sporadic MTCs for the presence of somatic mutations in the RET proto-oncogene. The mutation at codon 918ATG->ACG was found in 21/32 (69%) of MTCs. The mutation at codon 883GCT->TTT was found in 1/32 (3%) of MTCs. Ten MTCs did not have a mutation in codons 768, 883 or 918 of the RET proto-oncogene. Detection of the codon 918 mutation has been improved by the design of a new primer which introduces a restriction site for *RsaI* in the presence of the mutation. The presence or absence of the somatic mutation at codon 918 did not correlate clinically with: age at diagnosis, tumour size, presence or absence of metastases or MTC related morbidity. Patients with codon 918 mutation positive tumours had higher calcitonin levels at diagnosis ($p=0.03$) than patients without this mutation. The presence of a somatic mutation in the RET proto-oncogene in association with the absence of previously described germline mutations is highly suggestive of sporadic MTC.

# RESPONSE OF HEAT SHOCK GENES TO SELECTION FOR HEAT RESISTANCE IN *DROSOPHILA MELANOGASTER.*

Gawain M^cColl, Ary A. Hoffmann[1] and Stephen W. M^cKechnie

**Department of Genetics and Developmental Biology, Monash University, Clayton 3168, Victoria. [1]School of Genetics and Human Variation, La Trobe University, Bundoora 3083, Victoria.**

Replicate outbred lines of *Drosophila melanogaster* have been selected for their resistance to knockdown by a 37°C heat stress. Two selective regimes were initiated, one incorporating a mild acclimation treatment prior to each round of selection, the other without. Two stress gene candidate loci, heat shock RNA-omega (*hsr*-ω) and heat shock protein 68 (*hsp68*) were surveyed for polymorphism by the application of denaturing gradient gel electrophoresis to DNA fragments amplified from these genes for the control and selected lines. Samples of allele frequencies from both loci showed associations with the selection treatment that included an acclimating heat dose. The results suggest that adaptive molecular variation at these two loci contributes to the natural heritable variation in heat resistance that occurs in this species.

# Is parental genetic diversity a useful predictor of sugarcane cross performance?

C. Lynne McIntyre[1], Pascale Besse[1], Guillermo Galvez[2] and Phillip A. Jackson[3].

[1]CSIRO Division of Tropical Crops and Pastures, 306 Carmody Rd, St Lucia, 4067
[2]Sugar Ministry, Calle 23 No. 171, Vedado, Havana, Cuba.
[3]CSIRO Division of Tropical Crops and Pastures, Davies Laboratory, Townsville, 4810, and, CSR Technical Field Department, Macknade, 4850.

Variation in performance among crosses may be partitioned into that due to differences in general combining ability (gca) of the parents and that due to differences in specific combining ability (sca) among the pairs of parents combining to produce each cross. In sugarcane, variation due to specific combining ability is large and sca is difficult to predict. Apart from a general observation that parents that are closely related to each other show inbreeding depression, there is no other basis for predicting sca. The approach used by sugarcane breeders is, therefore, to try a large number of crosses in an attempt to find the rare good ones.

Molecular markers have been suggested as a means of predicting hybrid performance based on marker dissimilarity between parents. The approach has been attempted in several plant species, including rice, wheat, maize, and alfalfa, with mixed results. This project is attempting to use molecular markers to predict hybrid performance in sugarcane, to see if there is any association between genetic dissimilarity of pairs of parents and sca effects of crosses made with those parents.

Forty-two sugarcane clones utilised in the CSR breeding program were studied. Crosses incorporating these clones had previously been assessed for CCS (commercial cane sugar), cane yield and sugar yield. Levels of genetic diversity were determined for the 42 clones using 20 RAPD primers which generated approximately 100 RAPD bands. The correlation between diversity and performance was calculated and the implication of these results is discussed.

# NATURAL ALLELIC VARIATION IN A SODIUM CHANNEL GENE FROM *Helicoverpa armigera* DETECTED BY TEMPERATURE GRADIENT GEL ELECTROPHORESIS

**Stephen W. M<sup>c</sup>Kechnie, Nicola H. Stokes and [1]Neil W. Forrester**

**Department of Genetics and Developmental Biology, Monash University, Clayton, Vic, 3168 and [1]NSW Agriculture, Australian Cotton Research Institute, Narrabri, NSW, 2390, Australia**

*Helicoverpa armigera*, the cotton budworm, is a significant pest of Australian field and vegetable crops, and has a widespread incidence of resistance to pyrethroid insecticides. Here we report the development of an assay for detecting in *H. armigera* naturally occurring variation in a sodium channel gene. This gene is a suspected candidate locus for pyrethroid resistance in a related American pest *Heliothis virescens*. The frequency of molecular variations in this gene has also shown marked geographical discontinuity in *H. virescens* (M.Taylor, pers. comm.), suggesting that it may be a useful marker for studies of migration.

Eggs were collected from field sites in the Namoi valley, NSW, and St George, QLD, in February 1993, reared through to 30-40 mg 3rd/4th instar larvae, and subsamples from each site treated with a discriminating dose of the pyrethroid fenvalerate. Survival to the adult stage among treated individuals was 40% for the Namoi sample and 80% for the St George sample, and 100% for the untreated control groups. DNA preparations from adult survivors of the treated and control groups from both locations were used as template to amplify a sequence of about 890 bp from the sodium channel gene using primer oligonuleotides derived from the sequence of the *H. virescens* para Na channel gene. This fragment was cut into four with the restriction enzyme HpaII and the digest run on an ultrathin temperature-gradient electrophoretic gel. Allelic variation was detected by differential migration distances that resulted from melting point difference in the 3', ~250 bp, end fragment.

At least 16 alleles were detected, each unknown having been run adjacent to four 'standard' individuals chosen to represent 8 different alleles that evenly spanned the observed range of migration distances. Conspicuous homogeneity between fenvalerate treated and control groups suggests that the detected variation in this gene is not associated with any genetic basis to variation in resistance to fenvalerate. However significant heterogeneity in allelic frequencies occurred between the samples from Namoi and St George, suggesting that this may be a useful marker in future studies, when used in combination with other genetically independent markers, for detecting and quantifying migration levels and identifying the origins of these vagile pest invaders.

# Genetic Variation in *Aspergillus fumigatus* Strains Isolated from Ostriches

**Martin McLoon, Brian F. Cheetham and Margaret E. Katz**
**Department of Biochemistry, Microbiology and Nutrition,**
**University of New England, Armidale, N.S.W. 2351.**

Aspergillosis is reputed to be the cause of serious losses to the ostrich farming industry in Australia. However, very little is known about the prevalence or mode of transmission of aspergillosis infections in ostriches. Diagnosis of the disease is difficult and treatment of advanced cases is usually unsuccessful. *Aspergillus fumigatus* is the organism which is most frequently associated with aspergillosis in birds and humans though other *Aspergillus* species are occasionally found in infected individuals. It has been reported that environmental isolates of *A. fumigatus* may not be as virulent in birds as isolates from infected birds. This suggests that not all strains of *A. fumigatus* are virulent and that infections may be spread by direct contact with infected birds.

We have developed of a method to detect DNA from *Aspergillus fumigatus* using the polymerase chain reaction (PCR). We chose to use primers which would amplify a fragment from a single copy *A. fumigatus* gene which is similar to an *A. nidulans* gene which we have isolated. We tested the method on *A. fumigatus* isolates from infected ostriches in five states and we showed that the test was specific for *A. fumigatus.* One isolate (TF1) consistently failed to yield an amplification product. All of the isolates except TF1 were obtained from ostriches which have died of invasive pulmonary aspergillosis. TF1 was isolated from an ostrich which was successfully treated with anti-fungal drugs. Southern blot analysis showed that TF1 does contain the target gene but the size of the fragment which contains these sequences is different indicating that the DNA sequence of the TF1 alkaline protease gene differs from that of the other strains Six of the eight isolates which were analysed using Southern blots showed an identical pattern. In one case, isolates from two different ostriches in the same flock were different, which suggests that infection with *A. fumigatus* may not be the result of transmission between individuals.

Considerable genetic variation in the alkaline protease gene may exist among environmental isolates of *A. fumigatus.* If this is true, it may be significant that most of the isolates that we have analysed were identical. Sequence analysis of the alkaline protease gene of TF1 is in progress. The expression of the gene will also be studied to determine whether the variation which has been observed affects gene function.

# POPULATION SIZE AND GENETIC VARIATION

ME. Montgomery, LM. Woodworth, RK. Nurthen, DA. Briscoe and R. Frankham

School of Biological Sciences, Macquarie University, NSW 2109

As biodiversity is lost due to pollution, competition/predation by introduced species and hunting, more and more species are becoming threatened, endangered or possibly even extinct in the wild. The small, isolated populations that remain become susceptible to inbreeding depression and suffer a loss of genetic variation. Genetic variation is the raw material for adaptation to a changing environment. Thus in order to preserve a species for long term conservation it is necessary to maintain genetic variation.

The genetic variation in a population is expected to decline over time in proportion to the effective population size and the initial amount of heterozygosity within the population. This prediction was evaluated by measuring the changes in allozyme genetic variation in populations of *Drosophila melanogaster* maintained for 50 generations with effective population sizes of 25, 50, 100, 250 and 500. Our findings did not differ significantly from the predictions. Consequently, population size is expected to be a major factor determining levels of genetic variation in wildlife.

A literature survey revealed that in 11/12 studies in other species population size has a positive correlation with heterozygosity. Also, heterozygosity is lower in island populations than mainland populations of vertebrates. In plants the major variable explaining differences in heterozygosity is geographical range, which correlates with population size. Further, genetic variation is highest in plants, followed by invertebrates then vertebrates, this also correlates with population size. Thus, there is overwhelming evidence that population size is a major factor determining level of allozyme genetic variation in wildlife.

# Chaperonin 10: an Evolutionarily Conserved Multigene Family?

**Murphy R.M[1], Webb G.C[2], Peters G.B[2], Cassady A.I[3], Cavanagh A.C[1] and Summers K.M[1]**

**[1]Department of Surgery, University of Queensland, Royal Brisbane Hospital, Brisbane, Queensland**
**[2]Queen Elizabeth Hospital, Woodville, South Australia**
**[3]Centre for Molecular and Cellular Biology, University of Queensland, Brisbane, Queensland**

Chaperonin 10 (cpn10) is a heat shock protein which functions as a molecular chaperone. This protein has been identified in a multitude of bacteria, as well as yeast and a number of higher eukaryotes. Amongst higher eukaryotes, the degree of protein conservation (95-100%) is reflected in nucleotide conservation. A cDNA clone of human cpn10 was used to probe a blot of DNA from eight unrelated individuals, digested with a panel of restriction enzymes. At least nine bands of varying intensity were seen, suggesting that there is more than one sequence homologous to cpn10 in the human genome. In situ hybridisation to human metaphase chromosomes was used to localise the sequences to chromosomes 1, 2, 12, 14 and 16. Further investigation of these sequences in humans and other mammals was undertaken.

PCR amplification of human genomic DNA, using human cpn10-specific primers that encompass the open reading frame, resulted in detection of three bands, one of the expected size and two larger bands. Hybridisation of this gel with a cpn10-specific probe, as well as sequencing of PCR products, showed that there were many related sequences. Characterisation of two products has been completed and is continuing for the other PCR clones. A 600 bp product was found to result from an Alu sequence insertion within the open reading frame. A 350 bp product was characterised by the presence of a single base deletion resulting in the introduction of a new restriction site and a near perfect 30 bp direct repeat. It seemed likely that these products were amplified from pseudogenes as the putative translation products are truncated and have low amino acid sequence homology with cpn10. However RT-PCR of total RNA, isolated from rat and mouse, showed that the 350 bp product was transcribed in a variety of cell types. A cDNA clone isolated from a mouse bone marrow-derived macrophage cDNA library is the homologue of the human 350 bp PCR product, with considerable sequence homology, including the single base deletion which would result in premature termination of the protein.

These results show that cpn10, like other heat shock proteins, is a member of a large gene family which is highly conserved in mammalian species. Several cpn10 transcripts have been detected, suggesting that more than one of the genes are functional. However the translated proteins may be very different due to conserved mutations which alter the reading frame and result in truncated proteins. Further study of this interesting gene family will permit understanding of the functioning of proteins of this family.

**Exopolysacchardies play a role in biological control protection of *Pseudomonas* bacteria against the take-all fungal root pathogen of wheat.**

**K.A.E. Groom and M. Nayudu**

**Division of Botany & Zoology, Faculty of Science, Australian National University, Canberra, ACT 0200.**

The non-fluorescent *Pseudomonas* biological control strain, AN5, protects wheat against the "take-all" fungus, *Gaeumannomyces graminis* var. *tritici* (Nayudu et al., 1994). Two mucoidy mutants of strain AN5 that produce excess exopolysaccharide (EPS) were isolated from Tn5-*gus* transposon mutagenesis of AN5 (4.ZMuc, slightly more EPS; 4.ZMuc$^{+++}$, extreme overproducer of EPS derived from 4.ZMuc).

This excess EPS production was a consistent phenotype on different media with a diverse range of carbon sources. An assay of disease protection provided by the EPS mutants, through pot trials and agar bioassays, found that the mutants exhibited significantly reduced biological control against the take-all fungus when compared to the parent AN5. Factors thought to be important in the biological control of the take-all fungus by AN5 (eg. Growth rate or root colonisation ability) were shown not to be altered in these EPS mutants.

Molecular genetic analysis of the EPS mutants, found that two transposons were inserted in the genomes of each of the EPS mutants. From genetic analysis of these two regions of transposon insertion, through cloning out the regions from a cosmid bank, and then transferring them into the parent strain AN5 we have demonstrated that only one insertion site is responsible for the mucoidy phenotype. The results are consistent with a point mutation at this site in 4.ZMuc, and in 4.ZMuc$^{+++}$ there has been a deletion of an adjacent region next to the same insertion site. Wildtype DNA regions corresponding to the mutated region in these EPS mutants have been isolated and when transferred back into the parent AN5 reduce EPS production. These results are consistent with a negative regulatory model for EPS production, also proposed in closely related bacterial species. A model has been proposed to explain how excess EPS reduces the quantity of antibiotics that is released to the external environment, leading to a decrease in biological control production.

Reference

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**How *Pseudomonas* bacteria colonize the roots of wheat plants.**

**K.A.E. Groom and M. Nayudu**

**Division of Botany & Zoology, Faculty of Science, Australian National University, Canberra, ACT 0200.**

Some soil bacteria have the ability to colonize the roots of plants. Specific bacterial species such as *Pseudomonas* have been known for their ability to inhibit root pathogens such as *Gaeumannomyces graminis* var. *tritici* or the "take-all" pathogen. This has lead to the bacteria being able to significantly protect (called biological control protection) against the take-all disease, caused by this pathogen. We have also observed other bacterial species, from the *Bacillus* genera, are capable of biological control protection against the take-all pathogen. An essential part of this protection is dependent on the bacteria being able to colonize wheat roots. Although extensive work has been done on the effect of the take-all pathogen on wheat roots (Foster, *et al.*, 1983), very little is known about how biological control bacteria interact with wheat roots. In this study we have taken a sample of different biological control bacteria and by scanning electron microscopy tried to determine how they interact with wheat roots.

All biological control bacteria induce a specific response on wheat roots. *Pseudomonas* strain AN5 is found on the surface of the root where they degrade the outer surface of the root. Degradation is very localised next to the bacteria and may be due to the release of extracellular enzymes. This probably aids in the invasion of the epidermal layer of the root surface. Bacteria were observed inside the root epidermis. This effect was shown to be as a result of the inoculated bacterial strain using the spot inoculation method of Turgeon and Bauer(1983). Most *Pseudomonas* and *Bacillus* species tested have the same response describes for strain AN5. However one strain *Pseudomonas* strain Pf5 colonizes wheat roots in a similar fashion except that colonization occurs in distinctive clumps and not all over the roots, as for strain AN5.

Root colonization of wheat by bacteria is a specific response that can be characterised, and also host specific in nature. The specific nature of bacterial root colonization suggests host bacterial genes are involved.

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**Antibiosis is the key element in *Pseudomonas* bacteria's biological control protection against the take-all fungal root pathogen of wheat.**

**K. Turnbull and M. Nayudu**

**Division of Botany & Zoology, Faculty of Science, Australian National University, Canberra, ACT 0200.**

"Take-All" is a fungal root rotting disease of wheat which causes significant yield losses. *Pseudomonas* strain AN5 produce metabolites that are inhibitory to the growth of the take-All fungal pathogen *Gaeumannomyces graminis* var. *tritici* . To elucidate the role of antibiosis in biological control protection strain AN5 was mutated with two types of Tn5 -gus transposons. Over 10,000 mutants were generated and screened for antibiosis using a Potato Dextrose Agar (PDA) overlay assay. There were five class of antibiosis deficient mutants isolated. Only one class of mutants were not pleiotrophic in nature and thus were considered to be directly involved in antibiosis (Nayudu *et al.*, 1994).

Three bacterial mutants were isolated that were unable to inhibit the growth of the take-all fungus in agar plate bioassays. Investigation of these mutants showed that there are two regions in the genome that are important in antibiotic production. In particular, a single mutant, -7.37, was critically analysed to elucidate the exact role of antibiosis in biological control. The wild-type, AN5, and the antibiosis deficient mutant, -7.37, did not differ significantly in growth rate, or in their ability to colonise wheat roots. Furthermore -7.37 was not a significantly poorer competitor, compared to the wild-type, in mixed strain competition experiments. This suggests that the main difference between the strains was the inability of -7.37 to produce antibiotics. Glasshouse-based pot trials confirmed that the antibiosis-negative strain -7.37 could not protect wheat plants from infection by the take-All pathogen. Biological control protection on plates and in glasshouse experiments was restored to -7.37 by the transfer of a cosmid containing the corresponding complementary wild-type region of the parent strain AN5. The antibiosis mutants isolated still produced other metabolites such as Hydrogen Cyanide. This suggests that these mutations are not in a previously identified global regulatory region such as *gac* A (Laville *et al.*, 1992), but directly in a gene involved in structural antibiotic production. Two essential genomic DNA regions for antibiosis have been isolated and the DNA sequence of antibiosis genes are being determined. The significance of antibiosis mutants in biological control protection will be discussed.

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**Bioluminescence (using luciferase or *lux* ) a unique and cheap marker for detection of biological control *Pseudomonas* bacteria from the plant rhizosphere.**

**M.Nayudu, T. Murphy, P.T.W.Wong[1] and J. Ash.**

**Division of Botany & Zoology, Faculty of Science, Australian National University, Canberra, ACT 0200; [1]Biological & Chemical Research Institute, NSW Department of Agriculture, Rydalmere.**

We have isolated a non-fluorescent *Pseudomonas* species (strain AN5) from soils in the Cowra region of central New South Wales in Australia. This strain AN5 is able to effectively protect wheat against the take-all fungal pathogen, *Gaeumannomyces graminis* var *tritici*, in pot experiments and under controlled environment glasshouse conditions. It was also able to significantly increase wheat yield in biological control protection against take-all in field trials at dryland sites, where most wheat is grown in Australia. As a prelude to the introduction of genetically engineered *Pseudomonas* strain AN5 into the field (GEMS) we had to determine its effective colonization ability on a range of plant hosts, including native Australian species.

To test whether bioluminescence can be used as an effective and reliable marker for this purpose we used the transposon Tn*4431* which contains the promotorless *lux CDABE* cassette (Shaw *et al.*, 1987). We selected an insert of this transposon into the genome of *Pseudomonas* strain AN5 which generated strong light production, presumably from a native promotor. *Pseudomonas* strain AN5::Tn*4431* was selected and shown to colonize wheat as effectively as the parent and the insert shown to be relatively stable when selection was not imposed. This strain was used for further colonization experiments.

A number of plant species were selected for colonization experiments using cladistic analysis of their relative genetic distance from wheat. A cladogram was generated and all the plant species in this were tested for colonization using the bioluminescent *Pseudomonas* strain AN5. A suprising result was that this strain strongly colonizes a number of plants unrelated to wheat such as *Casuarina*. There were some significant differences observed in the case of plants such as *Persea gratissima* between washed and unwashed isolations from root samples. This suggested multiplication of the bacteria in the root vicinity and not colonization of the roots of these plants. This may be due to the ability of this strain to utilise carbon compounds released in the exudate of these plant roots. In conclusion the luciferase system is a cheap and specific method for identification of soil bacteria and in these experiments has been used to show that biological control *Pseudomonas* strain AN5 has a broad host range for root colonization.

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**The Molecular Evolution of SRY: An Analysis of Reproductive Isolation and Haldane's Rule in Rock Wallabies (*Petrogale*)**

Rachel J. Waugh O'Neill, R.H. Crozier and J.A.M. Graves
**Department of Genetics and Human Variation, La Trobe University, Bundoora VIC 3083**

Haldane's Rule states that in hybrid zones between closely related species, it is most often the heterogametic sex which is infertile or inviable (Haldane, 1932). This infertility/inviability may be involved in reproductive isolation leading to speciation. To date, none of the genes involved in this phenomenon have been identified. It has been suggested by Whitfield and others (1993) that the mammalian sex determining gene, SRY, could play a major role in reproductive isolation. The SRY gene encodes a protein with a domain known as the HMG box (Sinclair, 1990). Although the HMG box is highly conserved among mammals, sequences flanking the box have been shown to be evolving extremely rapidly, possibly due to positive directional selection (Whitfield, 1993). We are examining the relative rates of evolution of SRY flanking sequencing in a group of rapidly speciating mammals, rock wallabies (*Petrogale*), to determine whether there may be a correlation between SRY sequence divergence and hybrid fertility/viability between different species and subspecies. Sequence analysis has shown that SRY is not species specific in *Petrogale* and phylogenetic trees produced in this study contain nonsignificant bootstrap values due to the low number of informative sites. These results indicate SRY is probably not involved in reproductive isolation in rock wallabies.

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**The *hapC* gene of *Aspergillus nidulans*, a homologue of the yeast *HAP3* gene.**

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

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

DNA binding factors that recognise the core sequence CCAAT have been found in many eukaryotic systems and regulate a number of processes, ranging from respiration to immunoglobulin production. The 5' regulatory region of the *amdS* gene of *Aspergillus nidulans* contains a CCAAT sequence which is required for setting the basal level of *amdS* transcription. Mobility shift studies have identified a factor in *A. nidulans* nuclear extracts which binds to this CCAAT sequence which has been named *A. nidulans* CCAAT binding Factor (AnCF).

In *Saccharomyces cerevisiae* the *HAP3* gene encodes one component of a multisubunit complex which binds CCAAT sequences. Genes with homology to *HAP3* have been isolated from a number of species. A search of the Genbank database has found an *A. nidulans* sequence with significant homology to the *HAP3* gene adjacent to the previously cloned regulatory gene *amdR*. Sequencing of the remainder of this region has revealed a gene with extensive homology to *HAP3*. This gene has been named *hapC*. *hapC* cDNA clones have been isolated and comparison of cDNA and genomic sequence has revealed three introns in the coding region of *hapC* and one in the 5' untranslated region. A haploid carrying a *hapC* gene disruption has been created and is viable. A gene disruption has also been created in a diploid strain. This diploid was haploidised, but no haploid sectors carrying the gene disruption were observed. This phenomenon can be reversed by providing a complete copy of the *hapC* gene in *trans*. We are attempting to determine whether *hapC* is a component of AnCF.

**The Systematics and Hybridisation of two Species of Waratah, *Telopea mongaensis* and *T. oreades*.**

**A report from Honours thesis (Parrish, 1994) and current research.**

**Tracey Parrish**
**Dr Mike Crisp**
**Division of Botany and Zoology,**
**Australian National University**
**Canberra, 0200**

The status of *Telopea mongaensis* as a species distinct from *T. oreades* has been questioned. The species are identifiable only through continuous vegetative characters. Hence, variation observed in the field may be due to environmental parameters. This project aims to determine whether the two species are indeed distinct, using both morphometric and RAPD data. Hybridisation between the species is being examined.

Morphometric analysis of glasshouse grown material has shown that *Telopea mongaensis* occupies a multivariate space discrete from that of *T. oreades* ; hence characterising the taxon as a distinct species. RAPD analysis, in support of morphometric findings, has identified two fixed and five polymorphic markers which distinguish between the species by grouping them into discrete clusters.

Hybridisation at seed set has been detected by morphometric analysis of a population where the species grow in sympatry. Previously a single intermediate adult has been identified at this site (Crisp & Weston, 1993). Current research is using RAPD markers to further examine the extent of hybridisation at the seed set and adult level.

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Parrish, T L, 1994 *The Systematics of Two Species of Waratah,* Telopea mongaensis *F. Muell. and* T. oreades *Cheel (Proteaceae)*. Honours Thesis, Australian National University.

# Analysis of genetic variation within and among varieties of the outcrossing species *Lolium perenne* using RAPD

**Matthew K. Morell[1,2,3], Rod Peakall[1,4], Lynette R. Preston[1], Katrina Moore[1] and Rudi Appels[1,2]**

**[1]CRC for Plant Science, PO Box 475, Canberra, ACT 2601, [2]CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, [3]Directors Unit, Research School of Biological Sciences, ANU, PO Box 475, Canberra, ACT 2601, [4]Division of Botany and Zoology, ANU, Canberra, ACT 0200**

DNA-based genetic markers for plant species are increasingly being used as tools in plant population biology, genome analysis and in plant breeding. DNA markers are also emerging as important tools in practical applications such as the description and identification of plant varieties for Plant Breeder's Rights and quality assurance in agriculture. Much of the research has concentrated on developing markers for the analysis of clonally propagated or selfing plant varieties. In this study, we sought to establish the feasibility of using DNA profiling to examine genetic variation both within, and among, varieties of the predominantly outcrossing pasture species, *Lolium perenne* (perennial ryegrass). The study involved the separate RAPD (Random Amplification of Polymorphic DNA) analysis of 17 individuals from each of 13 European and Australasian *L. perenne* varieties, using 3 oligonucleotide primers. Analysis of the pairwise genetic distances of these individuals showed that there was considerable clustering of individuals into varietal groupings. Further analysis using an Analysis of Molecular Variance (AMOVA) allowed the apportioning of genetic variance within and among varieties and calculation of average pairwise genetic distances for the varieties. Based on random permutations of the data set, all pairwise genetic distances among the varieties were significantly different from random expectations. The study demonstrates the feasibility of using DNA profiling to analyse genetic variation both within and among varieties of outcrossing plant species.

**Australian Aboriginal Study: Alpha-globin Haplotype Analysis Supports a Common and Ancient Origin with New Guinea Highlanders**

J.M.Roberts-Thomson*, J.Norwich#, J.J.Martinson#, R.M.Harding#, J.B.Clegg# and B.Boettcher*

*** Department of Biological Sciences, The University of Newcastle, NSW.**
**# MRC Molecular Haematology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, England.**

The relationship between Australian Aborigines and the peoples of New Guinea, SE Asia and the Pacific regions remains controversial. The α-globin haplotypes of members of a central Australian tribe were studied to address some of the ambiguities of previous studies concerning the origins of Australian Aborigines.

The composition of the haplotype repertoire of the Australian Aborigines is similar to that of New Guinea Highlanders and strongly supports a common origin of these two populations. The set of α-globin haplotypes is different to that seen in present-day SE Asians, and there is a lower genetic diversity. The presence of new haplotypes in the Australian aboriginal population, and the reduced genetic diversity suggests there was a population bottleneck around the time of the early colonisation of Sahul, with subsequent recovery of diversity. These conclusions contrast with some previous genetic studies which suggested links between Australian Aborigines and coastal New Guineans and SE Asians. The discrepancy between the studies may be due to more recent SE Asian admixture on the north coast of Australia.

## Genetic evolution at three levels in the Australian Onychophora.

D.M. Rowell[a], D.M. Gleeson[a], N.N. Tait[b], D.A.Briscoe[b] and A.V. Higgins[a]

[a] Division of Botany and Zoology, ANU ACT 0200
[b] Biological Sciences, Macquarie University, North Ryde 2109

Although the Australian Onychophora is morphologically conservative, genetic techniques indicate that it has undergone an extensive radiation. We now recognise 110 distinct species, which is more than the currently described fauna for the rest of the world.

Chromosome numbers range from 2n=16 to 2n=42, and sex determination involves an XY system in some taxa, but is nonchromosomal in others. Among some species groups karyotypic differences can be attributed to a small number of recognisable chromosomal rearrangements, while in others major restructuring of the karyotype has occurred such that no evolutionary affinities can be inferred.

Similarly, levels of electrophoretic divergence among taxa are high; fixed gene differences of 70 - 90% among 20 loci are common. Nevertheless, polymorphism within populations is low, and average heterozygosity usually <1%.

We have recently sequenced a section of the cytochrome oxidase gene for 22 onychophora taxa. As with the chromosomal and electrophoretic data, the sequence data indicate high levels of divergence, although clear affinities between New Zealand and Australian taxa are evident. On the basis of estimates for the fragmentation of Gondwana and through comparison with the diptera, we estimate that the groups we have analysed resulted from a radiation over 100 million years ago.

# A CONSEQUENCE OF ILLEGITIMATE RECOMBINATION OF LINE 1 ELEMENT IN MAMMALS.

A. Ruvinsky[a], I. Rogozin[b],
University of New England, Armidale, 2351 NSW, Australia[a]
Institute of Cytology & Genetics, Novosibirsk 90, Russia[b]

Investigation of the *D17Leh80* like loci in the mouse has shown, that there are at least three very similar sequences with the level of homology about 80%, two of which contain common open reading frame (ORF) . Two fragments within the ORF, one homologous to the LINE1 element and the other to the first intron of the $C_\varepsilon$ gene of mouse immunoglobulin, were observed (Filippov et al. 1992). The comparison of these sequences suggest that the ancestor molecule might have arisen through illegitimate recombination between a LINE1 and the $C_\varepsilon$ gene in very narrow zone of high homology. Computer analysis gives a highly confident prediction that the studied sequence is an internal exon. Both canonic acceptor and donor splicing sites surrounding this sequence have been recognised. Central part of the sequences are quite conservative and only a few synonymous substitutions have been observed. The distal parts have lost homology significantly. The ORF has a high homology to ORF2 of LINE1 which codes for a protein similar to reverse transcriptase and possibly having functional role. Several possible functional sites and transcriptional factors have been found. Genomic blot hybridisation of the DNAs from different mammalian species indicates that they usually have 1-3 fragments homologous to D17Leh80. Very likely that the ancestral sequence arose no later than 27-33 Myr and spread across mammalian species. The data available support the possibility of new functional exons arisen and their involvement in following genes duplications.

# ARE THICKHEADS MONOPHYLETIC?: EVIDENCE FROM CYTOCHROME *B*

**Ian Scott[ad], Les Christidis[b], Dick Schodde[c] & Mike Westerman[d]**

**[a] Eukaryote Chromosome Organisation, Research School of Biological Sciences, ANU**
**[b] Department of Ornithology, Museum of Victoria**
**[c] Australian National Wildlife Collection, CSIRO Division of Wildlife and Ecology**
**[d] School of Genetics and Human Variation, La Trobe University**

The resolution of taxonomic relationships among the flycatchers, robins and thickheads (whistlers, shrike-thrushes and shrike-tits) has proved difficult utilising traditional morphological methods. It has been suggested that the flycatchers, robins, monarchs, fantails, whistlers and shrike-thrushes should be segregated as a distinct family, the Pachycephalidae[1]. However, on the basis of DNA-DNA hybridisation studies[2,3] it was concluded that the fantails, flycatchers, monarchs and their allies comprised a monophyletic grouping (subfamily Dicrurinae), while the whistlers, shrike-thrushes, pitohuis, shrike-tits, along with the sittellas, comprised another (subfamily Pachycephalinae). Moreover, on the basis of these data a pattern of relationships was proposed such that the sittellas (tribe Neosittini) are the descendants of the earliest branch, and sister to the clade including the Pachycepalini (whistlers, shrike-thrushes and pitohuis) and the Falcunculini (shrike-tits and allies).

We present results obtained from analyses of partial sequences (924 bp) of the mitochondrial gene, cytochrome *b*. The results indicate that the cytochrome *b* data are not consistent with the pattern of relationships among the taxa proposed by Sibley and Ahlquist. Nor are the cytochrome *b* data observed consistent with the suggestion that the "pachycephaline" taxa are monophyletic with respect to the fantails and their allies. Indeed it would appear on the basis of evidence from cytochrome *b* that the thickheads (*sensu* Sibley & Ahlquist) are polyphyletic.

Although it would appear that cytochrome *b* provides limited resolution of relationships among the taxa we interpret low bootstrap values and short internodal distances to represent rapid cladogenesis. These conclusions are consistent with previous results obtained using cytochrome *b* to estimate phylogeny among corvine taxa[4].

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2. Sibley, C. & Ahlquist, J. 1982. *Emu* **82**: 199-202
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# YEAST AND HUMAN UBIQUITIN SPECIFIC-PROTEASES

**K.E.Sloper and R. Baker.**

**The John Curtin School of Medical Research, The Australian National University, Canberra, A.C.T. 2601.**

Ubiquitin specific-proteases (Ubps) are thiol proteases which act on both ubiquitin-precursor molecules and ubiquitin-protein conjugates as part of the ubiquitin-dependent proteolytic pathway. The cDNA clones of two putative human Ubps were identified by screening DNA databases for similarity to two conserved sequence domains (containing conserved cysteine and histidine residues) characteristic of yeast Ubps[1].

Sequence analysis of the first cDNA clone, HSA35AO5 identified it's partial nature and confirmed the presence of the complete cysteine-containing conserved Ubp domain, and an incomplete copy of the histidine-containing conserved Ubp domain.

Protein expression of HSA35AO5 as a GST fusion protein was attempted and the resulting cell extracts analysed for Ubiquitin-cleavage activity. This produced negative or inconclusive results which were attributed to the partial nature of this cDNA clone.

Attempts to identify a human cDNA library suitable for screening for a full length clone of HSA35AO5 by using Northern and Southern hybridisation analyses were repeatedly unsuccessful. Based on this lack of success, a Southern blot analyses of yeast DNA was conducted to examine the possibility that HSA35AO5 originated from yeast, not humans. Finding this to be the case, HSA35AO5 was renamed *UBP13*.

The search for the full length clone of HSA35AO5 continued, using a process known as integrative transformation. Due the lack of success using this process, an alternative approach to PCR amplify, subclone and sequence the clone was applied. The 3' end of the cDNA was cloned using this procedure. Recently the entire sequence of *UBP13* has been determined by the yeast chromosome II sequencing project.

Phenotypic assays conducted on HSA35AO5 (*Δubp13::URA3)* mutants found that the UBP13 gene was non-essential for viability and exhibited no strong phenotypes under conditions of environmental stress.

Northern hybridisation analyses has confirmed the human nature of the second clone, HSAAADQEI and a human testis cDNA library is currently being screened for the full length clone. Complete sequence analysis of the partial HSAAADQEI cDNA clone is also being conducted.

Future research is directed towards obtaining full length copies of both the UBP13/ HSA35AO5 and HSAAADQEI genes and characterisation of their protein products.

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## USE OF FISH TO INVESTIGATE 3 ONCOLOGY CASES WITH COMPLEX CYTOGENETIC FINDINGS.

**Robson L (1), Lukeis R (2), Suter M (3), Forsyth C (4), Tembe V (1), Smith A (1).**

1. Cytogenetics Unit, Children's Hospital, Camperdown 2. Kanematsu Research Laboratory, Royal Prince Alfred Hospital 3. Haematology Department, St. Vincent's Hospital 4. Dept. of Haematology, Royal Prince Alfred Hospital, Sydney.

Complex karyotypic changes in the bone marrow of leukaemic patients are frequently difficult to resolve. FISH with library, unique sequence and centromeric probes has the potential to elucidate the origin of these rearrangements.

Routine cytogenetics on specimens from 3 oncology patients revealed complex karyotypes. Case 1 (AML M4EO) had apparent loss of chromosomes 7,12 and 16, structural rearrangements involving chromosomes 5, 8 and 13 and a marker (?16) chromosome. Case 2 (AML) had metacentric marker (? an isochromosome of 7q) and Case 3 (Ewings sarcoma) had a translocation between 3 chromosomes - t(11;22;13). Probes used included libraries of chromosomes 5,7, 8, 11, 12, 13, 16, 20 and X as well as unique sequence 7cen/tel and D22S75/S39. A total of 14 hybridisations was performed.

With FISH, the results in each case provided a different interpretation. In case 1, the marker was derived from chromosome 7 and multiple cell lines with numerous other rearrangements were detected; in case 2 the "isochromosome" was a translocation involving chromosome 7; in case 3 the rearrangement was more complicated than originally thought with 2 separate breakage and reunion events occurring sequentially.

The results obtained by FISH could not have been arrived at by routine cytogenetics and the abnormalities detected are not part of the published specific rearrangements in the different leukaemic subtypes.

**UTILITY OF FLUORESCENCE in situ HYBRIDISATION (FISH) IN THE EVALUATION OF THE CHROMOSOME 15(q11-13) REGION.**

**Smith A, Robson L. Cytogenetics Unit, Children's Hospital, Camperdown, NSW 2050, Australia.**

Detecting deletions in chromosome 15(q11-13) may be undertaken by different laboratory procedures. We commenced FISH in 1991 as a research project in PWS using unique sequence plasmid probes D15S18, D15S9, D15S13, RL6.1, D15S10, D15S12, D15S24. These were trialled on 34 PWS patients (pts) and 2 Angelman syndrome (AS) pts, with 139 hybridisations (hyb). FISH with these probes proved to be unsuitable for routine diagnosis and in gene mapping. Diagnosis was established by standard DNA techniques at that time.

In 1992, cosmid probes D15S11 and GABR$\beta$3 and in 1994 SNRPN and D15S10 became commercially available. Use of these probes facilitated microdeletion detection and we have experienced a steady increase in referrals of new PWS/AS pts to be tested by FISH with these probes (table 1). In addition D15S11 and GABR$\beta$3 were used to investigate 16 cases (31 hyb) of additional small markers of chromosome 15 origin. Clinically, 2 of these pts had PWS, 2 had AS and 2 had some features of both syndromes. Different structural rearrangements were present in a further 2 PWS and 4 AS pts and were investigated using 8 additional probes (25 hyb) from other chromosome regions. In all cases FISH was performed on slides prepared at the initial cytogenetic harvest.

**TABLE I - SUMMARY OF FISH STUDIES**

| | Research | Referrals | | | TOTAL |
|---|---|---|---|---|---|
| | 1991 | 1992 | 1993 | 1994 | |
| PWS | 34(137) | 1(9) | 3(12) | 20(30) | 58(178) |
| AS | 2(2) | 2(8) | 18(22) | 36(43) | 58(75) |
| MAR | | | | | 16(31) |
| | | | | | 132(284) |

Legend - number of pts (number of hybridisations).

The FISH results for PWS showed 3/24 with deletion all of whom had the classical features on clinical follow up. Of the nondeleted pts, the diagnosis was reviewed and withdrawn in 15 and DNA studies for uniparental disomy were recommended in the remaining 6 pts. For the structural rearrangements FISH provided useful information not available by routine cytogenetics or standard DNA studies.

Based on our experiences with FISH investigations in PWS/AS and other chromosome 15 abnormalities, FISH is an excellent screening test in conjunction with routine cytogenetics.

**Molecular characterisation of the *Drosophila stoned* locus**

M. Smith and L.E. Kelly
Department of Genetics, The University of Melbourne,
Parkville, Victoria 3052 Australia

The *stoned* (*stn*) gene of *Drosophila melanogaster* encodes a novel protein that is essential in the nervous system (Petrovich *et al.*, 1993). Three behavioural alleles, $stn^{ts1}$, $stn^{ts2}$ and $stn^{C}$, and seven lethal alleles of *stn* have been isolated. In combination with the endocytotic *shibire* mutation, $stn^{ts}$ alleles display synergistic lethality. This suggests a role for the stoned protein in the neuron-specific endocytotic event; synaptic vesicle recycling.

The $stn^{ts}/stn^{C}$ heterozygote displays interallelic complementation. This suggests there are two domains of the stoned protein, and that the $stn^{ts}$ and $stn^{C}$ mutations affect alternative domains. Sequence data supports this, in that the *stn* gene contains two open reading frames (ORFs). Localisation of the $stn^{C}$ and $stn^{ts}$ mutations have been carried out to identify functional regions of the stoned protein and facilitate some understanding of the two ORF structure of *stn*. From previous genetic and sequence data, the $stn^{C}$ mutation was predicted to be in a region of ORF1 encoding a four-repeat domain. This region was amplified using PCR, from $stn^{C}$ genomic DNA and control DNA, subcloned and sequenced. Independent PCR clones identified a single basepair difference in the $stn^{C}$ sequence (A to G transition) in the region encoding the first of the four repeats. This leads to a threonine to alanine substitution, and suggests this region may be significant in stoned protein function. A similar analysis is being performed for ORF2, to localise the $stn^{ts2}$ mutation.

Anti-*stoned* antibodies are being raised against fusion proteins of ORF1 and ORF2. This will enable us to determine if both ORFs are translated *in vivo*, if they occur as separate polypeptides, and also be used in cellular biology experiments to directly identify whether *stn* is involved in synaptic vesicle recycling.

Petrovich, T.Z., Merakovsky, J., and Kelly, L.E. (1993). A genetic analysis of the *stoned* locus and its interactions with *dunce*, *shibire*, and *Suppressor of stoned* variants of *Drosophila melanogaster*. *Genetics* **133:** 955-965.

**Localisation of IGF2 and SNRPN/SNURF in the Marsupial, the Tammar Wallaby.**

**Megan J. Smithwick, Stephen A. Wilcox, and Jennifer A. Graves.**
**School of Genetics and Human Variation, La Trobe University, Melbourne, Australia.**

Genomic imprinting is observed in several groups of organisms. This mechanism of parental imprinting ranges from whole genomes to several small regions on chromosomes being preferentially inactivated. In the vertebrate class, only mammals have been shown to have small regions of their genome to be imprinted. This includes the X chromosome in marsupials, and several regions on the autosomes in man and mouse. Within two of these regions, the genes, IGF2 and SNRPN, have been shown to be imprinted.

These two regions along with several imprinted genes, have been shown to be conserved between man and mouse. The IGF2 region is located on chromosome 11 in human and chromosome 7 in mouse, while the region encompassing SNRPN is on chromosome 15 in human and 7 in mouse, but on a different region than IGF2.

These genes have been cloned from marsupial cDNA, lambda genomic, and cosmid libraries. The clones obtained from these libraries, along with the human and mouse probes, were localised to the genome of a marsupial, the tammar wallaby.

# *CRABS CLAW*, a gene involved in carpel and nectary development in flowers of *Arabidopsis*

**John L Bowman, John Alvarez and David R Smyth**

**Department of Genetics & Developmental Biology, Monash University, Clayton, Vic 3168**

Mutants of the *CRABS CLAW* gene specifically disrupt two aspects of flower development in *Arabidopsis thaliana*. Firstly, the two carpels develop abnormally. Instead of being joined fully along their length, they are unfused in their upper regions and curl inwards, something like the pincers of a *crabs claw*. Secondly, the development of nectaries, outgrowths of secretory tissue at the base of the stamens, is completely abolished.

We have cloned the *CRABS CLAW* gene by chromosome walking. It codes for a small protein which contains one zinc finger motif. In wild type plants, *CRABS CLAW* expression is localised to developing carpels and nectaries. Expression in carpel primordia commences before any visible sign of morphological differentiation, and long before the stage at which physical disruption is seen in *crabs claw* mutants. Later, it becomes confined to the outer cell layer of the ovary wall. *CRABS CLAW* expression also commences very early in cells destined to generate nectary tissue, and persists throughout nectary development.

It is likely that the *CRABS CLAW* product controls the transcription of at least three genes or sets of genes. The first of these are required early for the specification of carpel identity. A role for *CRABS CLAW* in regulating such genes is supported by studies of carpel development in multiple mutants combining *crabs claw* with mutants of other genes involved in specifying carpel identity. *CRABS CLAW* also apparently regulates the expression of genes required later for the growth of certain specific regions of the developing carpel. Finally, the *CRABS CLAW* product also controls nectary development, presumably by regulating the expression of a third gene or set of genes.

**Population genetic effects of habitat fragmentation in *Hakea carinata*.**

**Gary Starr**

**Department of Environmental Science and Rangeland Management**

**University of Adelaide**

**Roseworthy SA 5371**

Fragmentation of native vegetation by changing land use has greatly altered the population distribution and abundance of many plant species. Population sizes have been reduced in many cases, and interpopulation distances have increased. This is particularly evident in South Australia where only a few percent of the land area remains uncleared. The effects of these processes on population genetic structure of species and genetic processes within and between populations warrant inspection. In particular, changes to genetic processes that result in the loss of genetic variation in species and increased risk of population extinction need to be more fully understood if species are to be conserved.

*Hakea carinata* is a widespread understorey shrub in South Australia that is now restricted to islands of remnant native vegetation in the Mt Lofty Ranges, Lower Flinders Ranges, and areas of the South-East of the state. Some populations are greatly reduced in size and area and others, particularly in the remote areas, are relatively undisturbed. This project is investigating the level and distribution of genetic variation in the species using isozyme electrophoresis. The data will be used to assess random drift and its relationship to population size, and gene flow between fragments. The plant breeding system is mixed selfing and outcrossing, and the effects of small population size on the predominant breeding system will also be investigated using the genetic data.

*Haloragodendron lucasii*: A case study where genetic studies have been critical to the conservation management of an endangered plant species.

Marita Sydes and Rod Peakall
Division of Botany and Zoology
The Australian National University
Canberra, ACT 0200.

With the increasing interest in the use of genetics in the conservation of our rare and endangered plant species it is becoming critical that we are able to define appropriate areas of use for these genetic markers so that money is spent efficiently. The use of genetic studies for plants with different or unusual reproductive characteristics has been identified as a key study area and is discussed using an example species. This study focuses on the use of the molecular technique of RAPDs to assess the extent and degree of clonality in the endangered plant, *Haloragodendron lucasii*. At present this species is recorded from only three sites in the northern suburbs of Sydney and estimating the number of individuals present has been difficult. Initial RAPD analysis suggests that the previous estimates of the number of individuals in the populations were severely over-estimated, with single clones potentially covering an area of up to 50m$^2$. Although there is little or no variation of individuals within a site, the between site differences are very marked. This finding highlights the need to conserve all populations in order to conserve the genetic diversity of the species, the loss of a single site could result in the loss of up to 33% of the genetic diversity of the entire species. I believe this example illustrates a case of the usefulness of genetic studies for rare plants that have unusual reproductive mechanisms, and in this example the findings will have important implications on the strategies for the conservation management of the species.

# P ELEMENT-INDUCED RECOMBINATION IN MALE *DROSOPHILA MELANOGASTER*: THE DISTRIBUTION OF RECOMBINANTS AND AN INTERPRETATION INVOLVING THE HYBRID EXCISION-INSERTION MODEL

Mark Tanaka, Yasmine Gray and John Sved; School of Biological Sciences, A12; University of Sydney, Sydney, NSW 2006.

Opposite end-deficient P elements homologously located and provided with a transposase source yield high frequencies of specific recombination (~30%) in male *Drosophila melanogaster*. The current study utilised two genetic marker loci, *cn* and *bw*, each flanking the target P element site, allowing for two parental and two recombinant genotypes with respect to these loci in the offspring of the males in question. We presently report a few notable features in the distribution of recombinant types in this system. Firstly, there is a significant tendency for one of the two recombinant classes to predominate over the other, according to the arrangement of markers and P ends. This bias is accompanied by the significant prevalence of a particular parental type over the other. This asymmetric distribution of genotypes is in agreement with predictions of the Hybrid Excision-Insertion model of P element induced recombination. Secondly, the frequency of recombination increased significantly as the males aged. A similar result was found with respect to complete (double ended) homologous elements; however these findings contrast with reports that where P elements are complete, hemizygous and present in multiple copies, recombination frequencies decrease or do not change with age. Thirdly, extreme heterogeneity was noted in the distribution of counts in the four offspring genotype classes, which is related to the clustering of recombinants found in the case of hemizygous elements. Two of the three possible patterns of association amongst the four offspring genotypes were observed to increase with age, while the other showed no systematic change. The two highly clustered associations are related to reciprocal and non-reciprocal clustering previously reported, while the grouping found to be relatively homogeneous reflects the negligible level of mitotic non-disjunction. A mathematical model and computer simulation were constructed to approximate the process of cells undergoing mitotic recombination, conceivably by the mechanism of Hybrid Excision-Insertion, through spermatogenesis in *Drosophila*. The theoretical consequences are consistent with the effects of aging on the distribution of recombinants observed empirically.

## IDENTIFICATION OF DNA MARKERS TIGHTLY LINKED TO GRAIN HARDNESS.

M. Turner 1,2, S. Rahman 2, P. Sharp 1

1. Plant Breeding Institute, Cobbitty, University of Sydney.
2. CSIRO Division of Plant Industry, Canberra.

Wheat grain hardness is an important economic consideration as wheats with different levels of hardness contribute to different end products. It is known that the main locus controlling grain hardness, Ha, is on the short arm of chromosome 5D but the biochemical basis of hardness is unknown. We have previously reported that some of the genes for a 15kD protein, GSP-1, were tightly linked to Ha. Here we report the isolation of different versions of the GSP-1 gene from Triticum tauschii, the donor of the D genome of wheat. In addition we have isolated DNA from 40 recombinant inbred lines differing in grain hardness (kindly supplied by Dr.John Snape at the John Innes Institute.) . This was used to produce two pools of soft and hard DNA. A set of 140 primers was used on these pools to generate random amplified polymorphic DNA (RAPDs) and the products analysed by denaturing polyacrylamide gel electrophoresis. A total of 20 markers were isolated from both bulk and Cot fractionated DNA and these were cloned. Similarly 10 RAPD markers were cloned after analysing DNA from another pool of soft and hard wheats obtained by combining soft Heron and soft Falcon and their near-isogenic counterparts. Furthermore markers from T.monococcum and barley that map to homologous regions in the corresponding chromosomes have also been obtained from other laboratories. All these DNA markers will be used to analyse the inheritance of grain hardness in a segregating population of 200 F2 lines produced by crossing near isogenic soft and hard Falcon lines . This should provide a clearer definition of the DNA sequences comprising the Ha locus.

**The Hermes Transposable Element from the House Fly, *Musca domestica* Transforms *Drosophila* with High Efficiency**

**William D. Warren[1]*, Peter Atkinson[2] and David O'Brochta1[1] Center for Agricultural Biotechnology, University of Maryland, College Park MD, USA.2 CSIRO Division of Entomology, Black Mountain, Canberra ACT.* Present address, Peter MacCallum Cancer Institute, East Melbourne, Vic.**

The house fly, Musca domestica, contains multiple copies of an active transposable element system called Hermes. Some strains of *M. domestica*contain only full length Hermes elements whereas others contain both fulllength and internally deleted elements of various sizes. Using PCR andinverse PCR we amplified and sequenced overlapping segments of severalHermes elements and from these data constructed a 2749 bp consensus HermesDNA sequence (GenBank accession L34807). Hermes termini consist of 17 bpimperfect inverted repeats that are almost identical to the invertedterminal repeats of the hobo element of *Drosophila*. Full length Hermeselements contain a single long open reading frame capable of encoding a612 amino acid protein which is 55% identical to the amino acid sequenceof the hobo element transposase. We have now demonstrated that genetically marked Hermes elements can bemobilized in both *M. domestica* and in *Drosophila*. Using a plasmid basedassay we have shown that Hermes is able to transpose from one plasmid toanother while being transiently maintained in *M. domestica* embryos. Onlysequences delimited by the terminal inverted repeats transposed andinsertion resulted in an 8bp duplication of the target site. To test thepotential of Hermes to act as a broad host-range insect transformationvector we replaced the transposase coding region of Hermes with the*Drosophila* white (w+) gene and constructed a "helper" plasmid containingthe Hermes transposase coding region fused to a heat shock promoter. Amixture of these plasmids was injected into preblastoderm embryos from aw- strain and eye pigmentation monitored in the G1. Three independentinjection experiments were performed and individuals with pigmented eyeswere recovered from the progeny of the injected animals in all threeexperiments. PCR analyses of DNA from these animals confirmed the presenceof Hermes sequences which were not present in their white-eyed siblings.An average of 35% of the fertile G0 adults that developed from theinjected embryos produced transgenic offspring. 88% of the G0 adultsproducing transgenic progeny had multiple inserts (as initially indicatedby varying levels of eye pigmentation and subsequently confirmed throughgenetic mapping, chromosome in situ and Southern analysis). 57% of the G0adults producing transgenic progeny yielded clusters of transgenics(wherein one eye phenotype comprised 10% of more of their total progeny)indicating that Hermes insertions occurred premeiotically in many cases. Surprisingly, two G0 adults produced 100% transgenic progeny, indicatingthat their all of their germ cells had one or more Hermes elements insertions.

## Progress on the DOGMAP and Ceroid Lipofucsinosis in Border Collies

**Alan N. Wilton**

**School of Biochemistry and Molecular Genetics, Univ. NSW, Sydney NSW 2052**

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 150 border collies including several known carriers and affected dogs.

CL in border collies is widespread in Australia 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. All cases can be traced back to a single dog imported in the 1950s so there unlikely to be heterogeneity of the disease. If this single founder hypothesis is true 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 are looking for RFLPs in dogs using these human genes as probes on Southern blots of dog DNA to determine whether their dog homologues are linked to the CL gene in dogs. The RFLPs will help determine whether CL in border collies is caused by the same gene as Batten's disease in man. We have tested a small number of published dinucleotide repeat markers for linkage to CL in dogs. Any linked polymorphic loci will allow pre-symptomatic diagnosis and carrier detection for CL.

It was reported at the DOGMAP conference in Cambridge in April that there are presently approximately 1,000 microsatellite markers available in dogs. There is a set of 10 reference pedigrees on which these will eventually be typed to generate a linkage map. Chromosome maps are also being constructed using FISH and comparative mapping using human chromosome paints.

## Gene Mapping in Queensland Fruit Fly *Bactrocera tryoni*

**Jing Ting Zhao Marianne Frommer John Sved**

**School of Biological Sciences**

**University of Sydney**

The Queensland fruit fly, *Bactrocera tryoni*, is the major pest of orchards in eastern Australia, causing losses of many millions of dollars each year.

The project is to establish the mitotic karyotype and a polytene chromosome map of *B. tryoni*, which can be used as basis for locating molecular markers, and eventually visible markers. As well, the project will establish the linkage groups for microsatellite markers and visible markers. Therefore, by locating the microsatellite sequences on the polytene chromosomes using *in situ* hybridization, we can define the actual chromosome which carries each linkage group of visible markers. This provides the start for an integrated physical and genetic map.

The metaphase spread of *B. tryoni* is presented. A composite photographic map of polytene chromosomes from the salivary glands of *B. tryoni* is constructed.

Laboratory stocks carrying visible mutations are typed for microsatellite variants by PCR. Microsatellite markers are then associated with visible mutations by typing the progeny of segregating crosses. So far two linkage groups have been established.

The attempt to carry out *in situ* hybridization using probes labelled with biotin-16-dUTP is quite promising. The *white* gene clone of *B. tryoni* and two microsatellite sequences, pDt-32 and pDt-15, have been located on specific polytene chromosomes. The linkage groups of *orange-eye* / pDt-15 and *white-mark* / pDt-32 are thus associated with specific polytene chromosomes.

In the future, it would be desirable to have a linkage group corresponding to each chromosome. The integrated genetic and physical map will be valuable in the Queensland fruit fly study to investigate genetical systems which will aid in the control of *B. tryoni*.

**Analysis of the atrial natriuretic factor gene as a candidate risk factor gene for hypertension in the spontaneously hypertensive rat.**

**Li Zhang, Kim M Summers, Malcolm J West.**
**Department of Medicine, University of Queensland, Prince Charles Hospital, Brisbane, Queensland.**

Atrial natriuretic factor (ANF) and its receptors have an important physiological role in the regulation of blood pressure. Variants of the genes coding for these proteins may therefore be involved in the genetic risk for essential hypertension in humans. In studies of animal models, the gene coding for one of the ANF receptors, guanylyl cyclase A, has been found to co-segregate with blood pressure in several crosses involving genetically hypertensive rats. We have now used the spontaneously hypertensive rat (SHR) to study the role of variation at the ANF gene in determination of blood pressure. Two segregating populations, F2 and backcross, were derived from crosses between SHR and normotensive Wistar Kyoto (WKY) rats. F1 animals were brother-sister mated to produce the F2 generation. The backcross generation was derived from matings between F1 females and SHR males. Microsatellite markers within and around the ANF region on chromosome 5 were amplified by PCR. Blood pressure was measured by the direct method in animals at 12 weeks of age.

Two hundred and seven F2 rats were analysed. The ANF allele derived from the SHR grandparent (ANF-S) was associated with higher blood pressure than the allele derived from the WKY grandparent (ANF-W) when results for both sexes were pooled ($P<0.004$). When each sex was analysed separately, the ANF-S allele strongly co-segregated with increased blood pressure in F2 males ($P=0.001$, $n=106$) but was only weakly linked to blood pressure in F2 females ($P=0.03$, $n=101$). In F2 males homozygous for the ANF-S allele, the systolic blood pressure was 178±15 (mean±sd) mmHg compared with 162±18 mmHg for males homozygous for the ANF-W allele. Heterozygotes had a blood pressure intermediate between these levels, indicating that each dose of the ANF-S allele was responsible for an increase of 8 mmHg in these animals, with no indication of dominance. SHR male animals had a mean systolic blood pressure of 206±14 mmHg and WKY rats had a mean systolic blood pressure of 149±13 mmHg. Variation at the ANF locus could thus account for about 25% of this difference. However, when backcross males were analysed, there was no association between ANF genotype and systolic blood pressure ($P=0.87$, $n=83$), indicating that the genetic background may also be important. Although SHR also develop enlarged hearts, thought to be due to the increased pressure load on the heart caused by the elevated blood pressure, there was no association between heart weight and ANF genotype. To establish whether the effect on blood pressure was due to the ANF locus or a linked locus, we determined the relationship between blood pressure and another marker on chromosome 5, the anonymous marker MIT-R1678, which is about 30cM from ANF. Weak linkage to systolic blood pressure was found in females ($P=0.03$). There was no association with blood pressure in males, nor with heart weight in either sex.

These results suggest that there may be a hypertension locus close to (or at) the ANF gene. Other authors, investigating a cross between Dahl salt sensitive hypertensive and Dahl normotensive animals, found a hint of linkage to ANF. Our results show that this may not be mediated through salt induced hypertension, since our animals were not salt loaded. The lack of association in the backcross animals suggests that the genetic background of the animals may be important, since these animals had a higher genetic contribution from the SHR strain than the F2 animals. Since there was a strong association with systolic blood pressure in males, but no linkage to heart weight, the effect of ANF gene variation may be primarily on blood pressure, with other factors interacting to cause the cardiac hypertrophy seen in SHR at this age. These data demonstrate that the relationship between genes and blood pressure is complex and dependent on many factors including genetic background. This suggests that it will be difficult to generalise from the rat models to the human situation.