



3-7 July, 2000  
Canberra



BREEDING PHYLOGENY MAPPING



**GSA 2000**  
**PROGRAM and ABSTRACTS**



## PAPER

MOLECULAR EVIDENCE FOR ONE SPECIES OF THE COMMON DOLPHIN, *DELPHINUS DELPHIS*, IN SOUTHERN AUSTRALIACatherine White<sup>1,2</sup>, Stephen Donnellan<sup>1</sup>, Catherine Kemper<sup>1</sup> and Peter Hale<sup>3</sup><sup>1</sup>South Australian Museum, Adelaide, Australia 5000<sup>2</sup>Department of Genetics, The University of Adelaide, Adelaide, Australia 5000<sup>3</sup>Centre for Conservation Biology, University of Queensland, St Lucia, Australia 4072

The common dolphin is a highly mobile cetacean distributed widely in tropical and temperate waters. There is notable morphological variation between and within populations resulting in more than 20 nominal species. Recent morphological and molecular studies of northern hemisphere common dolphins indicate just two species. *Delphinus capensis* and *D. delphis*, differentiated by beak length (long- versus short-beak), cranial and body proportions, body colour/pattern and mitochondrial DNA haplotypes. Common dolphins with long- and short-beaked morphologies are found also in southern Australian waters. We tested their species status with a phylogenetic analysis of mitochondrial DNA sequences that also included published sequences from the eastern Pacific and the Black Sea. Maximum likelihood methods indicated extensive polyphyly of the haplotypes of long- and short-beaked morphotypes. Population analyses showed no evidence of phylogeographic structure of *Delphinus* in southern Australia. These analyses provide no support for a two species hypothesis in southern Australia.

## POSTER

ALLOZYME POLYMORPHISM IN AN ENDANGERED AUSTRALIAN TERRESTRIAL ORCHID *PTEROSTYLIS GIBBOSA* R. BR. (ORCHIDACEAE), AND ITS IMPLICATIONS FOR CONSERVATION

I.K. Sharma, M.A. Clements and D.L. Jones

Centre for Plant Biodiversity Research, Division of Plant Industry CSIRO, GPO Box 1600, Canberra, ACT, 2601, Australia

A relatively high genetic diversity, in all known populations of the endangered Australian native terrestrial orchid *Pterostylis gibbosa* R.Br. was observed when investigated through starch gel electrophoresis. The percentage of polymorphic loci ( $P$ ), the number of alleles per locus ( $A$ ), observed ( $H_o$ ) and expected heterozygosity ( $H_e$ ) at population levels were 69%, 2.21, 0.210 and 0.261 respectively. The  $G_{st}$  value of 15% indicates that around 85% of variation resides within populations. High genetic variability along with low population divergence may be the result of recent population fragmentation or from extensive gene flow maintained by seed and pollen movement. Testing revealed high seed viability (range 68-90%) suggesting that poor seed viability is not the cause of its rarity. Although endangered, and restricted to only four geographical areas, *P. gibbosa* showed a higher level of genetic variation than other orchids with larger populations.



## POSTER

MENDELIAN ANALYSIS OF GENOTYPING DATA REVEALS MORE  
GENOTYPING ERRORS THAN CONVENTIONAL ERROR ANALYSIS

K.R. Ewen, W. Ward, D. Lucas, **M. O'Keefe**, J. Barlow and S. Foote

While it is clear that errors in genotyping data can lead to severe errors in analysis there is as yet no consensus for identifying these errors. Strategies suggested include running duplicate samples and duplication of allele calling. This study aimed to develop a better understanding of error types associated with microsatellite genotyping and so enabling a more rational strategy to be developed.

Two microsatellite marker sets, a commercial and a custom set, were used to generate 118,420 and 22,500 initial genotypes and then 10,088 and 8,328 duplicates respectively. Mendelian errors were identified using PedManager software and concordance determined for the duplicate samples.

Analysis of Mendelian errors identified two classes of errors, human and errors caused by mutation events while duplication only identified human errors. Mendel checking of the commercial marker data identified 0.25% errors (0.13% human and 0.12% mutation errors), while concordance of the duplicate samples only detected 0.08% errors. Similarly the custom set found 1.37% Mendel compared to 1.19% by concordance.

These data suggest it is more beneficial for error detection/reduction to Mendel check for errors rather than duplicate samples or calls ensuring the second class of errors (mutation events) are captured and so increasing the likelihood of linkage.



## UNRAVELLING SAMPLE SWAPS IN LARGE LINKAGE MAPPING PROJECTS

**Melanie Bahlo**<sup>1,2</sup>, Justin Rubio<sup>1,2</sup><sup>1</sup>*Genetics and Bioinformatics, The Walter and Eliza Hall Institute of Medical Research, Royal Parade, Parkville 3050 VIC*<sup>2</sup>*CRC for the Discovery of Genes for common Human Diseases*

Large Scale genome projects, such as those required to investigate complex diseases, are becoming more common place with the advent of faster and cheaper genotyping. The handling of such large collections bring with them an increased risk of sample swaps. These swaps can occur at the point of collection due to mislabelling of tubes or during DNA extraction and aliquotting. Loss of genotyping data is costly, particularly if the data is from a unique, large kindred. Although it is usually easy to infer which sample is in error it is usually very difficult to infer the sample's ID. Three perl programs are presented that reassign "lost" samples to their correct ID. Examples show which program is appropriate in a given situation and how to interpret the output. Perl is a scripting language available as shareware for MAC, PC and UNIX and is very suitable for the manipulation of genetic data.

INTERACTION OF *RHIZOBIUM LEGUMINOSARUM* bv. *TRIFOLII* STRAINS WITH NON-LEGUME RICE**F.M. Perrine**, J. Prayitno, M.A. Djordjevic, J.J. Weinman and B.G. Rolfe*Genomic Interactions, Research School of Biological Sciences**Australian National University, PO BOX475, Canberra ACT 2601, Australia*

We studied the colonization and growth of rice seedlings using rice-rhizobia strains R4 and E4 (Yanni et al., 1997; Prayitno et al., 1999), the characterized *Rhizobium leguminosarum* bv. *trifolii* clover strain ANU843 and its plasmid cured derivatives (Rolfe et al., 1980). Strain R4 stimulated, while strains E4 and ANU843 inhibited the growth of rice seedlings (Prayitno et al., 1999). Using the Green Fluorescent Protein (GFP) as a constitutive marker, our findings were that strain R4 associated only with the first anchor root of rice seedlings and exhibited a particular colonization pattern by forming intercellular long lines (ILLs) in lateral roots. In contrast, strains E4 and ANU843 infected at the lateral root junctions (LRJs). Because strain ANU843-inoculated seedlings were inhibited in their growth, we investigated whether the five plasmids ranging from 180 kb to ~700kb of strain ANU843 could affect the interaction between strain ANU843 and rice. Our results showed that strain ANU845, cured of its Sym-plasmid, pa, inhibited rice seedling growth. Derivative strains, however, lacking either the pb, pc, pd and pe plasmids respectively or both plasmids pa and pc or pa and pb, did not inhibit the seedling growth. Furthermore, strain ANU843 derivative, cured of pc, showed a stimulatory effect similar to strain R4. This result suggests that the presence of certain plasmids of strain ANU843 influence the effect of strain ANU843 on rice.



## POSTER

### STRATEGIES FOR CONVERTING AFLP MARKERS TO SIMPLE PCR BASED MARKERS IN WHEAT

**Wujun Ma**, Wes Keys, Mathew Morells, Rudi Appels and Kevin Gale

*CSIRO Plant Industry, PO Box 1600, Canberra, ACT, 2601*

AFLP procedure is a recently developed powerful molecular marker procedure. However, the technically demanding and time-consuming nature of this procedure inhibits the direct use of AFLPs in marker assisted selection (MAS). Converting AFLPs to simple PCR based markers will greatly enhance the use of molecular markers in MAS. Conversion of AFLP markers to simple PCR based markers is frequently difficult, and involves procedures such as TAIL PCR, inverse PCR or uneven PCR. We have developed novel strategies for the conversion of AFLP fragments into simple PCR markers. Examples of these strategies will be presented.



Please note that this talk will be held at 4:50pm on Tuesday 4<sup>TH</sup> July in the CSIRO Discovery Centre, in the place of the programmed talk by Luciano Behegaray.

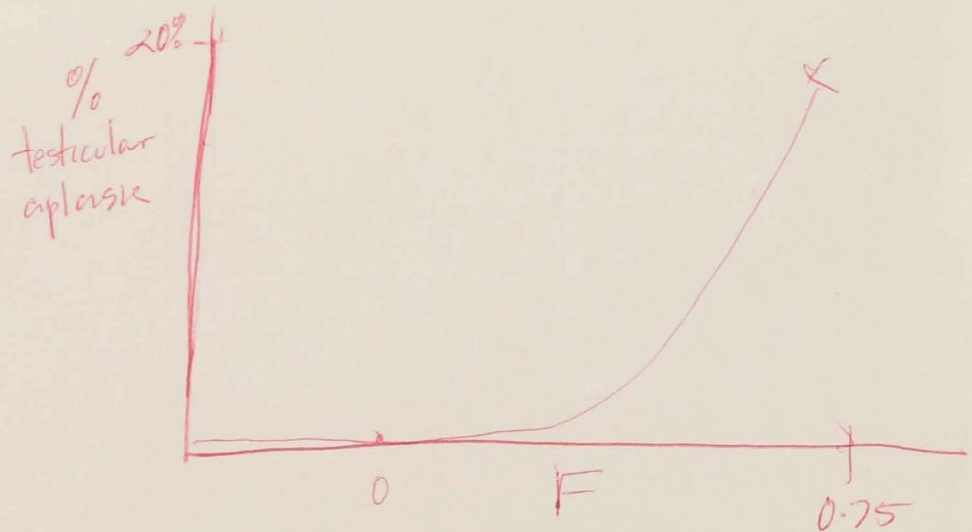
## INBREEDING DEPRESSION IN THE KOALA

M. E. Montgomery<sup>1\*</sup>, R. J. Duckett<sup>2</sup>, B. A. Houlden<sup>1</sup>, D. A. Taggart<sup>3</sup>

<sup>1</sup>*School of Biological Sciences, University of New South Wales*, <sup>2</sup>*Department of Anatomy, Monash University* <sup>3</sup>*Department of Zoology, Melbourne University*

A series of sequential founding events led to the establishment of koala populations on French Island, Kangaroo Island and the Eyre Peninsula in southern Australia. Recent studies have shown that there is a highly significant decrease in genetic variation between these southern populations and a large natural population of koalas from the Pilliga region of NSW. This study examined a variety of male reproductive parameters in these populations to determine if a correlation existed between male reproductive capacity and loss of genetic variation. Results indicated that significant changes occurred in the proportion of sperm head morphologies with a decrease in genetic variation. There was also a strong correlation between loss of genetic variation and an increase in testicular aplasia in these populations. The incidence of testicular aplasia ranged from ~0% in the non-inbred population to ~28% in the most inbred population. Testosterone levels ( $1.4 \pm 0.7 - 3.0 \pm 0.5$  ng/ml), sperm motility ( $71 \pm 7 - 87 \pm 3\%$ ) and sperm volume ( $0.88 \pm 0.1 - 1.75 \pm 0.3$  ml) did not vary significantly between inbred and outbred populations.

$$F_e = 1 - \frac{H_{\text{island}}}{H_{\text{mainland}}}$$





# **GENETICS SOCIETY OF AUSTRALIA**

## **47<sup>th</sup> Annual Meeting**

**Australian National University, Canberra. July 2000**

## **CONFERENCE INFORMATION FOR REGISTRANTS**

### **VENUE**

All paper and poster sessions will be held at the CSIRO Division of Plant Industry opposite the ANU on Clunies Ross St (see map). Morning paper sessions, posters, tea & coffee and trade displays will be in the CSIRO Discovery Centre. The two concurrent afternoon paper sessions will be split between Discovery lecture theatre and the lecture theatre in Building 1 which is a two minute walk from Discovery (see map). Both theatres have slide, overhead and MS-Powerpoint facilities. Your registration also allows you access to the CSIRO Discovery displays located in the lower level of the Discovery Centre.

### **ACCOMMODATION & PARKING**

Accommodation is at Burgmann College ANU (see map). You can check in from 2pm on the day you arrive. Parking is available at the ANU. You can either use any of the pay and display areas, or upon registration you can purchase parking tickets for \$2 per day. This will allow you to park in any of the blue permit parking areas on campus, provided that you display your ticket in clear view on your dashboard. Parking is not available on the CSIRO site.

### **REGISTRATION**

At Burgmann College 7.30-10.00 pm on Monday July 3<sup>rd</sup> (during the mixer - see below) and at the registration desk in the lower foyer of the Discovery Centre on the morning of Tuesday 4<sup>th</sup> of July 8.30am-10.30am. Day registration will also be available at the Discovery Centre registration desk each day of the conference from 8.30am-9.30am.

### **POSTERS**

Posters can be set up from the morning of Tuesday 4<sup>th</sup> July and will be displayed in the upper foyer of the Discovery Centre for the duration of the conference. Presenters should be available to answer questions during the poster session on the afternoon of Wednesday 5 July between 4.10 pm and 5.30 pm during which time the student prize team will judge poster presentations. All posters should be removed by Thursday afternoon.

### **MIXER**

A mixer will be held at Burgmann College 7.30-10.00 pm on Monday 3<sup>rd</sup> July. Finger food will be served and the bar will be open.



## MEALS

Lunch may be obtained at the CSIRO Discovery Centre Café, however this can become rather busy at lunchtime (especially with 150 extra people) so participants are encouraged to explore the ANU campus where there are a range of excellent eating options.

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 of these are within ten minutes walk of the Discovery venue in the general area of the main ANU quadrangle (see map). 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 a Vietnamese restaurant, two Italian restaurants, a vegetarian restaurant and a takeaway, as well as a bar, supermarket and bottle shop. 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 Wednesday 5<sup>th</sup> July 7.30-12.00pm in the dining room at Burgmann College. The dinner will consist of an entrée of either smoked salmon or avocado and tomato soup followed by a main course of either Beef Wellington or Baked Barramundi. Dessert will be Chocolate Charlotte or Melon Cocktail with Strawberry Liquor. Dinner includes red and white wine, orange juice and tea and coffee. There will also be a cash bar available. Music for dancing will be provided by the local Canberra Band "Barry Drive".

## LUNCH AT 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 Friday 7<sup>th</sup> July at 12.30 pm. The gardens are situated on the side of Black Mountain, five minutes walk from Burgmann College on the same side of Clunies-Ross Drive as the CSIRO. 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	6285 9222
ANU Health Service	6249 3598/4098
Canberra afterhours locum medical service (CALMS)	6288 17811



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

### **Vivaldi's Restaurant**

ANU Campus

6257 2718

### **Delicateating** (Italian cuisine)

O'Connor Shops (BYO\*)

62471314

### **All Bar Nun** (Bar and Food)

O'Connor Shops

62579191

### **Tu Do Vietnamese Restaurant**

O'Connor Shops (BYO\*)

6248 6030

### **Siamese Kitchen** (Thai cuisine)

14 Lonsdale St., Braddon (BYO)

6248 8802

### **Great Wall Chinese Restaurant**

113 Marcus Clarke St (Licensed)

6247 5423

### **Shalimar Indian Restaurant**

9 Tasman House Marcus Clarke St

(Licensed/BYO)

6249 6784

### **The Vietnam Restaurant**

8-10 Hobart Place Civic (BYO)

6248 7093

### **Three Mothers (Thai)**

34 Garema Place Civic (BYO)

6249 8900

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

There is also an excellent range of Restaurants in Woolley Street, near the Dickson Shopping Centre

## STUDENT PRIZES

Student prizes for the best paper (\$250) and poster (\$100) have been kindly donated by Bio-Rad and CSIRO. Prizes will be awarded after the last session of Friday.

### **Local Organising Committee:**

Dave Rowell

Geoff Clarke

Andrew Young



## **SPEAKER INSTRUCTIONS**

In the break prior to their session all speakers should meet in the lecture theatre with their session chair to ensure that everyone is present and that all slides, overheads and Powerpoint presentations are working properly. For those using Powerpoint it would be useful to provide a copy of your presentation on disk when you register so that it can be loaded onto the hard drive. Please label your disk with your name and session time. If choosing to use Powerpoint please remember to have a backup of overheads or slides!

## **TRADE DISPLAYS**

Twelve trade displays will be located near the tea and coffee area in the Discovery Centre for the duration of the conference. We thank these companies for their support:

Medos Company

Fisher-Biotec/Genset pacific

Genesearch

Integrated Sciences

Geneworks

ANGIS

Progen Industries

Carl Zeiss Pty Ltd

Pall Gelman

Pathtech

Promega Corporation

Biorad



West Basin

CONTRACTOR/CONSULTANT



PROJECT TITLE  
AUSTRALIAN NATIONAL UNIVERSITY

DRAWING TITLE  
SITE PLAN

Date: JUN 98	Scale: 1:6000
Drawn: Paul Sjoberg	Checked:
Approved:	Sheet Size: A8





The following companies have supported the Genetics Society of Australia Inc. by being Sustaining Members of the society in 2000.

**Advanced Labs**

**Annual Reviews**

**Beckman Coulter Australia**

**Bio-Rad Laboratories**

**Blackwell Science Asia Pty. Ltd.**

**Carl Zeiss Pty Ltd**

**Crown Scientific Pty Ltd**

**Genesearch Pty Ltd**

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**Promega Corporation**

**QIAGEN Pty Ltd**

**Quantum Scientific Pty Ltd**

**Selby-Biolab**

**Taylor Wharton Australia Pty Ltd**



# GSA 2000 Canberra Program

## MONDAY 3<sup>RD</sup> JULY 2000

7:30 – 10:00 Registration & Mixer (Burgmann College)

## TUESDAY 4<sup>TH</sup> JULY 2000

Morning Session – CSIRO Discovery

### Symposium 1 – Phylogenetics (Chair: Scott Keogh)

- 8:45 – 9:00 **Welcome** - Professor Sue Serjeantson, President of FASTS
- 9:00 – 10:00 **Keynote Address: Dave Swofford** - The coming of age of model-based methods in phylogenetic inference: new perspectives on consistency, efficiency, robustness, and philosophy
- 10:00 – 10:30 *Morning Tea*
- 10:30 – 11:00 **Scot Kelchner** - The reality of structured sequence data requires a rethinking of phylogenetic analysis procedures
- 11:00 – 11:30 **Dan Faith & John Trueman** – After cladistics: towards an inclusive philosophy for phylogenetic inference
- 11:30 – 12:00 **John Trueman & Dan Faith** - Corroboration 2000: measures of fit, probabilities of fit, and selecting a most corroborated tree
- \* 12:00 – 12:30 **Mark Gibbs** – Unscrambling eggs: what to do with recombinant sequences
- 12:30 – 2:00 *Lunch*

*parsimony = no common mechanism  
ML methods  
(evolution is time independent)*

\* *cf  
Jun Hori's  
recombinant  
PERVs.*

Afternoon Session - CSIRO Discovery

### Symposium 1 – Phylogenetics (continued) (Chair: John Trueman)

- 2:00 – 2:20 **Mark Blackett, Mark Adams, Carey Krajewski & Michael Westerman** – Genetic variation within the dasyurid marsupial genus *Planigale*
- 2:20 – 2:40 **Alan Wilton** – Monitoring hybridisation between dingoes and dogs in the wild
- 2:40 – 3:00 **J.G. Mant, F.P. Schiestl, R. Peakall & P.H. Weston** – Phylogenetic perspectives on a highly specialised pollination system
- 3:00 – 3:20 **Richard Newcomb, Tamara Sirey, Robyn Howitt, David Greenwood & Dianne Gleeson** – Tracing pheromone evolution in two native New Zealand genera of tortricid moths
- 3:20 – 3:40 **Josh Mackie, Les Cristidis & Mick Keough** – Colonial colonials – contrasting patterns of mitochondrial DNA variation in three introduced bryozoans suggest two different introduction histories
- 3:40 – 4:10 *Afternoon Tea*



## Symposium 1 – Phylogenetics (continued) (Chair: Dave Rowell)

- 4:10 – 4:30 **David Low**, Ben Oldroyd & Lars Jermiin – Incorporation of secondary structure information in a phylogeny of the stingless bees
- 4:30 – 4:50 **I.B. Jacobsen**, J.A. Saleeba, M. Poidinger & T.G. Littlejohn – TREEGENEBROWSER: a phylogenetic approach to sequenced databases
- 4:50 – 5:10 **Luciano Beheregaray** & Paul Sunnucks – A molecular evolutionary study of the fish genus *Odontesthes*: unravelling historical and recent biogeographic scenarios in South America
- 5:10 – 5:30 **Steve Cooper**, Jan Birrell & Agatha Labrinidis – Mitochondrial DNA phylogeography of the dunnart, *Sminthopsis crassicaudata* and the sleepy lizard *Tiliqua rugosa* from southern and central Australia

## Afternoon Session – CSIRO Building 1

### Contributed Papers 1 – Chair: Margaret Byrne

- 2:00 – 2:20 **C.H. Bock**, P.H. Thrall & J.J. Burdon – Epidemiology and variation of *Alternaria brassicicola* in naturally occurring populations of *Cakile maritima* along the NSW coast
- 2:20 – 2:40 **Stephen McKechnie** & Alisha Anderson – Geographic variation in frequency of the Thr-Gly repeat-length variants in the *Drosophila period* gene along the Australian east coast
- 2:40 – 3:00 **Jeremy Brownlie**, Charles Claudianos & Steve Whyard – A genomic survey of the *C. elegans* genome reveals distribution patterns and insertion site preferences for the Tc1/mariner/maT superfamily
- 3:00 – 3:20 Xiumei Liang & **John Sved** – Tests of the hybrid element insertion/repair model for P element-induced recombination in *Drosophila melanogaster*
- 3:20 – 3:40 **Steven Whyard** & Hilary Mende – Persistent gene silencing in *Drosophila melanogaster* using double-stranded RNA
- 3:40 – 4:10 *Afternoon Tea*

- really interesting  
? silencing of gal transferrase  
in pigs?

### Contributed Papers 2 – Chair: John Sved

- 4:10 – 4:30 **Megan Higgie**, Steve Chenoweth & Mark Blows – Experimental demonstration of the evolution of reproductive character displacement. I. Response of mate recognition to natural selection
- 4:30 – 4:50 **Mark Blows** & Megan Higgie – Experimental demonstration of the evolution of reproductive character displacement. II. Evolutionary change under natural selection and G matrix eigenstructure
- 4:50 – 5:10 **Kelli Gowland**, Julian Ash & Tristan Armstrong – The inheritance of traits in the *Ranunculus dissectifolius* – *R. millanii* hybrid complex
- 5.10 - 5.30 **Helen M. Stace** Genome evolution among basal taxa of angiosperms: austrobaileyaceae, trimeniaceae, and illiciales (the 'ita clade')



## WEDNESDAY 5<sup>TH</sup> JULY 2000

### *Morning Session – CSIRO Discovery*

#### **Symposium 2 - Molecular Genetics in Plant and Animal Breeding (Chair: Curt Brubaker)**

- 8:45 - 10:00**     *Keynote Address: Rudi Appels* - Defining key agronomic traits in breeding programs using molecular genetics
- 10:00 – 10:30**     *Morning Tea*
- 10:30 – 11:00**     E.S. Lagudah, **W. Spielmeyer**, O. Moullet, S. Seah, F. Ogbonnaya, R. Eastwood, H Bariana, J deMajnik and R. Appels - Molecular genetics of disease resistance in wild relatives of wheat and their application to wheat improvement
- 11:00 – 11:30**     **Steve Jefferies** – A QTL mapping approach to the dissection of a complex trait – boron tolerance
- 11:30 – 12:00**     **Ian Franklin** - Identifying QTL for wool production
- 12:00 – 12:30**     Allan Green & **Surinder Singh** - Molecular genetics of fatty acid desaturation in oilseeds and its relevance to oil quality modification
- 12:30 – 2:00**     *Lunch*

### *Afternoon Session – CSIRO Discovery*

#### **Symposium 1 – Phylogenetics (continued) (Chair: Scot Kelchner)**

- 2:00 – 2:20**     Ben Phillips, Stuart Baird & **Craig Moritz** – Vicariance and evolution in the Australian wet tropics
- 2:20 – 2:40**     **Remko Leys** & Steve Cooper – The origin and historical biogeography of the large carpenter bees (*Xylocopa*)
- 2:40 – 3:00**     **Belinda Cardinal** & Les Christidis – Systematics of the large bentwing bat (*Miniopterus schreibersii*) in Australia
- 3:00 – 3:20**     **M. Byrne** & B. Hines – Phylogenetic structure in the york gum complex, *Eucalyptus loxophleba*, *E. gratiae* and *E. blaxellii*
- 3:20 – 3:40**     **Tim Littlejohn** – Chopping down trees with bionavigator: a lumberjacks guide to phylogenetics
- 3:40 – 4:10**     *Afternoon Tea*
- 4:10 – 5:30**     **Poster Session and Trade Displays**
- 7:30 – 12:00**     **Annual Dinner (Burgmann College)**



**Contributed Papers 3 – Chair: Steve McKechnie**

- 2:00 – 2:20**     **Peter Hunt**, B Trevaskis, Richard Watts, Marc Ellis, Mark Hargrove, John Olson, Liz Dennis & Jim Peacock – Functional characterisation of plant hemoglobins analysis
- 2:20 – 2:40**     **Kylea Clarke**, Ben Oldroyd, J. Javier G. Quezada-Euan & Thomas Rinderer – Mitochondrial DNA analysis of honey bees from the Yucatan Peninsula
- 2:40 – 3:00**     **Kellie Palmer** & Ben Oldroyd – Multiple mating in the genus *Apis* as determined by microsatellite analysis
- 3:00 – 3:20**     **Keryn Wilkes** & Ben Oldroyd – QTL mapping of hygienic behaviour in honeybees
- 3:20 – 3:40**     **Stuart Gilchrist**, Jason Kennington & Linda Partridge – Comparing the genetics of clinal divergence of various *D. melanogaster* traits
- 3:40 – 4:10**     *Afternoon Tea*
- 4:10 – 5:30**     **Poster Session and Trade Displays**
- 7:30 – 12:00**   **Annual Dinner (Burgmann College)**

**THURSDAY 6<sup>TH</sup> JULY 2000**

**Symposium 3 – Gene Mapping (Chair: Alan Wilton)**

- 8:45 - 10:00**     *Keynote Address: Joe Terwilliger* - Feasibility and resolution of gene mapping experiments with complex traits
- 10:00 – 10:30**     *Morning Tea*
- 10:30 – 11:00**     **Curt Brubaker** - Comparative genome mapping identifies homologous recombination events in *Gossypium hirsutum* X *G. australe* alien chromosome addition lines
- 11:00 – 11:30**     **Graham Webb** - A cytogeneticist bands up with gene localisation: RISH and FISH to animal chromosomes
- 11:30 – 12:00**     **Chris Moran** - Progress in domestic animal gene and quantitative trait locus mapping
- 12:00 – 12:30**     **Dave Heckel** - Insect Gene Mapping in the pre- and post-genomic eras
- 12:30 – 2:00**     *Lunch*



Afternoon Session – CSIRO Discovery

CSIRO  
Forestry Forest  
Products

Symposium 3 – Gene Mapping (continued) (Chair: Chris Moran)

- 2pm start.
- 2:00 – 2:20 Penny Butcher, Emlyn Williams, Dave Whitaker, Shiyong Ling, Terry Speed & Gavin Moran – Mapping in *Acacia mangium*: application of OUTMAP – a package for linkage analysis in outcrossed forest trees
- 2:20 – 2:40 Amanda Chamberlain, Mick Carrick, Helen MacPartlan, Thamy Balasingham, Phil Bowman, Nicholas Robinson & Mike Goddard – Mapping QTL affecting milk composition traits in dairy cattle using a complex pedigree
- 2:40 – 3:00 Helen MacPartlan, Amanda Chamberlain, Thamy Balasingham, Mick Carrick, Nick Robinson & Mike Goddard – Fine scale mapping of genes involved in milk quality in cattle
- 3:00 – 3:20 J.H. Edwards & Leif Anderson – Sib-similarity in pigs
- 3:20 – 3:50 Afternoon Tea

VIAS / UMelb

VIAS / UMelb

Symposium 3 – Gene Mapping (continued) (Chair: Dave Heckel)

- 3:50 – 4:10 J.A. Lade, G. Guo, E.K. Moses, A.N. Wilton, M. Grehan, R. North, D.W. Cooper & S.P. Brenneke – Suggestive evidence from a genome-wide scan for pre-eclampsia/eclampsia susceptibility loci on at least four chromosomal regions
- 4:10 – 4:30 Gavin Huttley, Susan Wilson, Glenys Thomson, John Hopper, Deon Venter & Simon Eastaugh – BRCA1 adaptive evolution and intramolecular epistatic interactions contribute to breast cancer risk
- 4:30 – 4:50 Glenys Thomson – Meta analysis of relative penetrance rank order statistics with application to HLA DR-DO genes and type 1 diabetes

4:50 – 5:10 C. White, S. Donnellan, C. Kemper and P. Hale – Molecular evidence for one species of the common dolphin, *D. delphis*, in southern Australia

5:15 – 6:00 Annual General Meeting (CSIRO Discovery)

Afternoon Session – CSIRO Building 1

Contributed Papers 4 – Conservation and Population Genetics (Chair: Geoff Clarke)

- 2:00 – 2:20 Weijong Ji, Stephen Sarre, Nicola Aitken, Robin Hankin & Mick Court – Sex related dispersal in stable and recovering populations of possums as revealed by minisatellite DNA profiling
- 2:20 – 2:40 Adam Stow, Paul Sunnucks, David Briscoe & Michael Gardner – The impact of habitat fragmentation on dispersal in Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites
- 2:40 – 3:00 Gudrun Wells – Fine scale genetic structure in *Rutidosis leptorrhynchoides*
- 3:00 – 3:20 David Coates, Linda Broadhurst & Pete Whitaker – Population genetic structure and spatial/temporal mating system variation in two rare banksia species
- 3:20 – 3:50 Afternoon Tea



## Contributed Papers 5 – Conservation and Population Genetics (cont) (Chair: Andrew Young)

- 3:50 – 4:10 Susan Hoebee & Andrew Young – Low effective population size and high interpopulation differentiation in the endangered shrub *Grevillia iaspicula* McGillivray (Proteaceae)
- 4:10 – 4:30 Rod Peakall & David Lindenmayer – Genetic consequences of habitat fragmentation: unravelling the complexity in native bush rats
- 4:30 – 4:50 Geoff Clarke – Conservation genetics of the endangered golden sun moth, *Synemon plana*
- 4:50 – 5:10 Barry Brook, David Tonkyn, Julian O’Grady & **Richard Frankham** – Contribution of inbreeding to extinction risk in threatened species
- 5:15 – 6:00 **Annual General Meeting (CSIRO Discovery)**

## FRIDAY 7<sup>TH</sup> JULY 2000

*Morning Session – CSIRO Discovery*

### MJD White Presidential Address (Chair: Richard Frankham)

8:45 - 10:00 **Jim Peacock** - Apomixis

10:00 – 10:30 *Morning Tea*

### Contributed Paper 6 - Chair: Ross Crozier

- 10:30 – 10:50 **David Reed** & Richard Frankham – The relationship between molecular and quantitative measures of genetic diversity: a meta-analysis
- 10:50 – 11:10 **Carla Sgrú** & Linda Partridge – The cost of reproduction and the evolution of ageing
- 11:10 – 11:30 **Katrina McGuigan** & Mark Blows – Morphological evolution in a freshwater fish
- 11:30 – 11:50 **Kaska Hempel** & Rod Peakall – Cleistogamy – microsatellites reveal insights in an unusual plant mating strategy
- 12:00 **Student prizes (CSIRO Discovery)**
- 12:30 *Lunch (BBQ at Australian National Botanic Gardens)*

*Morning Session – CSIRO Building 1*

### Contributed Papers 7 – Chair: Phil Batterham

- 10:30 – 10:50 **Jason Fair**, Michael Bogwitz, Trent Parry, Kris Behan, John Pollock & Phil Batterham – The molecular structure of the *lozenge* gene of *Drosophila melanogaster*
- 10:50 – 11:10 **Nicole Siddall**, Ben Hogan, Sally Coutts, John Pollock & Phil Batterham – The identification of genes that interact with *lozenge* in *Drosophila* eye development



- 11:10 – 11:30** **H. Indrasamy**, C. Milton, J.A. McKenzie & P. Batterham – The contribution of Notch signalling pathway to bristle asymmetry
- 11:30 – 11:50** **Claire Milton**, John McKenzie & Phil Batterham – Effect of chaperone genes on asymmetry in *Drosophila melanogaster*
- 12:00** **Student prizes (CSIRO Discovery)**
- 12:30** *Lunch* (BBQ at Australian National Botanic Gardens)







## PAPER ABSTRACTS

### DEFINING KEY AGRONOMIC TRAITS IN BREEDING PROGRAMS USING MOLECULAR GENETICS

**Rudi Appels**

*CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601*

An overview of contemporary issues in genetic mapping and plant/animal improvement will be provided, emphasising the outcomes from programs targeting specific quality and agronomic attributes. A more detailed discussion on new technologies that are becoming available, including detailed genetic maps, sequencing all the genes in a tissue of interest and loss/gain of function experiments in transgenic organisms, will be presented in the context of enabling more efficient progress in plant and animal improvement. Some specific examples of gene discovery in relation to well established traits from the wheat improvement area will include data on defining traits accurately to provide the basis for QTL analyses and subsequent complementation studies using transgenics wheat lines. In addition new data illustrating epistatic interactions between chromosome regions in wheat are now providing DNA markers for an aspect of phenotypic variation that was previously difficult to analyze.

### A MOLECULAR EVOLUTIONARY STUDY OF THE FISH GENUS *ODONTESTHES*: UNRAVELLING HISTORICAL AND RECENT BIOGEOGRAPHIC SCENARIOS IN SOUTH AMERICA

**Luciano B. Beheregaray<sup>1</sup>** and Paul Sunnucks<sup>2</sup>

*<sup>1</sup>Dept. Biological Sciences, Macquarie University, Sydney NSW 2109, <sup>2</sup>Dept. Genetics, La Trobe University, Melbourne VIC 3083*

*Odontesthes* silversides comprise a widespread marine-freshwater group that has been affected by physical events ranging from relatively ancient (formation of the Andes) to recent (lagoons formed during the last sea level changes). Phylogenetic relationships of all major lineages of *Odontesthes* (24-26 species) were investigated using mtDNA sequences of the control region, *ATPase 6-8* and *cytochrome b*. Sequences from different genes yielded better resolution of relationships at particular levels of divergence. Two groups that differ in geographic distribution and general morphology were identified: one with deeply diverged species from southern South America, and another recently formed clade with several species endemic to a small coastal area in southern Brazil. In a next step, detailed population level analyses were conducted in 450 individuals from two closely related species groups from southern Brazil (one marine, the other freshwater) using nine microsatellite loci and control region sequences. Incipient speciation in estuarine populations associated with strong adaptive divergence was detected in the marine group. In freshwater a remarkably recent radiation involving a minimum of five species was revealed along a complex system of coastal lagoons formed between 60,000 and 5,000 years ago. High levels of haplotypic variation and low phylogenetic signal were observed in control region sequences. On the other hand, microsatellites proved to be very useful showing that the major phylogeographic events of this radiation are consistent with the geological evolution of the region during Pleistocene-Holocene. Historical and recent biogeographic scenarios that have shaped the evolution of this genus are discussed.



Mark J. Blacket<sup>1</sup>, Mark Adams<sup>2</sup>, Carey Krajewski<sup>1,3</sup> and Michael Westerman<sup>1</sup>

<sup>1</sup>Department of Genetics and Human Variation, La Trobe University, Bundoora, Vic. 3083, Australia.

<sup>2</sup>Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA 5000, Australia.

<sup>3</sup>Department of Zoology, Centre for Systematic Biology, Southern Illinois University, Carbondale, Illinois 62901-6501, USA.

Genetic variation within the genus *Planigale* was examined through analyses of 12S rRNA gene sequences and allozymes. The level of genetic divergence between the five currently recognised *Planigale* species was compared and the magnitude of divergence among populations assessed. This examination of molecular variation within the genus revealed that *Planigale* contains far more taxonomic diversity than is currently recognised. Specifically the Pilbara region of Western Australia probably contains two currently unrecognised *Planigale* species and there is substantial genetic heterogeneity within the widespread species *P. maculata*. Ambiguity over the status of several genetic and/or morphological forms within the genus indicates that further taxonomic changes are likely to be warranted in the future. This study also demonstrates that the currently accepted geographic ranges of many planigale species require careful re-evaluation and that many specimens in collections are apparently misidentified. This is especially true of *P. ingrami* which appears to have a much greater range than is currently recognised, being present in South Australia.

#### EXPERIMENTAL DEMONSTRATION OF THE EVOLUTION OF REPRODUCTIVE CHARACTER DISPLACEMENT. II. EVOLUTIONARY CHANGE UNDER NATURAL SELECTION AND G MATRIX EIGENSTRUCTURE.

Mark Blows and Megan Higgie

Department of Zoology & Entomology, University of Queensland, St Lucia, QLD 4072

The extent to which the direction of evolution is influenced by genetic constraint is a long-standing problem. Multivariate evolutionary change may be constrained by the genetic covariance matrix, *G*. In particular, the first principal component of *G*, *g*<sub>max</sub>, has been used to determine if populations have been constrained to evolve in the direction of greatest genetic variance. Here, we present a genetic analysis of the response to natural selection in a natural selection experiment investigating the evolution of reproductive character displacement. The cuticular hydrocarbons of field allopatric populations of *D. serrata* evolved to resemble field sympatric populations when exposed to experimental sympatry with *D. birchii*. We use the Flury method of covariance matrix comparison to determine if this repeatable evolutionary response in a multivariate character was a consequence of *G* eigenstructure.



## EPIDEMIOLOGY AND VARIATION OF *ALTERNARIA BRASSICICOLA* IN NATURALLY OCCURRING POPULATIONS OF *CAKILE MARITIMA* ALONG THE NSW COAST

C.H. Bock, P.H. Thrall and J.J. Burdon

Centre for Plant Biodiversity Research, CSIRO-Plant Industry, GPO 1600, Canberra, ACT 2601

Knowledge of host-pathogen relationships is needed to understand both natural and agricultural systems. Pathogen populations change temporally and spatially through the interaction between ecological and coevolutionary processes. Within and among population dynamics of *Alternaria brassicicola* on the naturalised, coastal vagrant plant *Cakile maritima* were assessed during the 1998-99 season. The studies were conducted in three sub regions - Durras, Moruya and Central Tilba. Results showed that within populations disease incidence varied with plant age, density, distance from the sea and assessment date. The severity and timing of the epidemic was different between the three sub-regions, although the overall progression of the epidemic was similar. Population turnover appears to be a significant component of the dynamics of this system, with extinction being more likely to occur on less sheltered beaches. Recolonisation by both host and pathogen appears to depend on long-distance dispersal of sea-borne fruits, and our analyses showed higher rates of recolonisation on beaches with greater access to the sea. These results also imply that this system exhibits the intermediate levels of connectedness typical of a metapopulation situation. Preliminary AFLP analysis of pathogen isolates from different sites confirmed the haploid pathogen *A. brassicicola* is quite variable. Isolates of *A. brassicicola* were also readily distinguishable from other species of fungus, including *A. alternata* and *Rhynchosporium secalis*. We are in the process of using this variation to study the relationship between different populations of *A. brassicicola* in the three sub-regions along the NSW coast, and the extent to which populations are spatially-structured.

## CONTRIBUTION OF INBREEDING TO EXTINCTION RISK IN THREATENED SPECIES

Barry W. Brook<sup>1,2</sup>, David W. Tonkyn<sup>3</sup>, Julian O'Grady<sup>2</sup> and **Richard Frankham**<sup>2</sup>

<sup>1</sup> Key Centre for Biodiversity and Bioresources, Macquarie University, Sydney, NSW 2109, <sup>2</sup> Key Centre for Tropical Wildlife Management, Northern Territory University, Darwin, NT 0909, <sup>3</sup> Department of Biological Sciences, Clemson University, SC 29634, USA

Wild populations face threats from both deterministic factors (habitat loss, over exploitation, pollution and introduced species) and stochastic events associated with small population size (demographic and environmental stochasticity, catastrophes, inbreeding and loss of genetic diversity). However, the role of genetic factors is controversial. Inbreeding reduces reproductive fitness in naturally outbreeding species and it increases extinction risk in captive populations, but its role in extinctions of wild populations is controversial. It has been suggested that 'non-genetic' factors such as demographic and environmental stochasticity and catastrophes will drive populations to extinction before inbreeding effects become important. To critically evaluate the role of inbreeding in extinction, we conducted population viability analyses (PVA) of 20 endangered and one hypothetical species - with and without inbreeding depression. This was done for population sizes of 50, 250 and 1,000 for 3, 5 and 25 generations and 100 years, encompassing the IUCN categories of critically endangered, endangered and vulnerable. The impacts of purging of deleterious alleles were included in our projections. Inbreeding depression had little impact in the short-term (3 generations), but significantly increased the probability of extinction in most endangered species after 5-25 generations, even in the face of demographic and environmental stochasticity. The risk was greatest at low initial densities, increased with successive generations, and was amplified by population fluctuations due to catastrophes. The prospects for survival of endangered species may be seriously overestimated if genetic factors are ignored.



A GENOMIC SURVEY OF THE *C. ELEGANS* GENOME REVEALS DISTRIBUTION PATTERNS AND INSERTION SITE PREFERENCES FOR THE *Tc1/mariner/maT* SUPERFAMILY.

Jeremy Brownlie<sup>1,2</sup>, Charles Claudianos<sup>3</sup>., Steven Whyard<sup>2</sup>

<sup>1</sup>Division of Botany and Zoology, Australian National University, Canberra ACT 0200, <sup>2</sup>CSIRO Entomology, GPO Box 1700, Canberra ACT 2601, <sup>3</sup>Department of Biology, Imperial College London SW7 2AZ U.K.

Most transposable elements (TEs) display some degree of target site specificity when they move. The *Tc1/mariner/maT* superfamily of TEs, for example, always insert adjacent to a TA dinucleotide. In vitro transposition assays have identified that certain TA dinucleotides appear to be more favoured insertion sites than others (Vos *et al.*, 1996). Previous efforts to examine sequences flanking the insertion sites have failed to detect any unique features conserved among favoured sites. Recently, certain DNA physical structures and general composition of the flanking sequences have been identified as potential signals that a TE might recognise as a favourable locus for insertion (Liao *et al.*, 2000). Here we present some preliminary findings, which indicate that certain DNA physical properties are associated with insertion sites of a number of *Tc1/mariner* elements in the *Caenorhabditis elegans* genome, including the related novel element CemaT1. Analysis performed on flanking sequences obtained from in vitro *Tc1* transposition assays (Vos *et al.*, 1996) has also revealed the same association between DNA structures and insertion sites that were found for the genomic *Tc1* elements, such as nucleosome positioning, bendability of DNA, and G+C% content. A survey of all *Tc1/mariner/maT* elements within the *C. elegans* genome, including an as yet undescribed miniature inverted terminal element (MITE), CeminimaT-1 was performed, and no apparent hotspots of insertion over the chromosomes was observed for any of these elements.

USING COMPARATIVE GENOME MAPPING TO IDENTIFY HOMOELOGOUS RECOMBINATION EVENTS IN *GOSSYPIUM HIRSUTUM* X *G. AUSTRALE* ALIEN CHROMOSOME ADDITION LINES

Curt L. Brubaker

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

Successful use of the wild Australian *Gossypium* species in cotton breeding rests on the ability to generate fertile hybrids between tetraploid cultivated cottons and the wild diploid species, but more critically, the occurrence of sufficient levels of homoeologous recombination between the donor and recipient chromosomes. Having generated fertile hybrids that combine the 13 *G. australe* chromosomes in a *G. hirsutum* background by hexaploid bridging, 698 *G. australe*-specific *EcoRI-MseI* AFLP alleles are being used to estimate the frequency of homoeologous recombination relative to chromosome loss in aneuploid (4N+) x tetraploid backcross progeny. Among 18 aneuploid BC1 progeny, 634 *G. australe* alleles belong to one of 13 cosegregating but unrecombined suites of loci corresponding to the 13 *G. australe* chromosomes. The 18 BC1 individuals inherited 3 to 8 (mean 7) of the 13 *G. australe* chromosomes. Variation in the transmission of *G. australe* chromosomes was more variable, ranging from 2 to all 18 individuals. The 13 suites of unrecombined suites of loci are largely consistent with 13 ordered linkage groups inferred from a complementary genomic map comprising 449 *G. australe* and 397 *G. nelsonii* alleles (94 *G. nelsonii* x *G. australe* F2 progeny). Comparison of the two genomic maps is coinformative. Because the aneuploid map is not sensitive to marker density, small linkage groups in the *G. nelsonii* x *G. australe* map could be linked to larger linkage groups despite the lack of intervening markers. Incongruent allele placements between the two populations identify false linkages or homoeologous interchanges and the recombinant individuals.



## MAPPING IN *ACACIA MANGIUM*: APPLICATION OF OUTMAP - A PACKAGE FOR LINKAGE ANALYSIS IN OUTCROSSED FOREST TREES

Penny Butcher<sup>1</sup>, Emlyn Williams<sup>1</sup>, Dave Whitaker<sup>2</sup>, Shiyong Ling<sup>3</sup>, Terry Speed<sup>3</sup> and Gavin Moran<sup>1</sup>

<sup>1</sup>CSIRO Forestry and Forest Products, PO Box E4008, Kingston ACT 2604, <sup>2</sup>Statistics Department, University of Waikato, Private Bag 3105, Hamilton, NZ <sup>3</sup>Department of Statistics, University of California, Berkeley, USA 94720-3860

Mapping in forest trees generally relies on outbred pedigrees where genetic segregation is the result of meiotic recombination from both parents. Inbreeding depression has precluded the development of inbred lines and long generation times have led to the use of pedigrees for mapping where the linkage phase between pairs of loci is not known *a priori*. This contrasts with mapping inbred crop lines where linkage phase is known. The currently available mapping packages are not optimal for outcrossed pedigrees as they either cannot order phase-ambiguous data (for example MULTIMAP, CRIMAP, MAPMAKER), or only use pairwise information when ordering loci within linkage groups (JOINMAP). In addition, they do not provide a means of comparing different marker orders. The advantages of a new package, OUTMAP, are demonstrated using linkage data from two outcrossed pedigrees of *Acacia mangium* Willd. Segregation data from 219 RFLP loci and 33 microsatellite loci were mapped in thirteen linkage groups. When ordering loci, OUTMAP uses a maximum likelihood algorithm which utilises information from many loci simultaneously in contrast to JOINMAP which uses pairwise information only. The marker orders produced using OUTMAP were consistently of higher likelihood than those produced by JOINMAP. Distances between markers often varied from those calculated by JOINMAP, resulting in an increase in the estimated genome size. OUTMAP successfully handles all segregation types, determines phase, provides a choice of four optimisation methods and can calculate the likelihood of alternative marker orders.

## PHYLOGENETIC STRUCTURE IN THE YORK GUM COMPLEX, *EUCALYPTUS LOXOPHLEBA*, *E. GRATIAE* AND *E. BLAXELLII*.

M. Byrne<sup>1</sup> and B. Hines<sup>2</sup>

<sup>1</sup>CALMScience, Department of Conservation and Land Management, Western Australia, <sup>2</sup>Department of Botany, The University of Western Australia

Species in the York Gum complex have a widespread distribution throughout the wheatbelt and goldfields regions of Western Australia. *Eucalyptus gratiae* and the three subspecies of *E. loxophleba* have adjacent but overlapping distributions, whilst *E. blaxellii* is restricted to the Moresby range area north of Geraldton. There is large morphological variation within the species complex, and taxonomic boundaries are unclear due to overlap in characters used to distinguish the taxa, and the presence of intergrade populations. Phylogenetic relationships between the five taxa were investigated using RFLP analysis of the nuclear and chloroplast genomes. The species have high levels of diversity within taxa and little differentiation between taxa for the nuclear genome. *Eucalyptus blaxellii* was clearly distinguished from the other taxa. The tree forms (*E. loxophleba* ssp. *loxophleba* and ssp. *supralaevis*) were separated from the mallee forms (*E. loxophleba* ssp. *lissophloia* and *E. gratiae*), but within these groups the taxa were not separated. Variation in the chloroplast genome was high with 22 mutations distributed over 15 haplotypes. The majority of the variation was maintained between populations with little variation within populations. The haplotypes were structured into two major clades, which were not correlated with taxa, but showed some geographical correlation. The southeastern clade, although present at a high frequency, had lower diversity and fewer haplotypes than the other clade suggesting that it represents a more recent radiation in this area. These results confirm that the taxa are closely related. The mallee and tree forms are separated, and *E. blaxellii* is distinct from the other taxa. There is no evidence that *E. gratiae* is genetically distinct from *E. loxophleba* ssp. *lissophloia*, or that *E. loxophleba* ssp. *loxophleba* and *supralaevis* are genetically distinct.



## SYSTEMATICS OF THE LARGE BENTWING BAT (*MINIOPTERUS SCHREIBERSII*) IN AUSTRALIA.

Belinda Cardinal<sup>1,2</sup> and Les Christidis<sup>1</sup>

<sup>1</sup> Museum Victoria, GPO Box 666E, Melbourne, Vic, 3001, <sup>2</sup> Department of Genetics, University of Melbourne, Parkville, Vic, 3010

A combined molecular and morphological analysis was undertaken to resolve the systematics of the *Miniopterus schreibersii* complex in Australia. The study of skull morphology and sequence analysis of two mitochondrial genes, NADH dehydrogenase subunit 2 and cytochrome-b, revealed three distinct Australian forms of *M. schreibersii* which are treated as subspecies. *M. s. oriana* occurs in northern Australia, *M. s. oceanensis* occurs in eastern Australia from Queensland through to central Victoria and *M. s. bassanii* sp.nov. occurs in western Victoria and eastern South Australia. The biogeographical history of the complex is discussed in light of this new revision.

## MAPPING QTL AFFECTING MILK COMPOSITION TRAITS IN DAIRY CATTLE USING A COMPLEX PEDIGREE.

Amanda Chamberlain<sup>1</sup>, Mick Carrick<sup>1</sup>, Helen MacPartlan<sup>1</sup>, Thamy Balasingham<sup>1</sup>, Phil Bowman<sup>1</sup>, Nicholas Robinson<sup>1</sup> and Mike Goddard<sup>1,2</sup>.

<sup>1</sup>Institute of Animal Science, Agriculture Victoria, Attwood, 3049, Victoria., <sup>2</sup>Institute of Land and Food Resources, University of Melbourne, Parkville, Victoria.

Mapping of quantitative trait loci, (QTL), affecting milk composition traits using microsatellite markers usually uses large half-sib families. In a previous experiment conducted at VIAS, using 91 markers, QTL affecting one or more of five milk composition traits were found on chromosomes, 2, 3, 6, 14, 20, 21, 23 and 28. However the bulls used in the experiment were not those currently used in breeding programs. To implement marker assisted selection (MAS) in today's Holstein population we must be able to detect QTL in complex pedigrees without large families. Therefore this experiment had 671 bulls represented in a complex pedigree with 501 bulls being genotyped for 13 microsatellite markers from 7 chromosomes, 3, 6, 9, 14, 20, 23 and 28. The probability that each pair of chromosomes carried QTL alleles that were identical-by-descent was estimated from the marker and pedigree data using a Gibbs sampling procedure. These probabilities, among all chromosomes in the pedigree, constitute a gametic relationship matrix. The average daughter yield of the bulls was analysed using a linear mixed model that included the polygenic breeding value of each bull and the effects of the 2 QTL alleles carried by each bull. The variance component associated with the QTL was estimated by REML and its significance tested. Results of the analysis confirm that there are genes still segregating on chromosome 14, 20 and 23. The method estimates the effects of all QTL alleles in the pedigree and hence can be used for MAS.



Geoffrey M. Clarke

CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601

Native temperate grasslands are the most threatened ecosystem in Australia, with less than 1% of the original cover still remaining. Remaining patches are typically small and heavily fragmented. We undertook allozyme electrophoresis to assess levels of genetic variation and diversity and investigate patterns of population structure in the endangered grassland feeding moth, *Synemon plana*. Forty populations were sampled from throughout the geographic range of the species. Levels of genetic variation within most populations were lower than that observed in other lepidopteran species. The evidence suggests that this level of variation may have resulted from population bottlenecks and founder events following habitat fragmentation. Five distinct groups of *S. plana* populations have been identified which correspond closely with geographic location. One of these groups may be sufficiently different genetically to be regarded as a separate subspecies or race. These five groupings should be treated as separate units for conservation management.

#### MITOCHONDRIAL DNA ANALYSIS OF HONEY BEES FROM THE YUCATAN PENINSULA.

Kylea E. Clarke<sup>1</sup>, Benjamin P. Oldroyd<sup>1</sup>, J. Javier G. Quezada-Euan<sup>2</sup> and Thomas E. Rinderer<sup>3</sup>

<sup>1</sup> School of Biological Sciences A12, University of Sydney, NSW 2006, Australia. <sup>2</sup> Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma de Yucatán. Apdo. Postal 4-116, Mérida, Yucatán, 97100, México, <sup>3</sup> Honey-Bee Breeding, Genetics and Physiology Research Laboratory, USDA-ARS, 1157 Ben Hur Road, Baton Rouge LA 70820, USA

Honey bees (*Apis mellifera* L.) sampled at sites in Europe, Africa and South America were analysed using a mitochondrial DNA RFLP marker. These samples were used to provide base-line information for a detailed analysis of the process of Africanisation of bees from the Neotropical Yucatan peninsula of Mexico. Radical changes in mitotype frequencies were found to have occurred in the 13 year period studied. Prior to the arrival of Africanised bees (1986) the original inhabitants of the Yucatan peninsula appear to have been essentially of south eastern European origin with a smaller proportion having north western European ancestry. Three years after the migration of Africanised bees into the area (1989), only very low levels of maternal gene flow from Africanised populations into the resident European populations had occurred. By 1998 however, there was a sizeable increase in the level of Africanisation of domestic populations (61%) with feral populations having 87% of mitotypes classified as African-derived. The results suggest that the early stages of Africanisation did not involve a rapid replacement of European with African mitotypes and that earlier studies probably overestimated the prevalence of African mitotypes.



## POPULATION GENETIC STRUCTURE AND SPATIAL/TEMPORAL MATING SYSTEM VARIATION IN TWO RARE *BANKSIA* SPECIES

David J. Coates, Linda Broadhurst and Peta Whitaker

CALMScience, Western Australian Herbarium, Department of Conservation and Land Management, Locked Bag 104, Bentley Delivery Centre, WA 6983

*Banksia cuneata* and *Banksia oligantha* are two rare and threatened species geographically restricted to heavily cleared areas of the south-west Australian wheatbelt. Along with *B. ilicifolia* they comprise a separate subgenus (*Isostylis*) within *Banksia* and are considered to be an evolutionary link between *Banksia* and the related south-west endemic genus *Dryandra*. Both species occur in small fragmented populations in remnants of uncleared vegetation along road verges and on private land. Population genetic structure was investigated in *B. cuneata* and *B. oligantha*, and compared with preliminary data from the more widespread close relative *B. ilicifolia*. Temporal and spatial mating system variation was investigated in *B. cuneata* with significantly lower levels of outcrossing and high correlated paternity found in some small disturbed populations. However, this pattern was not consistent over all populations. Mating system studies on five populations of *B. oligantha* revealed high outcrossing rates in all populations regardless of population size or level of habitat disturbance. In contrast significantly higher correlated paternity was evident in the two smaller populations. Despite some interesting inconsistencies, these data suggest that habitat disturbance and population size can have a significant influence on inbreeding within populations of these *Banksia* species.

## MITOCHONDRIAL DNA PHYLOGEOGRAPHY OF THE DUNNART, *SMINTHOPSIS CRASSICAUDATA* AND THE SLEEPY LIZARD *TILIQUA RUGOSA* FROM SOUTHERN AND CENTRAL AUSTRALIA

Steven Cooper, Jan Birrell and Agatha Labrinidis

Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide SA 5000

Here we present the initial results of a project that aims to investigate the biogeographic history of southern and central Australia using a comparative phylogeographic approach. The fat-tailed dunnart *Sminthopsis crassicaudata* and the sleepy lizard *Tiliqua rugosa* both have a widespread distribution across Australia, encompassing a variety of habitats found in southern mesic-coastal through to arid-central regions. We have carried out a survey of mitochondrial DNA (mtDNA) variation in each species to determine whether there are common patterns of population structure that may be associated with environmental and climatic changes over the past several million years. Phylogenetic and restriction enzyme analyses of control region sequence data from the dunnart revealed two major mtDNA clades, with one clade restricted to populations southeast of the Murray River and a second clade found throughout the remaining central to south-western range of the species. Phylogenetic analyses of ND4 sequence data from the sleepy lizard, revealed three geographically localised clades, one restricted to southeast of the Murray River, a second found exclusively in WA through to the Nullarbor plain, and a third found in the remaining southwestern and northern distribution of the species. The finding that both species share a similar phylogeographic pattern and mtDNA divergence (3.4-5%) between populations on either side of the Murray River suggests that common evolutionary forces have operated to subdivide each species. One potential historical barrier to gene flow that may be involved is Lake Bungunnia that persisted in the Murray basin over much of the Pleistocene.



J H Edwards<sup>1</sup> and Leif Anderson<sup>2</sup>

<sup>1</sup> Biochemistry Department, Oxford OX3 7LP., Department of Veterinary Science, University of Sydney. [jhe@bioch.ox.ac.uk](mailto:jhe@bioch.ox.ac.uk), <sup>2</sup>Department of Animal Breeding and Genetics Swedish University of Agricultural Sciences Box 597, SE-751 24 Uppsala [Leif.Andersson@bmc.uu.se](mailto:Leif.Andersson@bmc.uu.se)

Most recent sib-pair studies in human disorders are restricted to affected sibs and analysed on the assumption that the parental gametes deduced from genotypes are representative of those present before fertilisation, each allele having an equal chance of both achieving fertilisation and surviving to birth. Such equality is not to be expected in view of the advantages of selective fertilisation suppressing fusion between pairs of identical alleles at some loci, as in many plants, losses through some allelic pairs or overlapping deficiencies presenting as embryonic lethals. These will present as a deficiency of some homozygotes usually leading to a proportionate excess of other homozygotes. That is, sib-similarity will be increased beyond the assumed 50% expectation of identity. There are few data relating to sib-similarity in normal sibs, the 'controls' necessary for any secure inference based on affected sib-pairs. In humans, cows, sheep and pigs the data are consistent with losses between conception and birth of over 25%. We present estimates on pigs of the same: different ratio of paternal and maternal alleles in gametes present in piglets from 24 litters. These were from a Wild Boar x Large White intercross (Andersson *et al.* 1994, *Science* 263:1771). Tissue samples for DNA preparation were collected after slaughter at about 6 months. The genome was scanned with 298 markers and analysed by simple counts of alleles derived from heterozygous parents of different genotype.

## THE MOLECULAR STRUCTURE OF THE LOZENGE GENE OF *DROSOPHILA MELANOGASTER*

Jason Fair<sup>1</sup>, Michael Bogwitz<sup>1</sup>, Trent Perry<sup>1</sup>, Kris Behan<sup>2</sup>, John A. Pollock<sup>2</sup> and P. Batterham<sup>1</sup>

<sup>1</sup>Department of Genetics, University of Melbourne, Victoria 3010, <sup>2</sup>Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania, USA.

*lozenge* (*lz*) is a classic complex gene. The four *lz* sub-loci (*spectacle*, *krivshenko*, *lozenge* and *glossy*) span a 0.14 map unit region on the X chromosome. *lz* mutants are pleiotropic affecting the differentiation of eyes, antennae, ovaries, legs and blood cells. *lz* mutations define two cistrons (Batterham *et al.*, 1996). Cistron A mutants map to the *spectacle* sub-locus and affect only the development of the eyes and antennae. Cistron mutants map to all four loci and affect all characters. The *lz* gene has been isolated; a single cDNA clone was shown to encode a transcription factor of the Runt/Acute Myeloid Leukemia 1 family (Daga *et al.*, 1996). In studies aimed at understanding the structure and function of the *lz* gene, we sequenced 45kb of DNA incorporating the *lz* locus. The genetic and molecular maps have been aligned by the molecular characterisation of genetically mapped mutants. Further, an additional *lz* mRNA transcript has been identified through PCR analysis of additional cDNA clones.

## Reference

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## AFTER CLADISTICS: TOWARDS AN INCLUSIVE PHILOSOPHY FOR PHYLOGENETIC INFERENCE

Daniel P. Faith<sup>1</sup> and John W. H. Trueman<sup>2</sup>

<sup>1</sup>*Australian Museum, 6 College St., Sydney, NSW 2000, Australia* <sup>2</sup>*Division of Botany and Zoology, Australian National University, Canberra, ACT 0200, Australia*

The wide range of methods used today in molecular phylogenetics documents the vitality of phylogenetic inference. Paradoxically, these ongoing innovations flourish even in the face of cladistics' long-standing, dogmatic, prescriptions about proper methods. A cornerstone of cladistics has been its supposed exclusive justification under Popperian philosophy. The most parsimonious tree, in minimising homoplasy, is supposedly "least falsified" and so most "corroborated", and other methods are excluded as "denying" corroboration. An alternative, inclusive, interpretation of Popper views cladistics as just another candidate methodology for phylogenetic inference. Here, Popperian evidence is the degree of fit of the data to a tree hypothesis, as defined by any phylogenetic method. Corroboration assessment is applicable to any phylogenetic method (Faith, 1992); it assesses how improbable it would be to observe that method's evidence even without the hypothesis. Cladists' objections to this inclusive framework are shown here to be based on misrepresentation. Further, the weaknesses in the cladistic interpretation of Popper are demonstrated, invalidating the claimed equation of cladistic parsimony with Popperian corroboration, and providing a new "level-playing field" for phylogenetic methods. Recognising cladistic parsimony as simple goodness-of-fit, not Popperian corroboration, cancels the supposed philosophical justification behind cladistic directives to use "total evidence", to not weight characters, and to avoid models. Over different independent sources of evidence, overall corroboration is a product of individual improbabilities - this new "combined evidence" approach can replace the poorly justified "total evidence". Different kinds of data may call for different methods for defining evidence, and different background knowledge for calculating improbability-of-evidence.

## IDENTIFYING QTL FOR WOOL PRODUCTION

Ian Franklin

*CSIRO Animal Production, Locked Bag 1, Delivery Centre, Blacktown, NSW 2148*

Approximately 8 years ago, the Division began to develop reference and resource families to develop high resolution linkage maps in sheep. In addition, these flocks have been measured for a range of production traits, and are now being screened, by conventional means, for QTL. However, we have developed, in parallel, skin cDNA libraries, ESTs and expression arrays, in order to identify gene expression patterns associated with differences in wool growth or wool quality. Because the wool follicle is a well defined tissue, and the primary determinant of wool growth rates and wool properties, it is well suited to EST studies directed at identifying genes involved in wool production. In this talk, I discuss some preliminary results, the limitations of conventional QTL analyses, the potential application of expression arrays and, more generally, the use of DNA marker technology in animal breeding research.



Split decomposition: A technique to analyse viral evolution

→ network



doesn't tell about recomb sites or evidence for recombination

## UNSCRAMBLING EGGS: WHAT TO DO WITH RECOMBINANT SEQUENCES.

Mark Gibbs

Research School of Biological Science, Australian National University GPO Box 475 Canberra 2601  
mgibbs@rsbs.anu.edu.au

Recombination has played a part in the history of almost all sequences of any reasonable length and this is important because, depending on the scale of the comparisons, recombinant sequences may foul up attempts to discover the phylogeny. Different regions in recombinant sequences have different evolutionary histories and so trees found from these sequences may contain large errors. Recently many methods have been developed for detecting recombination ([http://grinch.zoo.ox.ac.uk/RAP\\_links.html](http://grinch.zoo.ox.ac.uk/RAP_links.html)). Most of these check the consistency of trees found using different regions in a sequence alignment. Some use likelihood trees, others parsimony or distance matrix-based trees. Methods that are more novel include one that finds networks rather than trees and some that use neither, e.g. a BLAST-alignment method and a non-phylogenetic distance correlation method. Comparisons of these methods have not yet been done but, as a guide, when dealing with entirely new sequences database searches should be done to detect inter-species recombination and when working on phylogeny using aligned sequences, window-based scans of tree-consistency or evolutionary distances should be done. These approaches may provide evidence of recombination but to confirm recombination has taken place the patterns of substitution in the dataset need to be tested. Adrian Gibbs, John Armstrong and I have developed a method for this using Monte Carlo randomisation. We have used our method to pick out signals against the background noise that exists in sets of viral sequences and found evidence of intra- and inter-species recombination as well as genomic regions that contain no signals and ones that contain misleading signals.

LARP Likelihood analysis of Recombination in DNA  
- exhaustive search for crossover position

## COMPARING THE GENETICS OF CLINAL DIVERGENCE OF VARIOUS *D. MELANOGASTER* TRAITS

Stuart Gilchrist<sup>1</sup>, Jason Kennington<sup>2</sup> and Linda Partridge<sup>2</sup>

<sup>1</sup>Fruit Fly Research Centre, School of Biological Sciences, University of Sydney, NSW 2006,

<sup>2</sup>Department of Biology, University College London, London NW1 2HE, UK

On different continents, *D. melanogaster* has evolved strikingly similar patterns of divergence in body size. Smaller flies are found nearer the equator, while larger flies are found in temperate regions. Using flies from three continents ("replicate" examples of phenotypic evolution), we compared the genetic basis of the clinal variation for body size on each continent. We also compared the genetic basis of divergence in desiccation and starvation resistance in two of the same clines. As a contrast, we also investigated the genetics of wing shape, as it is likely to be less closely connected to fitness. The results are discussed in the context of theories of how natural selection may shape genetic architectures depending on their relation to fitness.

Alternative

→ Sliding window alignment

ncbi web site for looking at HIV sequence (not applicable to PERV !!)

New method. - Monte Carlo simulation - randomise sequence within columns  
- calculate 3 score.

Sister scanning: a Monte Carlo procedure for assessing signals in recomb sequences  
Mark Gibbs, Armstrong & A Gibbs

Jon Hean most test for presence of recomb PERVs by test PCRing directly from genomic DNA.



## THE INHERITANCE OF TRAITS IN THE *RANUNCULUS DISSECTIFOLIUS* - *R. MILLANII* HYBRID COMPLEX

**Kelli M. Gowland**, Julian E. Ash and Tristan Armstrong

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

Five morphologically distinct species of Australia's alpine *Ranunculus* section *Chrysanthae* have been found to freely hybridise producing viable hybrids. Previous work has suggested that the vegetative morphology of these five species confer selective advantages within their respective habitat niches, as intermediates are selected against within species' habitat. However, in order for the species' to remain distinct despite potentially disruptive introgression, either barriers to interbreeding, gene linkage or adaptive gene complexes can be expected to evolve. Thus, an investigation was conducted on the *Ranunculus dissectifolius* - *R. millanii* complex, to identify barriers to interbreeding, and determine the inheritance pattern of particular traits thought to confer adaptive advantages in the field. No intrinsic barriers to interbreeding were identified as viable F<sub>1</sub> and F<sub>2</sub> generation hybrids and backcrosses were produced. The pattern of traits suggested both simple and complex modes of inheritance. The inheritance of laminar surface hairs indicated simple, major gene control with traits nearly fixed within the parental populations with about 10-16% recessives. By contrast, a number of the leaf size and shape traits suggested quantitative inheritance patterns, with some evidence of complex epistatic interactions present and a net dominance by *R. millanii* genes. Whilst there was some correlation between some of the size and shape traits, there was little evidence to suggest strong linkage and it was concluded that the genes were inherited independently. Thus it is proposed that as selection eliminates unfit phenotypes, and that genes appear to be inherited independently, the lack of barriers to gene flow may allow beneficial genes to spread, such that interbreeding and lack of linkage may be beneficial.

## MOLECULAR GENETICS OF FATTY ACID DESATURATION IN OILSEEDS AND ITS RELEVANCE TO OIL QUALITY MODIFICATION

Allan Green and **Surinder Singh**

*CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601*

Vegetable oils constitute one of the world's most important plant commodities with current annual production in the region of 100 million metric tons. There is an ongoing interest in modifying oil fatty acid composition to produce new and potentially more valuable vegetable oils. An extensive effort in generating fatty acid biosynthesis mutants, in particular fatty acid desaturation mutants, has resulted in a good understanding of seed lipid biosynthesis pathways. This has enabled the use of conventional and mutational breeding and, more recently, genetic engineering tools to produce many desirable fatty acid modifications in seed oils. The potential of this last approach and some of our recent results will be discussed.



**David G. Heckel**

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

In recent years there has been a significant increase in the number and diversity of insect genetic linkage mapping studies. These now include two or more species from each of the Diptera, Coleoptera, Hymenoptera, and Lepidoptera, and at least one Orthopteran. Most maps are built primarily using a single type of high-throughput molecular marker such as RAPDs, AFLPs, or other PCR-based techniques. In several cases the maps have been immediately applied to the analysis of the genetic basis of complex phenotypes such as behaviour, wing colour patterns, insecticide resistance, parasite vectorial competency, or various developmental mutants. This approach has obvious utility in evaluating candidate genes underlying the traits but it remains to be seen how quickly it will lead to the cloning of truly novel genes. A major limitation at present is that most mapped markers are not easily comparable across species borders. There is thus a need for concerted development of anchor loci for insects, so that questions of genome and chromosomal evolution can be addressed as they have been successfully within mammals and grasses. We will also consider how insect linkage maps can best be employed to aid the fledgling genomics approaches now underway for many of these species, and how the recently-obtained *Drosophila* sequence is most likely to be useful in these endeavours.

## CLEISTOGAMY - MICROSATELLITES REVEAL INSIGHTS INTO AN UNUSUAL PLANT MATING STRATEGY

**Kaska Hempel and Rod Peakall**

*Australian National University, Canberra ACT 0200*

The plant reproductive strategy of cleistogamy (CL) combines normal, open and potentially cross-pollinated flowers (OP) with modified flowers that self-fertilise obligately in buds. The study of CL provides a unique perspective on the evolution of selfing, an important topic in plant evolutionary biology. For CL species, the maintenance of OP is a puzzle, because reproduction via CL flowers provides reproductive assurance and reduces resource allocation to reproduction. Here I use a population genetic approach applying microsatellite markers to determine the relative importance of CL and OP in *Glycine clandestina*. To assess the range of mating patterns, inbreeding levels of 7 populations in the Canberra region were determined from five loci. Significant differences in inbreeding coefficient between populations suggested a range of selfing from 60 to 90%. Such variation indicated that populations differed in their reproductive strategy, either in the proportion of CL, the proportion of OP selfing, or both. Two populations with divergent inbreeding coefficients were studied in detail. Genetic estimates of OP selfing were combined with estimates of the proportion of CL in the field. The more inbreeding population had significantly higher selfing rates in OP flowers and a greater proportion of CL. The results indicate that: the contribution of CL to reproduction varies among populations; CL is a predominant source of inbreeding; and CL contributes substantially to seed production in *G. clandestina*. Application of microsatellites will be extended to the study of inbreeding depression and its role in the evolution of CL in *G. clandestina*.



# EXPERIMENTAL DEMONSTRATION OF THE EVOLUTION OF REPRODUCTIVE CHARACTER DISPLACEMENT. I. RESPONSE OF MATE RECOGNITION TO NATURAL SELECTION.

Megan Higgie<sup>1</sup>, Steve Chenoweth<sup>2</sup> and Mark Blows<sup>1</sup>

<sup>1</sup>Department of Zoology & Entomology, University of Queensland, St Lucia, QLD 4072, <sup>2</sup> Faculty of Environmental Sciences, Griffith University, Nathan, QLD 4111

A direct role for natural selection in the generation of reproductive isolation during speciation is highly controversial. Natural selection may rapidly increase divergence in mate recognition between sympatric populations of speciating taxa by selecting against hybridisation between heterotypic individuals. The reinforcement of mate recognition by this process will result in the pattern of reproductive character displacement, where sympatric populations of closely related species have diverged in mate recognition to a greater extent than allopatric populations. Field populations of *Drosophila serrata* display reproductive character displacement in cuticular hydrocarbons (CHCs) when sympatric with *Drosophila birchii*. To test if natural selection on mate recognition was responsible for generating this pattern of reproductive character displacement, we exposed three independent field sympatric populations and three independent allopatric populations of *D. serrata* to experimental sympatry with *D. birchii* for nine generations. Cuticular hydrocarbons of field allopatric *D. serrata* populations evolved to resemble the field sympatric populations, whereas field sympatric *D. serrata* populations remained unchanged. We show that natural selection operated not on reduced hybrid fitness as is commonly assumed, but on the ability of field allopatric males to efficiently discriminate conspecific females in sympatry. Our evolutionary manipulation indicates that natural selection on mate recognition resulted in the field pattern of reproductive character displacement.

# LOW NEIGHBOURHOOD SIZE AND HIGH INTERPOPULATION DIFFERENTIATION IN THE ENDANGERED SHRUB *GREVILLEA IASPICULA* (PROTEACEAE)

Susan Hoebee and Andrew Young

Centre for Plant Biodiversity Research, CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601 and Department of Forestry, Australian National University, Canberra ACT 0200

Mating system parameters and genetic diversity were examined for five of the eight known populations of the endangered shrub *Grevillea iaspicula* (Proteaceae). Controlled pollinations show that *G. iaspicula* has an effective self-incompatibility system and little potential for agamospermy. This is reflected in uniformly high multilocus outcrossing rates ( $t_m=0.96-1.00$ ). However, average paternal diversity within open pollinated sibships is low ( $r_p=0.31-0.54$ ), suggesting that mating within populations is quite restricted. Despite the small size of most populations, four of the five populations studied have fewer than 20 reproductive individuals, the species maintains a moderate to high allelic richness ( $A=1.6-2.5$ ). Interpopulation genetic differentiation is high ( $D=0.04-0.32$ ), suggesting that gene flow is limited, even among populations separated by a few kilometres. Paternity analyses using microsatellites will be undertaken to further explore the restricted mating patterns within, and limited gene flow among, the populations.



## FUNCTIONAL CHARACTERISATION OF PLANT HEMOGLOBINS.

**Peter Hunt<sup>1</sup>**, B. Trevaskis<sup>1</sup>, Richard Watts<sup>1</sup>, Marc Ellis<sup>1</sup>, Mark Hargrove<sup>2</sup>, John Olson<sup>3</sup>, Liz Dennis<sup>1</sup> and J. Peacock<sup>1</sup>

<sup>1</sup>*CSIRO Division of Plant Industry, Black Mountain Laboratories, Canberra, ACT 2601*, <sup>2</sup>*Dept. of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA 50010, U.S.A.*,

<sup>3</sup>*Dept. of Biochemistry and Cell Biology, Rice University, Houston TX 77005-1892, U.S.A.*

Plant haemoglobin genes belong to two phylogenetically distinct families. GLB1 genes are represented in Monocotyledons and Eudicots and can be induced by hypoxic stress. GLB2 genes have only been observed in Eudicots and are expressed in response to developmental signals or by cytokinin application. Both GLB1 and GLB2 genes have been independently recruited in separate Eudicot groups for new functions in nitrogen fixing symbioses. A possible GLB3 gene family with close affinities to predicted hemoglobins from various bacteria has also recently been identified. We have used *Arabidopsis* as a model to study the expression and function of GLB1 and GLB2. GLB1 transcription increases during hypoxic stress and when using sucrose supplemented growth medium. GLB1-GUS reporter transgenes are expressed strongly in roots and cotyledons in response to these inducing conditions when the GLB1-3'UTR is used. At germination, GLB1-GUS is strongly expressed in cotyledons and hypocotyl tissues. Plants over-expressing GLB1 have enhanced survival following hypoxic stress. GLB2 responds to entirely separate inducing conditions. GLB2-GUS expression is activated in young plants in response to the cytokinin 2IP. During normal growth, GLB2-GUS is not expressed until after flowering, when it is expressed in roots, leaves, bolt stem and developing seeds. We have expressed both hemoglobins in *E. coli* and measured oxygen binding in the purified proteins. Oxygen binding affinities are high compared to animal hemoglobins or symbiotic hemoglobins, and the kinetics are influenced by the existence of two alternate deoxyhaemoglobin states.

## BRCA1 ADAPTIVE EVOLUTION AND INTRAMOLECULAR EPISTATIC INTERACTIONS CONTRIBUTE TO BREAST CANCER RISK

**Gavin A. Huttley<sup>1</sup>**, Susan R. Wilson<sup>2</sup>, Glenys Thomson<sup>3</sup>, John L. Hopper<sup>4</sup>, Deon J. Venter<sup>5</sup> and Simon Easteal<sup>1</sup>

<sup>1</sup>*Human Genetics Group, John Curtin School of Medical Research, ANU, Canberra, ACT 0200*,

<sup>2</sup>*Centre for Mathematics and Its Applications, ANU, Canberra, ACT 0200*, <sup>3</sup>*Department of Integrative Biology, MC#3140 3060 Valley Life Sciences Building, University of California, Berkeley, CA 94720-3140, USA*, <sup>4</sup>*Departments of Pathology and Research, Peter MacCallum Cancer Institute, East Melbourne, VIC 3002*, <sup>5</sup>*Centre for Genetic Epidemiology, Carlton, VIC 3053 Australia*

Some of the most fascinating questions in biology concern human adaptation. In particular, what genes are involved in human adaptive evolution, and what is the mode and tempo of molecular adaptation at these genes? We have recently shown that the breast cancer susceptibility gene BRCA1 has been subject to positive Darwinian selection in both humans and chimpanzees. This suggests that modifications to BRCA1 have been one important component of human molecular adaptation. The joint involvement of BRCA1 in human disease and adaptive evolution led us to hypothesise that some diseases may be an inextricable consequence of our adaptive evolution. We present evidence implicating variants at two BRCA1 polymorphic sites as candidates for the operation of selective sweeps affecting contemporary Europeans. The current operation of adaptive evolution at BRCA1 enables us to indirectly assess whether this process contributes causally to breast cancer. Specifically, do interactions between the ancestral and putative adaptive variants contribute to risk of breast cancer? We detected significant epistatic interactions between the candidate selected sites that distinguished breast cancer cases and controls. Consistent with our hypothesis, there was an enrichment of haplotypes consisting of either solely derived or ancestral amino acids in controls while the combination haplotypes (ancestral and derived amino acids) were over-represented in cases. One of the interesting implications of these results is that the high proportion of BRCA1 nonsynonymous substitutions throughout human evolution may have arisen in part from selection affecting BRCA1 intramolecular epistatic interactions.



## THE CONTRIBUTION OF NOTCH SIGNALLING PATHWAY TO BRISTLE ASYMMETRY

H. Indrasamy, C. Milton, J.A. McKenzie and P. Batterham

*CESAR - Centre for Environmental Stress and Adaptation Research, Department of Genetics, University of Melbourne, Victoria, Australia 3010*

Developmental stability is the ability of an organism to buffer genetic and environmental perturbation. Asymmetry has been used as a measure of developmental stability for bilaterally symmetrical organisms. Departures from symmetry have been used as a measure to address a wide range of biological problems. These include association between environmental degradation, mate choice, loss of heterozygosity and the impact of parasitism on host development. However, in most cases, the underlying genetic and environmental factors responsible for the observed asymmetry phenotype are largely undefined. The Notch signalling pathway genes control bristle development in *Drosophila melanogaster*. The contribution of Notch pathway mutants to asymmetry was studied for six different bristle characters. The impact of Notch pathway mutants on asymmetry is bristle character specific. Not all bristle classes are affected. This is an important contribution because there is much talk in the literature about 'asymmetry genes' of general effect. However, there is currently no evidence for such genes and the concept receives no support from our data. This has real implications for character choice in other studies where asymmetry may be used for biomonitoring. Unless a suitable range of characters is scored, asymmetry may not be detected. When the asymmetry scores of individual characters are pooled, the significant asymmetry levels observed may be due to a limited number of individual characters being significantly asymmetric. Thus caution is necessary when using the asymmetry phenotype of specific characters to draw organism wide conclusions about developmental stability.

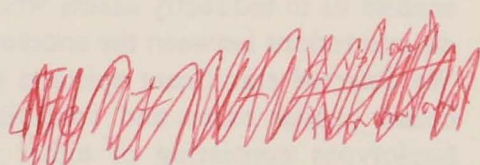
## TREEGENEBROWSER: A PHYLOGENETIC APPROACH TO SEQUENCE DATABASES

I.B. Jakobsen<sup>1</sup>, J.A., Saleeba<sup>2</sup>, M. Poidinger<sup>3</sup>, T.G. Littlejohn<sup>1,3</sup>

<sup>1</sup>*Australian Genomic Information Centre;* <sup>2</sup>*School of Biological Sciences, University of Sydney;*

<sup>3</sup>*eBioinformatics, inc.*

As sequence databases continue to grow, there is a need for new tools for searching and accessing this data. A useful biological approach is to consider sequences from a phylogenetic viewpoint: not merely in terms of the species an individual sequence is from, and that species' taxonomic status, but relating it to other sequences from other species. We have implemented such an approach for GOBASE (the Organelle Genome Database, <http://megasun.bch.umontreal.ca/gobase/gobase.html>), which includes curated organellar sequences that have been assigned to genes (loci). The TreeGeneBrowser allows selection and editing of a phylogenetic tree of interest to the researcher, and genes from GOBASE are selected and scored based on their location in the chosen tree. This approach is useful for identifying genes that have been widely sequenced within the phylogeny of interest, and for identifying 'holes' in the distribution of a particular gene, producing maximal phylogenetic data for minimum laboratory time. Additionally, the 'sequence richness' of each taxonomic branch can be viewed, allowing the identification of species groups with a lack of sequence data irrespective of the gene. This method can in principle be applied to any database with homologous genes.





## A QTL MAPPING APPROACH TO THE DISSECTION OF A COMPLEX TRAIT - BORON TOLERANCE

S.P Jefferies, A.R. Barr, M.A Pallotta , K.J. Chalmers and P. Langridge

*Department of Plant Science, Waite Campus, University of Adelaide, SA 5064, Australia and CRC Molecular Plant Breeding, Waite Campus, SA 5064, Australia*

Boron (B) is an essential plant micronutrient which can be phytotoxic to plants if present in soils in high concentration and has been recognised as an important problem limiting cereal production in the low-rainfall regions of southern Australia, West Asia and North Africa. The most obvious symptoms of B toxicity in wheat and barley are chlorosis and necrosis extending from leaf tips and leaf margins. In barley, brown lesions form initially at the leaf margins but extend over the distal half or more of the leaf. In severe situations symptoms can appear on leaf sheaths, stems and awns. Boron toxicity can also inhibit root and shoot growth. In southern Australia, B toxicity is most frequently observed in low rainfall environments and consequently, restricted root growth can inhibit access to sub-soil moisture reserves and subsequently reduce water use efficiency. As B tends to accumulate in toxic concentrations in sub-soils, soil amelioration is generally considered impractical. A genetic solution may, therefore, be the only practical and environmentally sustainable solution. Genetic variation for B tolerance in both wheat and barley has been reported. Boron tolerance is defined as the ability of a genotype to produce high relative grain yield in B toxic situations. Elucidation of genetic factors contributing to a specific adaptation of this type is often confounded by other, important but possibly unrelated, genotype by environment interactions. In this paper we report on the use of a trait dissection - QTL mapping approach to obtain an improved understanding of the genetic and physiological mechanisms involved in the control of boron tolerance in both wheat and barley. The number, location and proposed function of QTL/genes involved in the control of B tolerance in both wheat and barley will be reported.

## SEX RELATED DISPERSAL IN STABLE AND RECOVERING POPULATIONS OF POSSUMS AS REVEALED BY MINISATELLITE DNA PROFILING

Weihong Ji<sup>1</sup>, Stephen Sarre<sup>2</sup>, Nicola Aitken<sup>2</sup>, Robin Hankin<sup>1</sup> and Mick Clout<sup>3</sup>

<sup>1</sup>*School of Environmental and Marine Sciences, Auckland University, Auckland, New Zealand.*

<sup>2</sup>*Institute of Molecular Biosciences, Massey University, Palmerston North, New Zealand,* <sup>3</sup>*School of Biological Sciences, Auckland University, Auckland, New Zealand.*

Natal dispersal can have important effects on mammal population structure and dynamics. Such dispersal is of practical significance when applied to the control of pest species because dispersal may significantly, and undesirably, reduce the population recovery time following a control operation. The relative dispersal rate of the sexes is also critical because that too will affect the rate of population increase. Here, we use genetic similarity, as estimated by minisatellite DNA profiles to investigate dispersal in undisturbed and recovering populations of the Australian brushtail possum (*Trichosurus vulpecula*). Our results show that the genetic similarity in an undisturbed possum population was significantly lower between males than between females ( $P=0.012$ ). Conversely, genetic similarity between males and females in two recovering population was not significantly different, while relatedness among males was significantly higher ( $P<0.0009$ ) in a recovering population when compared with those in the pre-control population. These data indicate two important characteristics of dispersal in possums. (1) that dispersal in established populations is sex biased towards males, and (2) that within the first three years following population control, &#8220;the vacuum effect&#8221;, whereby individuals from areas adjacent to a control area, expand their home range and invade the depopulated area, is the most important source of re-colonisation for possums.



# THE REALITY OF STRUCTURED SEQUENCE DATA REQUIRES A RETHINKING OF PHYLOGENETIC ANALYSIS PROCEDURES

Scot A. Kelchner

*Centre for Plant Biodiversity Research, CSIRO Plant Industry; and Division of Botany and Zoology, The Australian National University*

All phylogenetic methods assume independence of site mutations for each nucleotide in a DNA sequence matrix. This assumption influences every level of conventional analysis, from alignment of gaps to tree construction and measures of clade support. Consequently, phylogeny estimation is directly dependent on the validity of this supposition. However, all DNA and RNA sequences contain structured elements, and a rethinking of conventional analysis procedures is necessary to cope with the invalidated assumption of character independence. The chloroplast *rpl16* intron is an excellent model for defining structured elements in DNA sequence matrices because secondary structure and tertiary interactions in this molecule are empirically well-defined. Sequences from this intron may be considered aggregates of structural elements evolving under different evolutionary constraints. Recognition of these differing constraints by application of more accurate molecular evolution models would, in principle, lead to improvement of phylogenetic techniques in recovering hierarchical signal in the data. Alignment can be enhanced by identification of structural components and mutational mechanisms, and subsequent analyses may be improved by adjustment of model parameters to counter the measurable biases inherent in this type of data. Highly structured sequences are often approached with apprehension, but they hold great promise for improving accuracy of evolutionary models, and thus accuracy of hierarchical signal detection that results in topologies.



# SUGGESTIVE EVIDENCE FROM A GENOME-WIDE SCAN FOR PRE-ECLAMPSIA / ECLAMPSIA SUSCEPTIBILITY LOCI ON AT LEAST FOUR CHROMOSOMAL REGIONS

**JA Lade**<sup>1,2</sup>, G Guo<sup>3</sup>, EK Moses<sup>1</sup>, AN Wilton<sup>3</sup>, M Grehan<sup>1</sup>, R North<sup>4</sup>, DW Cooper<sup>5</sup> and SP Brennecke<sup>1</sup>.

<sup>1</sup> *Department of Perinatal Medicine, Royal Womens Hospital, Melbourne, Australia;* <sup>2</sup> *Department of Genetics, LaTrobe University, Bundoora, Australia;* <sup>3</sup> *School of Biochemistry and Molecular Genetics, University of NSW, Sydney, Australia;* <sup>4</sup> *National Womens Hospital, Auckland, New Zealand;* <sup>5</sup> *School of Biological Sciences, Macquarie University, NSW, Australia.*

Pre-eclampsia/eclampsia (PE/E) is the most common serious medical disorder complicating human pregnancy, which impacts on both mother and child. Although the aetiology of this disorder is not completely understood, the placenta is fundamentally involved. Familial clustering of PE/E demonstrates a strong genetic component, but the paradoxical inheritance pattern exhibited suggests that PE/E is a complex trait. Here the results for chromosomes 3, 4, 7, 8, 12, 13, and X of a genome-wide scan, using Australian and New Zealand pedigrees are reported. Both parametric (using one simple and one affecteds only analysis recessive and dominant inheritance model) and non-parametric analyses were used. Four chromosomal regions were identified which conferred suggestive evidence for PE/E susceptibility loci. A suggestion of linkage was identified at D4S1535 on 4q35 using two-point LOD score analysis (maximum LOD of 1.78 at  $2=0.10$ ), and multipoint analysis (maximum LOD score of 2.13; NPL score of 1.84,  $p$ -value=0.01). Suggestive linkage on 7q36 was observed at D7S1805 by two-point LOD score analysis (maximum LOD score 2.14 at  $2=0.14$ ), APM analysis ( $f(p)=1/\sqrt{T}$ ,  $T=3.53$ ,  $p$ -value=0.009) and multi-point analysis (maximum LOD score of 2.87). These findings are consistent with earlier reports from our group. However, two novel chromosomal regions were also identified. Firstly, 13q33 gave suggestive evidence of linkage for two-point LOD score analysis (maximum LOD score of 1.92 for nuclear families) and sib pair analysis (LOD score of 2.25; mean test = 12.66,  $p$ -value=0.0006) at D13S1265, and a maximum multipoint NPL score of 1.86 ( $p$ -value=0.01). This is of particular interest given the significant association between PE/E and trisomy 13 fetuses. Secondly, Xq27-28 gave suggestive evidence of linkage for two-point analysis (maximum LOD score of 1.94 at  $2=0.16$ ) at DXS8091, and APM analysis over three consecutive markers DXS297, DXS8091 and DXS1073 ( $f(p)=1/\sqrt{p}$   $p$ -values=0.005, 0.017 and 0.0006, respectively), with a corresponding multipoint NPL score of 1.51 ( $p$ -value=0.05). Given the suggestive nature of these linkages, these findings should be considered as hypothesis generating, and await further verification or exclusion to these regions from independent data sets.



# MOLECULAR GENETICS OF DISEASE RESISTANCE IN WILD RELATIVES OF WHEAT AND THEIR APPLICATION TO WHEAT IMPROVEMENT

E.S. Lagudah<sup>1</sup>, **W. Spielmeyer**<sup>1</sup>, O. Moullet<sup>1</sup>, S. Seah<sup>1</sup>, F. Ogonnaya<sup>2</sup>, R. Eastwood<sup>2</sup>, H. Bariana<sup>3</sup>, J. deMajnik<sup>1</sup> and R. Appels<sup>1</sup>

<sup>1</sup>CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, <sup>2</sup>Department of Natural Resources and Environment, Horsham VIC., <sup>3</sup>University of Sydney, Plant Breeding Institute, Cobbity, NSW 2570

*Aegilops tauschii* (syn. *Triticum tauschii*) is the diploid D genome progenitor of hexaploid bread wheat. The wide genetic variation, diploid genome, high density genetic map, availability of a large DNA insert library and the ability to transfer genes of agronomic value by homologous recombination into bread wheat are the basis for exploiting *Ae. tauschii* in wheat improvement. Expression of disease and pest resistance phenotypes transferred from *Ae. tauschii* into hexaploid wheat ranges from complete, partial and suppressed phenotypes. Partial and suppressed phenotypes are commonly associated with foliar diseases, whereas resistance to insect and nematode species exhibit similar resistance levels in both the diploid and allohexaploid genome. Gene sequences encoding nucleotide binding site and leucine rich repeats (NBS-LRR) at the Cre3 cereal cyst nematode (CCN) resistance locus in wheat derived from *Ae. tauschii* identify homologues in non-syntenic regions of the wheat genome that correspond to other CCN resistance genes (Cre1, Cre6 and CreX). The Cre3-derived sequences are the basis of "perfect markers" currently being used for selecting and pyramiding these CCN resistance genes in wheat breeding in Australia. A leaf rust resistance gene, Lr21, also derived from *Ae. tauschii* is present on the distal end of wheat chromosome 1D. A disease resistance gene candidate, also from the NBS-LRR superfamily, for a stripe rust resistance gene on wheat chromosome 1B was used to identify homologues in *Ae. tauschii* that cosegregate with Lr21 in a highly recombinogenic region of the genome. A combination of chemical mutagenesis and plant transformation approaches are being used in order to ascertain the structure of the Lr21 gene. Resistance gene analogues based on NBS-LRR sequences have also been used to tag an introgressed segment derived from another wheat relative, *Ae. ventricosa*, which carries three rust resistance genes, Yr17, Lr37 and Sr38 used in wheat breeding programs in Australia and Europe.



THE ORIGIN AND HISTORICAL BIOGEOGRAPHY OF THE LARGE CARPENTER BEES  
(*XYLOCOPA*)

Remko Leys<sup>1,2</sup> and Steve Cooper<sup>1</sup>

<sup>1</sup>*Evolutionary Biology Unit, South Australian Museum, North Tce. Adelaide, SA 5000,* <sup>2</sup>*School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001*

Large carpenter bees (*Xylocopa*) comprise a worldwide genus of which the species have tropical, semitropical or occasionally temperate distributions. Molecular phylogenies show good support for a major division of the genus into three groups of subgenera and one sister lineage. These groups consist of subgenera with distinct distribution patterns in South America, Africa and India-South East Asia, and of a group of subgenera with mixed distributions. Here we test the alternative hypotheses that the distinct South American and African distributions have originated (1) by vicariance (breaking up of Gondwanaland), or (2) by relatively more recent dispersal from one of the continents. To test these hypotheses we applied molecular clocks, based on ant subfamily data (Crozier *et al.* 1997) and calibrated using fossil carpenter bees. Estimated divergence times for the major carpenter bee divisions and historical geography data (Smith *et al.* 1994) were then used to deduce the most likely area of origin of the carpenter bees. Our carpenter bee results show that the disjunct Asian/Oriental - South American distribution pattern, which is a general trend in many bee groups (eg. Meliponid bees) and that have been explained by vicariance and Gondwanan origins, are more likely the result of Palaeogene dispersals across northern landbridges (Bering Strait, Greenland route), with subsequent disappearance from the northern continents due to Pleistocene ice ages. The occurrence of carpenter bee subgenera in Africa and Australia are best explained with dispersals from Eurasia not earlier than Late Oligocene, when these southern continents had drifted close enough to the Eurasian continent to allow so.

Crozier *et al.* (1997). *Naturwissenschaften* 84: 22-23.

Smith *et al.* (1994). Cambridge University Press, Cambridge.



## TESTS OF THE HYBRID ELEMENT INSERTION/REPAIR MODEL FOR P ELEMENT-INDUCED RECOMBINATION IN *DROSOPHILA MELANOGASTER*

Xiumei Liang and John Sved

*School of Biological Sciences, University of Sydney, NSW 2006*

Gray *et al.* (Genetics 144: 1601-1610, 1996) have produced a model which provides at least a partial explanation of how P elements generate recombination. It is assumed that P elements normally replicate by excising, leaving a gap to be refilled by copying from the sister strand, followed by insertion elsewhere in the genome (cut, copy and paste). The excision step presumably starts with association of the left and right ends of the element. Gray *et al.* showed that if the left and right ends which associate are from sister chromatids, rather than from the same chromatid, the expected result is often a recombination event. The principal evidence supporting the model comes from genotypes which combine elements lacking either the right or left end (end-deleted elements). The model in this situation has asymmetrical predictions, with one class of recombinants attributable to the insertion event (Hybrid Element Insertion - HEI) and expected to have chromosomal changes, and the reciprocal class being attributable to repair (HER), and expected to lack chromosomal changes. In agreement with the model, most chromosomal changes came in the first class. However slightly more than 50% of the first class of recombinants lacked any sign of chromosomal change. These recombinants could either have been HEI events where the insertion was very close to the original site, or alternatively HER or some other type of repair events. The latter result suggested the possibility that P element ends which ought to be associated in a 'transpososome' structure, might lose their association, and precipitate a repair event which leads to recombination. We have studied the recombinants produced by end-deleted elements using a set of closely linked RFLP markers developed by Preston and Engels (Genetics 144: 1611-1622, 1996). We succeeded in jumping one of the end-deleted elements into a genetic background which enabled the recombination in the region of the element to be measured. We found that, as expected, the recombination of outside markers was accompanied by recombination of the most distal RFLP markers, placing the point of recombination within an interval of a few kb. However the full results are difficult to explain on the basis of insertion events, and suggest that there may be a novel mechanism for joining of P element-containing ends. We also report on other experiments which follow the fate of novel elements, 'head-to-head' and 'head-to-tail' elements generated by insertion from the end-deleted elements. One frequent outcome from the head-to-tail elements is the precise excision of one of the element ends. This result again indicates that some elaboration of the HEI model may be needed.

## CHOPPING DOWN TREES WITH BIONAVIGATOR- A LUMBERJACKS GUIDE TO PHYLOGENETICS

Tim Littlejohn

*Head, ANGIS, Sydney, Australia*

Genomics has catalysed the rebirth of genetics, and bioinformatics tools are now a part of modern genetics research. Bioinformatics tools are required for the management, processing and visualisation of genetic, genomic and functional genomic data. Researchers face a plethora of difficulties in making the most of this data and the bioinformatics tools- phylogenetic analysis being among the more complex and integrated problem spaces. Researchers typically struggle with learning how to use and stay on top of bioinformatics tools for the analysis of data. The problems of data and application integration, high throughput processing, managing information flow from raw to functional data, repeatability and publication of bioinformatics analyses still create significant bottlenecks in research. The "BioNavigator" bioinformatics system is based on over a decade of research into Internet-based bioinformatics application and service delivery at ANGIS and it's spin-off company eBioinformatics. Scientific and engineering issues including useability, reuseability, process flow, throughput, scalability, integration, project management and domain knowledge of bioinformatics resources, especially phylogenetics, will be addressed.



## INCORPORATION OF SECONDARY STRUCTURE INFORMATION IN A PHYLOGENY OF THE STINGLESS BEES

David Low<sup>1</sup>, Ben Oldroyd<sup>1</sup>, Lars Jermini<sup>2</sup>

<sup>1</sup>*School of Biological Sciences, and* <sup>2</sup>*Australian Genomic Information Centre, University of Sydney*

Phylogenetic analysis of nucleotide sequences often requires the user to choose a nucleotide substitution model. This choice may affect the phylogenetic result and it is therefore important that the model should not be violated by signals in the data. While generally recognised, this principle is rarely adhered to, and information on the secondary structure of ribosomal RNA and proteins is mostly ignored. Given that the secondary structure is closely associated with functional constraints, these constraints may also affect the rate of change of the encoding sequences. Here we investigate a poorly understood phylogeny, that of the stingless bees (Hymenoptera; Apidae; Meliponini), and determine whether inclusion of structural details for the mitochondrial encoded cytochrome b and c improves phylogenetic inference. In our analysis we have partitioned nucleotide sequence by both codon position and secondary structure of the encoded polypeptide, where amino acids (and hence the corresponding codons) were classified as membrane-spanning, inter-membrane or extra-membrane. Nucleotides of the same codon position in each partition of the secondary structure are thought to be under similar selective pressure. Assigning similar weighting to nucleotides partitioned in this way may improve corrections for homoplasy in the inferred trees. Our analysis employed maximum likelihood and an iterative optimisation of the weighting parameters, and the results show a significantly better fit of the data to the more realistic structural model.

## MORPHOLOGICAL EVOLUTION IN A FRESHWATER FISH

Katrina McGuigan and Mark Blows

*Department of Zoology and Entomology, University of Queensland, Brisbane 4072, Australia*

*Melanotaenia eachamensis*, the Lake Eacham Rainbowfish, is endemic to lakes and streams on the Atherton Tablelands of far north Queensland. We sampled *M. eachamensis* from two lake and two stream populations, from independent catchments. Body shape was quantified using a 10 landmark truss network. Multivariate analyses of morphology indicated that the axis of greatest variation discriminated between the lake and stream habitats. Divergence across habitats was primarily a consequence of changes in tail morphology. This suggests that natural selection has independently resulted in the same shape characteristics within each of the habitat types. The same habitat-based morphology was found in two other species of rainbowfish (*M. splendida australis* and *M. duboulayi*). To determine whether morphological convergence in similar habitats is a consequence of a genetic constraint, we determined the genetic (co)variance matrix underlying the traits, *G*. The genetic (co)variances amongst traits suggest that the tail of the fish is free to evolve independent of the rest of the body. We assess if the direction of phenotypic divergence between habitats is associated with the direction of greatest genetic variance.



GEOGRAPHIC VARIATION IN FREQUENCY OF THE THR-GLY REPEAT-LENGTH  
VARIANTS IN THE *DROSOPHILA PERIOD* GENE ALONG THE AUSTRALIAN EAST COAST

Stephen W. McKechnie and Alisha R. Anderson

*Department of Biological Sciences and Centre for Environmental Stress and Adaptation Research  
(CESAR), Monash University, 3800, Australia*

The clock gene, *period*, of *Drosophila melanogaster* contains a segment encoding a threonine-glycine (Thr-Gly) repeat that is polymorphic for the number of repeats, and in Europe the variation shows significant latitudinal association. This has been attributed to differential adaptation to temperature, with the high repeat-number allele (Thr-Gly)<sub>20</sub> that predominates in the colder regions, being better able to maintain the same free-running circadian period when growth temperatures are different; the (Thr-Gly)<sub>20</sub> allele shows better "temperature compensation". We have sampled this same polymorphism along a latitudinal transect down the east coast of Australia where several other single-gene and inversion polymorphisms, and a number of quantitative traits, show strong latitudinal/climatic associated clines. Our data are inconsistent with that reported from the northern hemisphere, in terms of latitudinal and temperature associations, and therefore indicate that the putative selective factors need to be re-examined.

COLONIAL COLONIALS - CONTRASTING PATTERNS OF MITOCHONDRIAL DNA  
VARIATION IN THREE INTRODUCED BRYOZOANS SUGGEST TWO DIFFERENT  
INTRODUCTION HISTORIES.

Josh Mackie<sup>1,2</sup>, Les Christidis<sup>1</sup>, Mick Keough<sup>2</sup>

<sup>1</sup>*Molecular Lab, Museum of Victoria, GPO Box 666E, Melbourne, 3001.*, <sup>2</sup>*Zoology Department,  
University of Melbourne, 3010*

Bryozoa are colonial marine invertebrates, with a sessile adult stage and a short-lived planktonic dispersal stage. A number of species have a global distribution in ports and harbours, suggesting that they have been dispersed over the inter-continental by ships. C01 sequence variation was sampled from southern Australian populations of three introduced bryozoans to test for evidence of multiple source populations. Sequences of *Bugula neritina*, introduced > 120 y ago, and *Watersipora subtorquata*, a recent arrival, were monomorphic, whereas 5% of sites were variable across an initial set of sequences from 18 *Watersipora arcuata* individuals. In *W. arcuata*, also a recent arrival, a consistent pattern of co-occurrence of divergent haplotypes across urban centres suggests that these centres have received common introduction events, or that high levels of gene flow have followed several isolated introductions. These possibilities are discussed with reference to data from endemic species.



## FINE SCALE MAPPING OF GENES INVOLVED IN MILK QUALITY IN CATTLE.

**Helen McPartlan<sup>1</sup>**, Amanda Chamberlain<sup>1</sup>, Thamy Balasingham<sup>1</sup>, Mick Carrick<sup>1</sup>, Nick Robinson<sup>1</sup> and Mike Goddard<sup>1,2</sup>

<sup>1</sup>*Victorian Institute of Animal Science, 475-485 Mickleham Road, Attwood, Victoria,* <sup>2</sup>*Institute of Land and Food Resources, University of Melbourne, Parkville, Victoria*

Initial genome screens at VIAS to detect Quantitative Trait Loci (QTL) affecting milk composition utilised six sires and their daughters. The daughters were selectively genotyped ie those with very high or very low fat percentage or protein percentage were chosen. The six sires were selected because they had higher than usual within sire segregation variance for fat percentage or protein percentage and thus were likely to be heterozygous for a gene affecting milk composition. The families were genotyped for 91 markers, 10 of which were found to be linked to QTL affecting milk composition traits. The aim of our current work has been to find microsatellite markers in closer linkage to the QTL on two of these chromosomes (chromosomes 14 and 20). The same families as in the first experiment, as well as animals from a more complex pedigree, were genotyped for a number of additional microsatellite loci mapping to the regions of interest. Animals in the complex pedigree were genotyped for all loci (six on chromosome 14 and 12 on chromosome 20). The fine scale mapping method uses linkage disequilibrium between marker haplotypes and QTL affecting milk quality. The analyses and results will be discussed.

## PHYLOGENETIC PERSPECTIVES ON A HIGHLY SPECIALISED POLLINATION SYSTEM

**Mant, J.G.<sup>1,2</sup>**, Schiestl, F.P.<sup>1</sup>, Peakall, R.<sup>1</sup> and Weston, P.H.<sup>2</sup>

<sup>1</sup>*Division of Botany and Zoology, The Australian National University, Canberra, ACT, 0200* <sup>2</sup>*Royal Botanic Gardens Sydney, Mrs Macquaries Road, NSW, 2000*

Orchids of the genus *Chiloglottis* are pollinated by the sexual deception of male thynnine wasps mainly from the genus *Neozeleboria* (Tiphiiidae). The orchids mimic both the appearance and sex pheromone chemistry of wingless female thynnines, but provide no reward to the deceived male. Pollinator experiments have previously established the highly specialised nature of this interaction. Specific forms of *Chiloglottis* are recognisable by their attraction of distinct pollinators, although there can be little or no morphological difference between the forms. Specialisation at both the generic and specific level suggests the possibility of coevolution among these terrestrial orchids and their pollinators. Molecular phylogenies of both orchids and wasps are presented to investigate the degree of specialisation at different taxonomic levels and to test for patterns of co-speciation. Sequence data on *Chiloglottis* reveal three clearly diverged species groups, yet fail to resolve whether the orchid forms within each of the groups are genetically distinct. Sequence data on the thynnines indicate all but three of the ca. 25 pollinators are closely related members of one genus, *Neozeleboria*. Considerable congruence between orchid and wasp phylogenies is evident below the generic level, however branch length comparisons suggest divergences between the two groups are not coincident. Comparative data on floral scent chemistry suggests similar attractive volatiles are used by different *Chiloglottis* taxa. Together, these molecular and floral odour results are used to propose an alternative hypothesis explaining phylogenetic congruence in the absence of co-speciation.



Claire Milton, John McKenzie and Phil Batterham

*CESAR - Centre for Environmental Stress and Adaptation Research, Department of Genetics,  
University of Melbourne, Victoria 3010*

Bristle asymmetry is used to measure developmental stability, the ability of an organism to buffer disturbances that occur during development. Mutations in several different *Drosophila* developmental genes result in an increase in asymmetry for the thoracic bristles. One of these genes, Hsp83, encodes the heat-shock protein Hsp90, a molecular chaperone which assists other proteins in reaching their native state. The targets of Hsp90 are mainly signal transducers: Hsp90 stabilises these signalling proteins until they are activated through signal transduction. Mutations in Hsp83 result in a range of leg, wing, bristle and eye deformities in heterozygotes (Rutherford and Lindquist, 1998). It has been proposed that Hsp90 buffers underlying silent genetic variation which affects developmental signalling pathways. When Hsp90 function is impaired this variation is uncovered; if enough of this variation is expressed the phenotype of the fly is altered. We have shown that mutations in Hsp83 result in increased asymmetry for the thoracic bristles. When a specific Hsp90 inhibitor, geldanamycin, is added to the fly food, thoracic bristle asymmetry is significantly increased.

Reference:

Rutherford, S.L and Lindquist, S. (1998). Hsp90 as a capacitor for morphological evolution. *Nature* 396: 336-342.

PROGRESS IN DOMESTIC ANIMAL GENE AND QUANTITATIVE TRAIT LOCUS MAPPING

Chris Moran

*Department of Animal Science, University of Sydney NSW 2006*

During the last decade, the genetic maps of domestic animal species, including pigs, cattle, sheep and chickens, underwent a period of rapid development from virtually a zero base. Detailed linkage maps containing thousands of highly informative microsatellite markers, studded with conserved gene markers, are now a valuable resource for gene hunting and QTL mapping in cattle, sheep, pigs and poultry. However many QTL results are being withheld from publication in order to protect intellectual property of potential commercial significance. Among companion and recreational animal species, including horse, dog and cat, genetic maps are being developed, often driven by a desire to characterise models of human disease. In addition to the strong growth of the linkage maps, there has been enormous progress in development of physical maps and mapping resources in most domestic species. FISH and somatic hybrid panels are routinely used to map gene loci and physical markers. The most significant new physical mapping resource are the radiation hybrid panels developed for example for cattle and pigs. These resources provide mapping with a resolution of about 100-200 kbp with only slightly more than 100 PCR reactions. Detailed comparative maps, generated by ZOO-FISH and refined by mapping of conserved gene markers, have revealed the detailed syntenic relationships between domestic animal genomes and those of human and mouse. In the case of the pig, bidirectional ZOO-FISH has provided a high degree of confidence and resolution in the comparative maps. Domestic animal genome mappers and geneticists are now well placed to mine the huge volume of human genome sequence and to identify and analyse positional candidate genes for their previously identified QTLs. Example from the author's laboratory of linkage, physical and QTL mapping in the pig will be used to illustrate the great strides being made in domestic animal genomics.



## TRACING PHEROMONE EVOLUTION IN TWO NATIVE NEW ZEALAND GENERA OF TORTRICID MOTHS

**Richard Newcomb**<sup>1</sup>, Tamara Sirey<sup>1</sup>, Robyn Howitt<sup>2</sup>, David Greenwood<sup>1</sup> and Dianne Gleeson<sup>2</sup>

<sup>1</sup>*HortResearch, Private Bag 92169 Auckland, New Zealand,* <sup>2</sup>*Landcare Research, Private Bag 92170 Auckland, New Zealand*

Moths of the genera *Ctenopseustis* and *Planotortrix* use tetradecenyl acetates with a double bond along the carbon chain. Sibling species are morphologically similar but differ in the position of the double bond in their pheromone. We have attempted to order speciation and pheromone evolution events within these genera using a phylogenetic analysis of the neutral COI and II mitochondrial gene region. This analysis has revealed that many of the species evolved very recently and that similar pheromones have evolved twice independently (eg Z5-14:OAc) within the genera. Pheromone Binding Proteins (PBPs) are present in male antennae and are thought to transport incoming pheromones to membrane-bound receptors. Comparison of PBP genes of these species reveals little variation, with a similar pattern to the neutral tree. One instance of convergent evolution is however noted, where PBPs of species contained within the different genera are each others closest relatives in the PBP tree. These species use the same pheromone and are allopatric in their distributions. Using this type of analysis we hope to be able to determine whether genes are that are involved in pheromone production in the female and pheromone discrimination in the male drive the speciation process in these species.

## MULTIPLE MATING IN THE GENUS *APIS* – AS DETERMINED BY MICROSATELLITE ANALYSIS

**Kellie Palmer** and Benjamin Oldroyd

*School of Biological Sciences, Macleay Building A12, University of Sydney, NSW 2006, AUSTRALIA*

Honey bee queens (*Apis*) are extremely promiscuous, with some species mating up to 70 times. This behaviour is unexpected for two reasons. First, mating is expected to be costly, exposing queens to predation risks, diseases and inclement weather. Second, multiple mating reduces intracolony relatedness which is counter to expectations of kin selection theory, the most widely accepted explanation for the evolution of sociality. Many hypotheses have been suggested to explain queen promiscuity, but for honey bees a definitive explanation remains elusive. Comparative analysis has been suggested to study the evolutionary pressures driving promiscuity. This analysis relies on accurate estimates of mating frequency for each species within the genus. Microsatellite analysis has enabled the most precise estimation of mating frequency to date. The validity of the estimates depend on the sample size, the number of polymorphic loci, allelic diversity and allele frequency at each locus studied.



## GENETIC CONSEQUENCES OF HABITAT FRAGMENTATION: UNRAVELLING THE COMPLEXITY IN NATIVE BUSH RATS

Rod Peakall<sup>1</sup> and David Lindenmayer<sup>2</sup>

<sup>1</sup>*Division of Botany and Zoology and* <sup>2</sup>*Centre for Resources and Environmental Studies, Australian National University Canberra ACT 0200*

Despite much theory on the genetic consequences of habitat fragmentation, empirical studies are often confounded by historical factors, choice of genetic marker and scale of sampling. In an attempt to overcome confounding factors, we are using hypervariable microsatellite loci (9 loci with > 90 alleles) to assess genetic patterns within and among replicate remnant and 'control' populations of Bush Rats *Rattus fuscipes*, at multiple spatial and temporal scales. This study is part of a larger multi-disciplinary study of habitat fragmentation at Tumut, NSW. At a scale of 10 x 15 km, significant genetic differentiation among populations has been detected (mean  $F_{st}$  = 0.044, range of  $F_{st}$  = 0 to 0.13), with some proximate pairs of remnant populations (<0.5 km apart), showing above average genetic differentiation, relative to other more distant populations. Genetic heterogeneity was also detected amongst populations in continuous native forests. Thus neither random mating nor isolation by distance explain the genetic patterns, nor can habitat fragmentation alone account for the findings, suggesting that other processes have contributed to the patterns. Early indications from our ongoing investigation show heterogeneity between years within sites can be as great as that between sites. Thus both spatial and temporal heterogeneity may confound attempts to understand the consequences of habitat fragmentation. For this reason, we are now combining genetic methods with the ecological manipulation of populations that will enable us to quantify the patterns and extent of dispersal within fragmented habitats. We will extend these findings by statistical modelling and computer simulation.

## VICARIANCE & EVOLUTION IN THE AUSTRALIAN WET TROPICS

Ben Phillips, Stuart Baird and Criag (sic) Moritz

*Department of Zoology and Entomology, The University of Queensland, Brisbane, Qld. 4072, Australia*

Comparative phylogeography, when combined with paleo-ecological modelling and analysis of phenotypic variation, has the potential to provide a historical framework for studies of evolutionary processes. For the wet tropics rainforests of Australia, previous studies of paleo-ecology and phylogeography provide strong evidence that rainforests underwent a series of contractions and expansions from the Pliocene to the mid-late Holocene, and that rainforest-dependent fauna tracked these changes in habitat distribution. Despite long (>2 Myr) periods of isolation, there is little evidence for phenotypic divergence between populations of lizards on either side of major historical barriers, in contrast to substantial morphological difference between rainforest and open-forest populations that are connected by recent or ongoing gene flow. This raises the question of whether allopatric or parapatric (selection-driven) processes of speciation are more applicable to this fauna. More explicitly, is there evidence for reproductive isolation (despite the lack of morphological divergence) when the historically isolated population merge? We are examining this question through genetic analyses of multi-species contact zones at locations where the rainforests have re-established contact, probably during the cool-wet period of the mid-Holocene. A detailed examination of a secondary contact zone in the skink *Carlia rubigularis* has revealed a narrow and congruent clines across multiple genes and suggests some selection against hybrids. This suggests that morphological divergence is a poor predictor of reproductive isolation and that both vicariant (allopatric) and parapatric processes shape diversity in this system.



## THE RELATIONSHIP BETWEEN MOLECULAR AND QUANTITATIVE MEASURES OF GENETIC DIVERSITY: A META-ANALYSIS.

David H. Reed and Richard Frankham

*Key Centre for Biodiversity and Bioresources. Department of Biological Sciences, Macquarie University*

The ability of populations to undergo adaptive evolution depends on their having quantitative genetic variation for ecologically important traits. However, the trend for the past 25 years has been for molecular methods to be used as surrogates for quantitative genetic measures, and a clear relationship between the two has not been established. To resolve this issue, we carried out a meta-analysis, based on 64 data sets. The mean correlation between molecular and quantitative measures of genetic variation was found to be weak ( $r = 0.21$ ). There was no relationship between the two measures for life history traits ( $r = -0.09$ ) or for the quantitative measure generally considered as the best indicator of adaptive potential, heritability ( $r = -0.05$ ). Consequently, molecular measures of genetic diversity have only a limited ability to predict quantitative genetic variability present in a population. When information about the evolutionary forces acting on a population or a populations short term adaptive potential is required, direct measures of quantitative genetic variation are needed.

## THE COST OF REPRODUCTION AND THE EVOLUTION OF AGEING

Carla M. Sgrú<sup>1</sup> and Linda Partridge<sup>2</sup>

<sup>1</sup>*Department of Zoology & Entomology, University of Queensland, St Lucia QLD 4072,* <sup>2</sup>*Department of Biology, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK*

Reproduction increases the death rate in many organisms. Mortality rates usually increase rapidly at the onset of ageing, but can decelerate at later, often post-reproductive, ages. To test the idea that earlier reproduction contributes to both an increase in death rates and a later deceleration, the level of mortality produced by increased egg production was measured in female *Drosophila melanogaster*. Reproduction resulted in a delayed wave of mortality, corresponding to the sharp increase in death rates seen at the onset of ageing, and the resultant deceleration of mortality later in life. These results imply that ageing has evolved primarily as a result of the damaging effects of reproduction early in life rather than because of the accumulation of mutations with deleterious effects only at late ages.



## THE IDENTIFICATION OF GENES THAT INTERACT WITH *LOZENGE* IN *DROSOPHILA* EYE DEVELOPMENT.

Nicole Siddall<sup>1</sup>, Ben Hogan<sup>1</sup>, Sally Coutts<sup>1</sup>, John Pollock<sup>2</sup> and Phil Batterham<sup>1</sup>

<sup>1</sup>Department of Genetics, University of Melbourne, Victoria 3010, <sup>2</sup>Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania, USA

The compound eye of *Drosophila melanogaster* is composed of about 800 ommatidia. Each of these contains an identical set of cells including 8 photoreceptor neurons, 4 lens-secreting cone cells, pigmented supporting cells and a mechanosensory hair nerve group. The eye is derived from the eye imaginal disc, a simple monolayer epithelium created during embryogenesis. In the developing eye, individual cell fates are specified when general signalling mechanisms are interpreted in the context of cell-specific transcription factors. *Lozenge*, a Runt/AML1/CBFA1-like transcription factor, determines the fate of a number of neuronal and non neuronal cells by regulating the expression of multiple fate determining transcription factors. *lz* negatively regulates *svp* in R7 and cone cells and positively regulates *Bar* in R1/R6 in the developing eye disc. *lz* also has been implicated in positively regulating *pros* in R7 and cone cells, and *spa* in cone and primary pigment cells. It has been proposed that *lz* functions as a pre-patterning factor for all cells which differentiate after the five cell precluster. We aim to identify genes upstream and downstream of *lz* in this process. The results of interaction screens and candidate gene analysis will be presented.

## THE IMPACT OF HABITAT FRAGMENTATION ON DISPERSAL IN CUNNINGHAM'S SKINK (*EGERNIA CUNNINGHAMI*): EVIDENCE FROM ALLELIC AND GENOTYPIC ANALYSES OF MICROSATELLITES.

Adam Stow<sup>1</sup>, Paul Sunnucks<sup>2</sup>, David Briscoe<sup>1</sup> and Michael Gardner<sup>3</sup>

<sup>1</sup>Department of Biological Sciences, Macquarie University, NSW 2109, <sup>2</sup>Department of Genetics & Evolution, La Trobe University, VIC 3083, Australia. <sup>3</sup>Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, South Australia 5000

The effects of habitat fragmentation on processes within and amongst populations is important for conservation management. Despite a broad spectrum of lifestyles and the conservation significance of many reptiles, very little work on fine-scale population genetics has been carried out on this group. This study examines dispersal patterns of a rock crevice-dwelling lizard, Cunningham's skink (*Egernia cunninghami*), in a naturally vegetated reserve and an adjacent deforested site. Both genotypic and genic approaches were employed, using microsatellite loci. The spatial organisation of individuals with respect to pairwise relatedness coefficients and allele frequencies along with assignment tests, were used to infer dispersal characteristics for both sexes in the natural and cleared area. The distribution of relatedness in both habitats was spatially structured, with *E. cunninghami* showing relatively high pairwise relatedness within their rocky retreat sites. Analysis of relatedness over different spatial scales, spatial autocorrelation of alleles, and assignment tests, all indicated that both sexes in the cleared area show less dispersal than their counterparts in the reserve. Furthermore, deforestation inhibits female dispersal to a greater extent than males. The geographic structuring of allele frequencies for adults in the cleared area, but not the reserve, indicates that habitat fragmentation has the potential to alter at least the microevolution of *E. cunninghami* populations.



## THE COMING OF AGE OF MODEL-BASED METHODS IN PHYLOGENETIC INFERENCE: NEW PERSPECTIVES ON CONSISTENCY, EFFICIENCY, ROBUSTNESS, AND PHILOSOPHY

**David L. Swofford**

*Laboratory of Molecular Systematics, Smithsonian Institution Museum Support Center, Suitland,  
Maryland 20746 USA*

Improved algorithms and faster computational power have produced an explosion in the use of maximum-likelihood and other model-based methods in empirical phylogenetic studies. The increased application of these methods has in turn stimulated renewed challenges from those who advocate cladistic parsimony as the preferred method of phylogenetic analysis. While some of the criticisms are reasonable, others are simply based on misunderstandings or misrepresentations of model-based methods. In this presentation, I will address some of these criticisms, summarising recent results from my own work and that of others. I will specifically focus on issues pertaining to the relevance and importance of statistical consistency, the relative efficiency of likelihood versus parsimony methods in "zones" where parsimony is subject to artifactual behaviour, and the performance of statistical methods in the face of weak to strong violation of their underlying assumptions. I will also touch on some philosophical issues that have been used to defend parsimony methods, and demonstrate that the so-called "total evidence" method can also be conducted in a likelihood framework.

## META ANALYSIS OF RELATIVE PENETRANCE RANK ORDER STATISTICS WITH APPLICATION TO HLA DR-DQ GENES AND TYPE 1 DIABETES

**Glenys Thomson**

*University of California, Berkeley, CA, USA*

The direct involvement of the HLA DR-DQ genes (DRB1, DQA1, and DQB1) in type 1 diabetes is well established. These genes display a complex hierarchy of predisposing, intermediate, and protective effects at the genotype and haplotype levels. The ratio of the observed frequency of a genotype (haplotype) in patients over the frequency in controls, referred to as the P/C (patient/control) ratio, is an MLE of the relative genotype (haplotype) penetrance values. A novel test has been developed to compare the relative rank orders of predisposing through protective P/C ratios of genotypes and haplotypes across ethnic groups. The algorithm developed to determine the probability of an observed rank order in a population compared to a putatively "known" rank order, allows for the fact that not all genotypes (haplotypes) will be found in every population. The key to development of the algorithm is use of a recurrence relationship to determine the probabilities when an additional genotype (haplotype) is added to the analysis. Meta analysis of the resulting p values across populations is weighted by sample size. Consistency in rank order of P/C ratios of HLA DR-DQ genotypes and haplotypes in type 1 diabetes is seen across ethnic groups. This allows investigation of the specific amino acids at the HLA DR-DQ genes involved in type 1 diabetes. The rank order method developed is applicable to other genetic regions besides HLA.



## CORROBORATION 2000. MEASURES OF FIT, PROBABILITIES OF FIT, AND SELECTING A MOST CORROBORATED TREE

John W. H. Trueman<sup>1</sup> and Daniel P. Faith<sup>2</sup>

<sup>1</sup>*Division of Botany and Zoology, Australian National University, Canberra, ACT 0200, Australia*

<sup>2</sup>*Australian Museum, 6 College St., Sydney, NSW 2000, Australia*

Phylogenies can be estimated by any of several optimality criteria (cladistic parsimony, maximum likelihood, 3ta, etc.), any of which will lead, in its own terms, to a best-fit tree. In the Combined Evidence approach (contrast the Total Evidence approach of conventional cladistics), hypotheses are evaluated at the level of corroboration, avoiding arbitrary weighting of different forms of evidence at the level of goodness-of-fit. Under this protocol each class of data may be analysed using a fitness criterion appropriate for that class. The degree of corroboration then is assessed, and the 'winning' tree becomes the tree with greatest corroboration over all tests and all data. Here these principles will be illustrated in the context of (1) Profile Parsimony, a new criterion of fit for character-state data, (2) PP-TPTP, a permutation-style corroboration test for PP, (3) Likelihood Severity Testing, a corroboration framework for the maximum likelihood criterion, and (4) an example in which sequence data are combined with other data at the level of corroboration (severity) analysis.

## A CYTOGENETICIST BANDS UP WITH GENE LOCALIZATION. RISH AND FISH TO ANIMAL CHROMOSOMES.

Graham C. Webb

*Departments of Animal Science and Obstetrics and Gynaecology, The University of Adelaide, Waite Campus, Glen Osmond SA 5064, and The Queen Elizabeth Hospital, Woodville SA 5011*

Examples of the usefulness of physically localising genes and other DNA sequences to the banded chromosomes of humans, mice and a variety of other mammals, over a period of 17 years, will be presented. Fluorescent *in situ* hybridisation (FISH) is now most commonly used but the use of radioactive (tritium) *in situ* hybridisation (RISH) can still be justified. Physical localisation will be compared with the more usual genetical mapping of genes in the mouse. The sometimes surprising detection of pseudogenes will be shown, including one which is read as antisense in early embryogenesis. The information which can be gained from localisation of human and mouse homoeologues of DNA sequences originating in *Drosophila melanogaster* will be illustrated. Localisations of repetitious sequences to chromosomes and sperm heads using two colour FISH will also be shown. Finally, my most recent headache, the localisation of the porcine endogenous retroviruses, will be discussed, particularly for the benefit of the prior speaker at the symposium.



Gudrun Wells

*Division of Botany and Zoology, ANU, 0200 and CSIRO Plant Industry, GPO Box 1600 ACT 2601*

This study examined fine-scale spatial genetic structure (SGS) in *Rutidosia leptorrhynchoidea* in a range of population sizes. *R. leptorrhynchoidea* is an endangered grassland herb from south-eastern Australia, which now exists in only 24 known populations, most of which consist of under 200 individuals. It has a sporophytic self-incompatible mating system. This study sought to answer three main questions: 1) is there fine-scale SGS in *R. leptorrhynchoidea* populations? 2) does the level of SGS change with population size? 3) what causes the observed levels of SGS? Spatial autocorrelation analysis of *fij* (a coancestry based coefficient) based on five allozyme loci showed that there was significant SGS in large *R. leptorrhynchoidea* populations, but that the magnitude and extent of SGS decreased with population size. Strong correlation between *fij* and seed dispersal indicates that the SGS seen in large populations is probably due to limited seed dispersal and high levels of relatedness within seed arrays (here approximately 30% of seeds are full siblings). The lower SGS in small populations could be due to higher mate limitation, which leads to increased effective pollen dispersal, coupled with lower levels of seeding survivorship. Taken together, the results of this study indicate that microevolutionary processes affecting the amount and distribution of genetic variation are disrupted in smaller *R. leptorrhynchoidea* populations. This could influence the long term viability and evolutionary potential of these smaller populations.

PERSISTENT GENE SILENCING IN *DROSOPHILA MELANOGASTER* USING DOUBLE-STRANDED RNA

Steven Whyard and Hilary Mende

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

Double-stranded RNA (dsRNA) can induce highly specific, homology-dependent suppression of gene expression in a wide array of organisms (1). In addition to its use as a powerful tool for gene function studies, RNAi may have a biological role as a general response mechanism of eukaryotic organisms to either viral invasion or to the production of aberrant transcripts, such as those caused by proliferating transposons. RNAi is highly potent, as only a few molecules of dsRNA are required in a cell to suppress target gene expression (2). This suggests that either the dsRNA is acting in a highly catalytic manner to reduce transcript levels, or the RNAi signal is amplified. In this study, delivery of dsRNA into *Drosophila melanogaster* was observed to specifically reduce, and in some cases, virtually inhibit the expression of a reporter gene. Embryos were injected with either in vitro transcribed dsRNA or DNA constructs that produced sense, antisense, or double-stranded RNAs specific to the reporter genes. The strongest gene silencing was observed following delivery of a genetic construct capable of producing hairpin RNAs. Gene silencing was observed to persist for at least three generations, albeit at progressively reduced levels. The silencing phenotype appears to be predominantly maternally inherited, suggesting that the silencing signal is carried in the cytoplasm. These results indicate that dsRNA is not only effective at gene silencing, but that the silencing signal can persist in an insect long after the initial production or introduction of the dsRNA molecules.

1. Sharp, P.A. (1999) *Genes Dev.* 13: 139-141
2. Fire, A. et al. (1998) *Nature* 391: 806-811



## MONITORING HYBRIDISATION BETWEEN DINGOES AND DOGS IN THE WILD.

Alan Wilton

*School of Biochemistry and Molecular Genetics, University of New South Wales, Sydney NSW 2052*

Introduction of domestic species into a unique environment with its own flora and fauna can have many far reaching deleterious effects. In Australia one result is hybridisation between the local members of the *Canis* family, the dingo, with the domestic variety, the dog. Dingoes in the wild are being gradually replaced by domestic dogs. The unique characteristics of dingoes are being lost due to introduction of genetic material from the dog through hybridisation. This problem has been recognised for some time but the tools to assess the extent of the problem have not been available. No loci are totally diagnostic for dog or dingo origin but distinct differences between dingoes and dogs in allele distributions at several canine microsatellite loci have been identified. Using 20 of these loci we have examined several hundred wild canids from several areas across the country and examined the amount of introgression of domestic dog. Large differences are evident between populations as might be expected since some populations are close to populated areas. The results demonstrate a practical application of allele frequency information to monitor populations.



## POSTER ABSTRACTS

### MOLECULAR GENETICS IN CANINE COPPER TOXICOSIS

**Hyun Changbaig**

*Immunogenetics Department, School of Veterinary Sciences. The University of Queensland, St Lucia Brisbane 4072*

Canine copper toxicosis is an autosomal recessively inherited genetic disease. Human Wilson's disease has many genetic and pathological similarities to copper toxicosis in Bedlington Terriers dog. The causal gene in Wilson's disease codes for a P-type ATPase (ATP 7B) which is involved in copper transportation within the liver cell. However the causal gene has not been isolated yet in dogs. In this study, the genetic analysis has been done by several ways to identify the defective gene in Bedlington Terriers affected with copper toxicosis.

### A MYSTERY GENE ANTISENSE TO IP3-KINASE IN *DROSOPHILA MELANOGASTER*

**Bronwyn Dixon**

*Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra ACT 2601*

In *Drosophila melanogaster* a number of overlapping genes have been reported. Two functions of overlapping genes are protein production and non-coding RNA. Overlapping genes have been found to be involved in a large range of biological functions including production of heat shock proteins, hormonal response, control of proliferation, and development. My Honours project is to investigate the structure and function of a gene that overlaps a well-conserved kinase gene, the inositol-1,4,5-trisphosphate-3-kinase gene (IP3kinase) in *D. melanogaster*. The overlapping gene of unknown function has been called the mystery gene (*mys*) as almost nothing is known about it. IP3kinase and *mys* overlap at their 3' ends. The most 3' exon of the IP3kinase gene is entirely overlapped by the mystery gene, more than 900 bases of overlap. EST database information reveals that differential splicing occurs in the RNA transcripts of *mys*, and that this gene is more highly expressed than IP3kinase. We are interested in *mys* as more than an example of gene overlap. We need to know whether this gene is involved in the antisense regulation of IP3kinase or whether *mys* encodes its own protein. This knowledge is important as it will contribute to our understanding IP3kinase and overlapping genes in general.



## SCAN OF HUMAN CHROMOSOME 1 FOR SUSCEPTIBILITY GENES FOR PRE-ECLAMPSIA/ECLAMPSIA

Guanglan Guo<sup>1,2</sup>, Alan N. Wilton<sup>1</sup>, Eric K. Moses<sup>2</sup>, Jennifer A. Lade<sup>2</sup>, Madonna Grehan<sup>2</sup>, Thomas Kaiser<sup>2</sup>, Kate Freed<sup>2</sup>, Robyn North<sup>3</sup>, Desmond W. Cooper<sup>4</sup> and Shaun P. Brennecke<sup>2,5</sup>

<sup>1</sup>*School of Biochemistry and Molecular Genetics, University of New South Wales, Sydney NSW 2052,*

<sup>2</sup>*Department of Perinatal Medicine, Royal Women's Hospital, Melbourne VIC 3010, <sup>3</sup>Department of Obstetrics and Gynaecology, University of Auckland, New Zealand, <sup>4</sup>School of Biological Sciences, Macquarie University NSW 2109, <sup>5</sup>Department of Obstetrics and Gynaecology (Royal Women's Hospital) University of Melbourne, VIC 3010*

Pre-eclampsia/eclampsia (PE/E) is a complex disorder of human pregnancy. It is clinically characterised by high blood pressure, proteinuria and oedema in late pregnancy and it is life threatening to both mothers and babies. The aetiology of PE/E is not clear. Poor placental blood perfusion and a genetic predisposition might be involved in the development of PE/E. The renin-angiotensin system (RAS) plays a critical role in the control of blood pressure. Some RAS genes, which are on the chromosome 1, are candidate genes for PE/E. As a part of a whole genome-wide scan to search PE/E susceptibility genes, 31 microsatellites were typed on 34 Australian and New Zealand PE/E affected families to give coverage of chromosome 1 with 5-10 cM intervals. The GENEHUNTER program was used for non-parametric analysis (NPL) and parametric multi-point analysis. No significant linkage was found between chromosome 1 markers and PE/E. The existence of a PE/E susceptibility gene was implied by an NPL score of 2.07 ( $p = 0.0063$ ) at 144 cM. Further typing with higher density markers in this area may help to reveal whether there really is a susceptibility gene for PE/E on chromosome 1.

## PATTERNS OF EXPRESSION OF THE CARPEL DEVELOPMENT GENE SPATULA IN *ARABIDOPSIS*

Marcus G. B. Heisler, Yasmin M. Bylstra and David R. Smyth

*Department of Biological Sciences, Monash University, Victoria 3800*

Mutant studies of the SPATULA gene of *Arabidopsis* indicate that it acts to promote the growth of carpel margins and development of transmitting tract tissues derived from them (1). SPT has been cloned by chromosome walking and shown to encode a transcription factor of the basic helix-loop-helix family. Its expression in the developing gynoecium is confined to those regions that are disrupted in spatula mutants. When the gynoecium arises at stage 6, SPT expression is strongest in those regions where carpels are congenitally fused. Later, expression occurs in the growing septum and at the apex of the gynoecium where stigmata and transmitting tract tissues develop. Thus SPT is a regulatory gene that apparently acts autonomously to control the development of specific sub-regions of the gynoecium. ETTIN is another gene involved in gynoecium development. ettin mutant gynoecia display a reduction in valve tissue, the appearance of a gynophore and the over-proliferation of stigmatic and septal tissues (2). Significantly, spt is almost completely epistatic to ettin with regard to gynoecium development, indicating that ETTIN may confine SPT activity to subregions of the wild-type gynoecium (3). Confirming this proposal, SPT expression expands to almost completely encompass the gynoecium by stage 7 in ettin mutants, and later it becomes concentrated in those regions that develop into ectopic outgrowths. SPT transcripts were also detected within subregions of other meristematic tissues, including the apical meristem, young floral buds, and developing petals, stamens and ovules. These organs are apparently unaffected in spt mutants, suggesting that SPT plays a redundant role in their development.

1. Alvarez, J and Smyth, D. R. (1999) *Development* 126:2377-2386
2. Sessions, A. and Zambryski, P. C. (1995) *Development* 121:1519-1532
3. Alvarez, J and Smyth, D. R. (1998) *Journal of Plant Research* 111:295-298



# MOLECULAR CHARACTERISATION OF *ss*, AN ELEMENT THAT MODULATES RECOMBINATION AT THE *NIT-2* LOCUS IN *NEUROSPORA CRASSA*

Steven Henderson & David E. A. Catcheside

*School of Biological Sciences, Flinders University, Adelaide, South Australia 5042, Australia*

*ss* is one of four classes of genetic element that control recombination in *Neurospora crassa*. Any heterozygous combination of the three known *ss* alleles (*ss*<sup>S</sup>, *ss*<sup>E</sup> & *ss*<sup>C</sup>) reduces recombination in *nit-2* by 2-20 fold. Genetic analysis indicates that *ss* is within 0.2cM of *nit-2*. *nit-2* is the major regulatory gene of nitrogen metabolism in *N. crassa*, yet the majority of the gene is not required for regulatory function (Fu & Marzluf, 1987). The polymorphic *trans*-acting gene *rec-1* also regulates recombination in *nit-2*, acting multiplicatively with *ss*, achieving regulation of recombination over a 100-fold range. We speculate that *ss* enables preservation of polymorphism for regulation of nitrogen metabolism by impeding the shuffling of *nit-2* sequence between strains. Alternatively, the *ss* phenotype could result from sequence divergence between *ss* alleles which inhibits recombination although meiotic recombination in *Neurospora* is tolerant of a substantial degree of sequence heterology (Yeadon & Catcheside, 1995). We are testing these hypotheses by characterising the molecular basis for the *ss* phenotype by mapping *ss* with respect to molecular markers within and flanking *nit-2*.

Fu, Y. & Marzluf, G. A. 1987, 'Characterization of *nit-2*, the Major Nitrogen Regulatory Gene of *Neurospora crassa*', *Molecular and Cellular Biology*, vol. 7, pp 1691-1696.

Yeadon, P. J. & Catcheside, D. E. A. 1995, 'The Chromosomal Region which Includes the Recombinator *cog* in *Neurospora crassa* is Highly Polymorphic', *Current Genetics*, vol. 28, pp 155-163.

## MAPPING OF GENES AFFECTING MILK SYNTHESIS IN DAIRY CATTLE USING SELECTIVE DNA POOLING

Maxy Mariasegaram<sup>1</sup>, Nick Robinson<sup>2</sup>, Ben Hayes<sup>1</sup>, Mike Goddard<sup>12</sup>

<sup>1</sup> *Institute of Land and Food Resources, University of Melbourne, Parkville, Vic 3052*, <sup>2</sup> *Victorian Institute of Animal Science, Dept. of Natural Resources and Environment, Attwood, Vic 3049*

Several genes or quantitative trait loci (QTL) and environmental conditions control traits of economic importance, such as milk yield and composition. The aim of this project is to map genes that affect milk yield and composition of dairy cattle. Prior studies have scanned the bovine genome and identified a number of chromosomal regions thought to contain such genes. These studies however, lack the power needed to detect QTLs with an effect of 0.2 standard deviations. Meta-analysis of published genome scans suggest that genes with effects  $\geq 0.2$  standard variation explain  $>50\%$  of the variance of typical quantitative traits. Our aim is to increase the power of genome scans so that a majority of the genetic variance can be explained by the mapped QTL. Power can be increased by selectively genotyping daughters of bulls, from the extremes of the phenotypic distribution. Two pools of DNA will be formed from each tail of the distribution trait of the Australian Selection Index (ASI) - which incorporates milk volume, protein and fat yields. The pools will be genotyped with up to 100 markers and the allele frequencies estimated from each pool using peak areas. A significant difference in sire marker allele frequency between the pools would indicate the presence of a linked QTL. The approach of allele image pattern difference (delta-AIP) between the two pools will be taken as a measure of the allele content difference.



## EVIDENCE FROM ALLOZYMES CLARIFIES RELATIONSHIPS AMONG SPOTTED GUM EUCALYPTS (CORYMBIA, SECTION POLITARIA: MYRTACEAE)

M. W. McDonald, P. A. Butcher and J. C. Bell

CSIRO, Forestry and Forest Products, PO Box E4008, Kingston, Canberra, ACT 2604, Australia

Allozymes were used to examine the level and distribution of genetic variation within and among species in *Corymbia* section *Politaria*. *Corymbia citriodora*, *C. maculata*, *C. henryi* and *C. variegata* are widespread endemic forest species in eastern Australia and are the focus of recent breeding programs for high-quality sawlogs. Twenty eight populations representing their range-wide distributions were studied using 18 allozyme loci. Results indicate these species are closely related as only 15% of the total genetic diversity was due to differences between species. Two distinct genetic alliances were evident: *C. maculata*-*C. henryi* and *C. citriodora*-*C. variegata*. *Corymbia citriodora* and *C. variegata*, however, could not be distinguished by their allozyme profiles. The lack of genetic differentiation between these species, supported by morphological evidence, suggests they represent races of a single taxon. They primarily differ in the chemistry of their leaf oils. By contrast, the *C. maculata*-*C. henryi* alliance comprise closely allied but genetically distinct taxa. *Corymbia henryi* had the highest genetic diversity in the group despite having the narrowest geographic range and the lowest genetic differentiation among populations. The more widely distributed *C. maculata* had the lowest diversity and the highest genetic differentiation in the group. There was evidence of isolation-by-distance among populations of *C. citriodora*, *C. maculata* and *C. variegata* but not in *C. henryi*. Phylogenetic analysis, using maximum likelihood and with *C. torelliana* as an outgroup, gave the same tree topology as cluster analysis of genetic distances.

## ALLIANCE MEMBERSHIP AND KINSHIP IN FREE-LIVING MALE BOTTLENOSE DOLPHINS

Luciana Muller<sup>1</sup>, Luciano Beheregaray<sup>2</sup>, Robert Harcourt<sup>1</sup> and Michael Krützen<sup>3</sup>

<sup>1</sup>MMRG, Graduate School of the Environment, Macquarie University, Sydney, NSW, 2109. <sup>2</sup>Dept. of Biological Sciences, Macquarie University, Sydney, NSW, 2109. <sup>3</sup>Dept. of Biological Sciences, University of New South Wales, Sydney, NSW, 2052.

Male bottlenose dolphins, *Tursiops aduncus*, form stable alliances that cooperate to herd females for mating, and combine into coalitions to defend against or attack other alliances while competing for females. Kin selection is a potential explanation for alliance formation because alliances typically herd single females, and fertilisation of the single offspring is not divisible. However, the importance of kinship has never been explicitly tested. We examined whether male bottlenose dolphins form alliances with their kin, and whether alliances associate preferentially with other related males in two southeastern Australian populations. Between 1997 and 2000, the composition of over 300 dolphin groups was determined by photographic identification of individuals, and biopsy samples were obtained from 106 animals using a biopsy system. Associations between pairs of dolphins were calculated using the simple ratio index. Sex of individuals was determined using sex-linked markers; genetic relatedness was estimated with eight microsatellite loci; and maternal kinship was assessed by sequences of the mitochondrial DNA control region. There was no significant relationship between alliance membership and kinship, nor was there any relationship between kinship and associations with non-alliance members. Thus, in these two bottlenose dolphin populations, kinship is not the primary factor involved in male alliance formation nor in associations with potential coalition partners. Since alliances may last many years, likely alternative explanations for the maintenance of non-kin male alliances are mutualism or reciprocal altruism, while for coalitions pursuit of self-interest may also be a possibility.



## RIBOSOMAL DNA SEQUENCES RESOLVE THE PLANT GENUS *ASTROTRICHA* (ARALIACEAE).

Jenny Saleeba, Kathi Downs, Kinnie Ho, Bruce R. Lyon and Murray Henwood

*School of Biological Sciences, The University of Sydney, Sydney, NSW 2006.*

*Astrotricha* is a morphologically distinctive genus of endemic Australian Araliaceae (the ivy family). The genus ranges from far north Queensland to the Grampian Range of south-western Victoria with a disjunct species (*A. hamptonii*) restricted to the Hammersley Range of Western Australia. There are 16 formally recognised species and a further 10 taxa of *Astrotricha* are recognisable on the basis of a combination of fruit and leaf characters (Henwood and Makinson, 1992). Within the geographical range of the genus, taxa are distributed in diverse patterns: parapatric, allopatric, sympatric and disjunct. Many of the morphologically distinct taxa appear to hybridise naturally. The use of a limited number of morphological characters, the lack of reproductive isolation and the relatively complex spatial distribution of the putative taxa has hampered not only the utilitarian recognition of taxa, but also prevented the investigation of the infrageneric phylogenetic and biogeographical relationships of this genus. The utility of morphological and molecular data for phylogenetic inference is discussed.

## DEVELOPMENT AND IMPLEMENTATION OF A MOLECULAR MARKER FOR EARLY-MATURITY (KU) IN *LUPINUS ANGUSTIFOLIUS*

M.N. Scobie<sup>1</sup>, W.A. Cowling<sup>2</sup>, R.H. Potter<sup>1</sup>, M.G.K. Jones<sup>1</sup> and S.J. Brien<sup>1</sup>

<sup>1</sup>WA State Agricultural Biotechnology Centre, Murdoch University, Perth, Western Australia 6150,

<sup>2</sup>University of Western Australia, Nedlands, Perth, WA 6009

Sweet narrow-leaved lupin (*Lupinus angustifolius*) is the main grain legume crop grown in Australia but has only been grown on a large scale for twenty years. Little is known of the genomic organisation of this species but gene mapping and molecular markers are now being developed to support traditional breeding methods. A partial molecular linkage map has been generated in *L. angustifolius* from the F2 population of a cross between an early maturing advanced breeding line (83A:476, maternal) and a late maturing wild type (P27255, paternal) using, predominantly, amplified fragment length polymorphisms (AFLPs). From this map, an AFLP locus linked to early-maturity (Ku) has been identified. Using the sequence of the AFLP fragment a specific codominant test, using PCR to produce a 237bp band followed by an *Mse I* restriction digest, has been developed that allows plants in this cross to be characterised for early/late-maturity. The Ku marker has been tested against 20 other current commercial lupin varieties. Although the 237bp fragment was amplified in all the varieties, the *Mse I* site was not conserved in all of the late maturing varieties. Sequence data of these fragments has been compared, and exhibits less than 2% variation. No polymorphism was found that could be linked to Ku in all the varieties. Work is proceeding to use the Ku marker fragment as an RFLP probe to identify larger fragments that can be sequenced using inverse PCR. A polymorphism may be found that relates to maturity across these varieties. A simple PCR test will then be designed which will be implemented in the lupin breeding program.



ALLOZYME POLYMORPHISM IN AN ENDANGERED AUSTRALIAN TERRESTRIAL ORCHID  
*PTEROSTYLIS GIBBOSA* R. BR. (ORCHIDACEAE), AND ITS IMPLICATIONS FOR  
CONSERVATION.

I.K.Sharma, M.A.Clements & D.L. Jones

*Centre for Plant Biodiversity Research, Division of Plant Industry CSIRO, GPO Box 1600, Canberra, ACT, 2601, Australia.*

A relatively high genetic diversity, in all known populations of the endangered Australian native terrestrial orchid *Pterostylis gibbosa* R.Br., was observed when investigated through starch gel electrophoresis. The percentage of polymorphic loci ( $P$ ), the number of alleles per locus ( $A$ ), observed ( $H_o$ ) and expected heterozygosity ( $H_e$ ) at population levels were 69%, 2.21, 0.210 and 0.261 respectively. The  $G_{st}$  value of 15% indicates that around 85% of variation resides within populations. High genetic variability along with low population divergence may be the result of recent population fragmentation or from extensive gene flow maintained by seed and pollen movement. Testing revealed high seed viability (range 68-90%) suggesting that poor seed viability is not the cause of its rarity. Although endangered and restricted to only four geographical areas, *P. gibbosa* showed a higher level of genetic variation than other orchids with larger populations.

GENOME EVOLUTION AMONG BASAL TAXA OF ANGIOSPERMS: AUSTROBAILEYACEAE,  
TRIMENIACEAE, AND ILLICIALES (THE 'ITA CLADE')

Helen M. Stace

*Department of Botany, University of Western Australia, Nedlands, WA 6907*

Recent molecular phylogenies found as basal angiosperms three clades of families, one being the 'ITA clade' (=Austrobaileyaceae (Trimeniaceae ((Illiciales))). The two basalmost taxa are Australian but Illiciales are distributed in Asia and disjunctly in south-eastern North America. Extant species of these taxa have large chromosomes ('genomic obesity') and considerable diversity for chromosome number. It is of interest to examine what information, if any, these may convey regarding the early angiosperm genome. Genomic conservation is a diploid model that utilises structural genomic units to explain chromosome number change. Austrobaileyaceae ( $x=22$ ), Trimeniaceae ( $x=8$ ), Illiciaceae ( $x=14,13$ ) and Schisandraceae ( $x=14$ ) have disparate karyotypes that are interpreted as genomic conservation based on a heuristic of  $FN=24$  small genomic units arranged and rearranged onto different numbers of centromeres. This analysis illustrates diploid cytoevolution spanning a wide array of chromosome numbers in one early clade of angiosperms.

Symposia = Gene Mapping



## CONSERVED 5' AND INTRONIC ENHANCERS IN GPDH LOCUS AND TRANSVECTION IN *DROSOPHILA MELANOGASTER*/*DROSOPHILA SIMULANS* HYBRIDS

Elly Tchoubrieva and John Gibson

Research School of Biological Sciences, GPO Box 475, Australian National University, Canberra ACT 2601

In *Drosophila melanogaster* the expression of sn-Glycerol-3-phosphate dehydrogenase (*Gpdh*) is controlled by two main enhancer elements, one located upstream of the promoter region and the other at the 5' end of the second intron. Both elements contain (CT)<sub>n</sub> repeats, which could be binding sites for regulatory proteins. Comparative analyses of these (CT)<sub>n</sub> blocks among different alleles within *D. melanogaster* and in melanogaster sibling species show that there is a significant conservation of these stretches of DNA sequence. It has been suggested that in *D. melanogaster* the enhancers are involved in a transvection effect in the *Gpdh* locus, which occurs in heterozygotes between low activity alleles with a KP- element inserted between the promoter region and the transcription start site, and normal activity alleles. We have investigated whether the same effect operates in *D. melanogaster*/*D. simulans* hybrids.

## REARRANGEMENTS OF THE CATTLE AND SHEEP X CHROMOSOMES FROM THE "REGULAR" EUTHERIAN TYPE.

Graham Webb<sup>1</sup>, Irene Koukoulas<sup>2</sup>, Cynthia Bottema<sup>1</sup>, Peter Aldred<sup>3</sup>

<sup>1</sup>Department of Animal Science, The University of Adelaide, Waite Campus, Glen Osmond SA 5064,

<sup>2</sup>Howard Florey Institute, The University of Melbourne, Parkville Vic 3052, <sup>3</sup>School of Science, The University of Ballarat, Mt Helen, Ballarat Vic 3350

For the submetacentric human X chromosome, the short, Xp, arm terminates with the pseudoautosomal region, which pairs with the Y chromosome. The X chromosomes of many eutherian mammals have the same shape and very similar banding pattern to the human X, leading to the probability that this "regular" X chromosome is the primitive type for eutherians. We localised the sheep angiotensin receptor 2 gene, *AGTR2*, to band q34 on the long arm of the acrocentric X chromosomes of sheep and goats, and to band p24, the tip of the short arm of the X in cattle. The distance of (*AGTR2* (*italics*))) from the centromere in the three species indicates that the X of cattle cannot be derived from that of sheep and goats by a single pericentric inversion. The submetacentric X chromosome in cattle was found to pair with the Y chromosome by its long arm, during male meiosis, indicating that it is not a regular eutherian X chromosome. From our results, and studies in the literature, we predict that the pseudoautosomal region of sheep and goats will be at the tip of the short arm of the acrocentric X chromosome.



# Genetics and Conservation of Australian Flora

## A Special Issue of *Australian Journal of Botany*

This volume (Vol. 48, no. 3, to be published July 2000) follows a symposium held at the Society for Conservation Biology Conference (Macquarie University, Sydney) on 13–16 July 1998. It incorporates a selection of papers presented at that symposium and other invited papers. Topics include a range of approaches used in plant conservation genetic studies from many different Australian plant groups.

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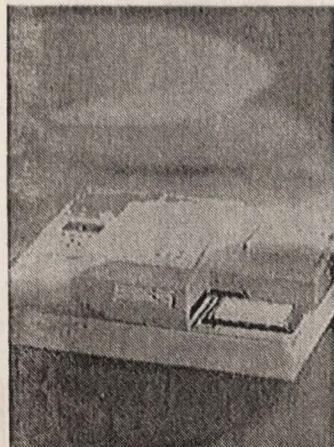
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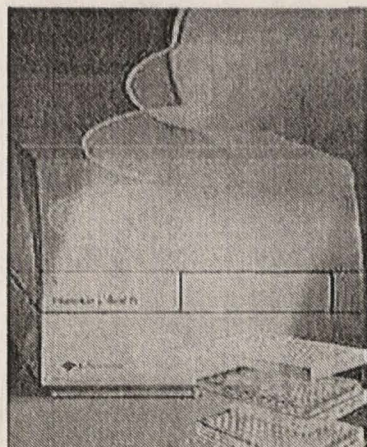
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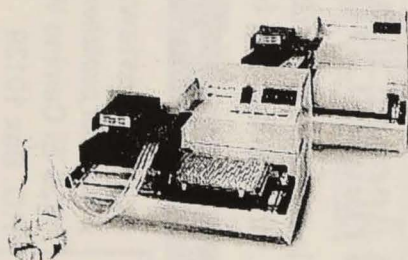
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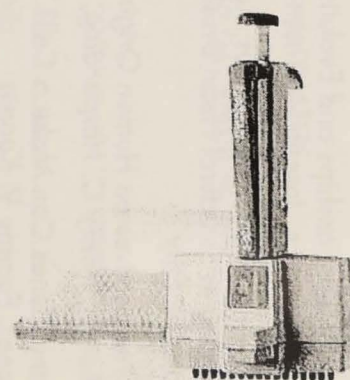
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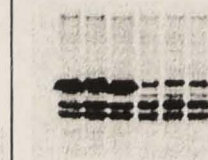
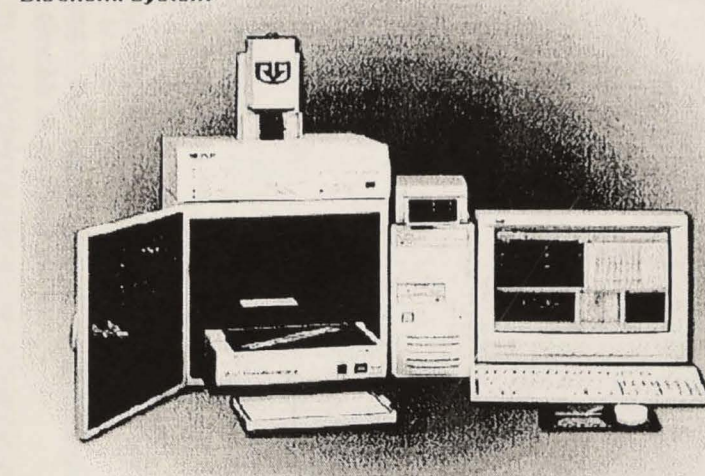
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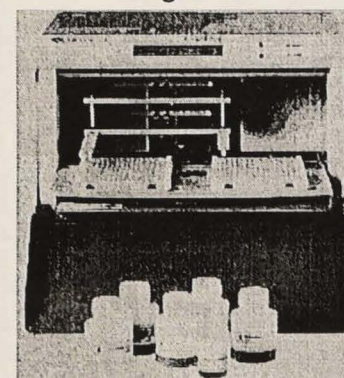
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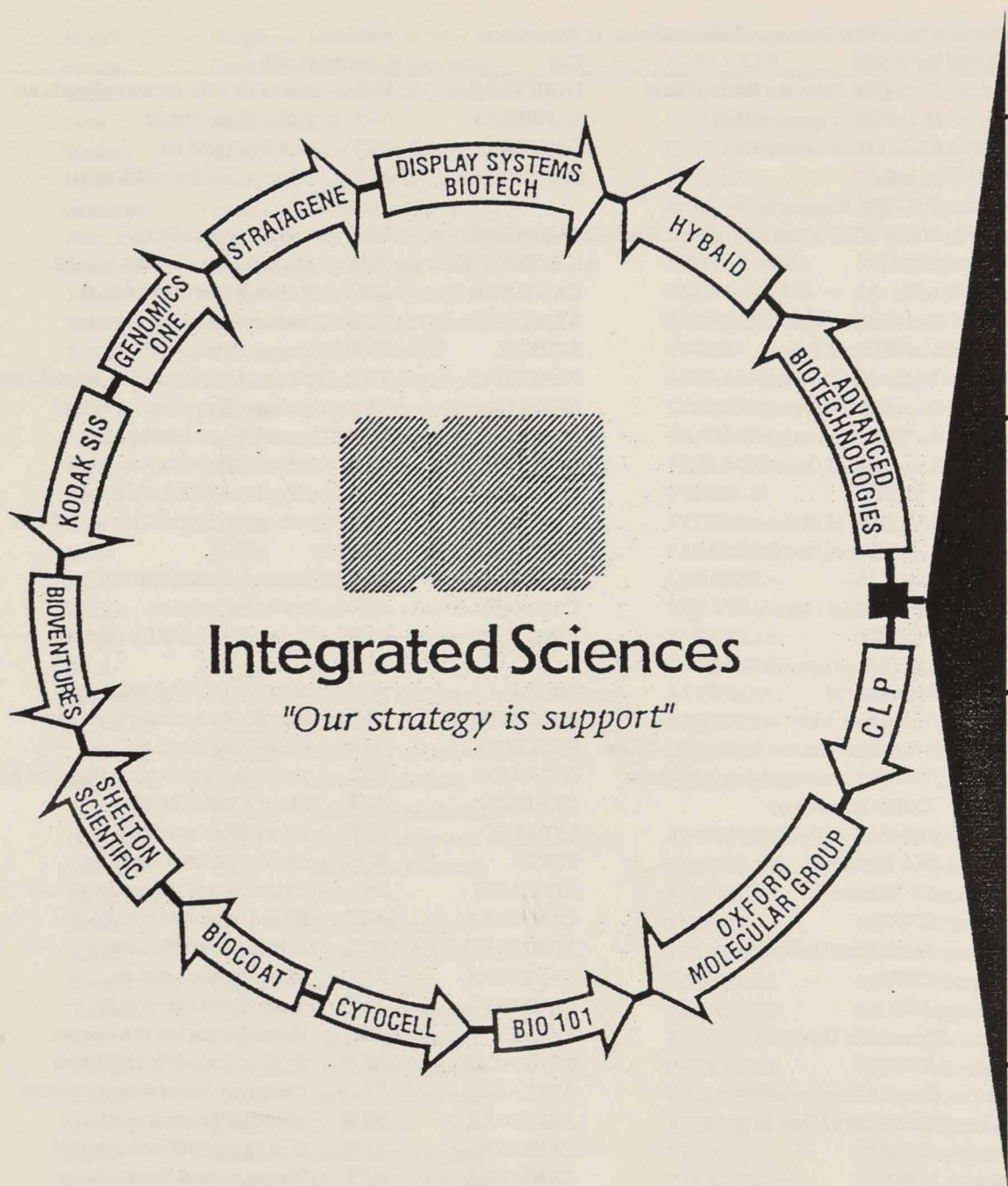
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Allen	Gillian	IP Australia representative	CANBERRA	ACT	gallen@ipaustalia
Appels	Rudi	CSIRO Plant Industry	CANBERRA	ACT	rudi@pi.csiro.au
Arioli	Tony	Aventis	CANBERRA	ACT	Tony.Arioli@aventis.com
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Baker	Rohan	ANU	CANBERRA	ACT	Rohan.Baker@anu.edu.au
Barker	Stuart	University of New England	ARMIDALE	NSW	sbarker@metz.une.edu.au
Barron	Andrew	University of Sydney	SYDNEY	NSW	abarron@bio.usyd.edu.au
Batterham	Philip	Melbourne University	PARKVILLE	VIC	p.batterham@genetics.unimelb.edu.au
Beheregaray	Luciano	Macquarie University	NORTH RYDE	NSW	lbehereg@ma.bio.mq.edu.au
Blacket	Mark	La Trobe University	BUNDOORA	VIC	genmb@gen.latrobe.edu.au
Blows	Mark	University of Queensland	ST LUCIA	QLD	mblows@zoology.uq.edu.au
Bock	Clive	CSIRO	CANBERRA	ACT	clive.bock@pi.csiro.au
Bogwitz	Michael	University of Melbourne	PARKVILLE	VIC	.mbogwitz!pgrad.unimelb.edu.au
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Brownlie	Jeremy	ANU	CANBERRA	ACT	jeremyb@ento.csiro.au
Brubaker	Curt	CSIRO Plant Industry	CANBERRA	ACT	curtb@pi.csiro.au
Butcher	Penny	CSIRO	KINGSTON	ACT	Penny.Butcher@ffp.csiro.au
Byrne	Margaret	Dept of Conservation and Land Management	PERTH	WA	margaretb@calm.wa.gov.au
Cardinal	Belinda	Museum of Victoria	ABBOTSFORD	VIC	bcardin@museum.vic.gov.au
Cattley	Sonia	Australian Genomic Information Centre	EVELEIGH	NSW	sonia@angis.org.au
Chamberlain	Amanda	Victorian Institute of Animal Science	ATTWOOD	VIC	Amanda.Chamberlain@nre.vic.gov.au
Clarke	Geoff	CSIRO Entomology	CANBERRA	ACT	Geoff.Clarke@emto.csiro.au
Clarke	Kylea	University of Sydney	SYDNEY	NSW	kclark@bio.usyd.edu.au
Coates	Bruce	WA Herbarium	PERTH	WA	davidc@calm.wa.gov.au
Cooper	Steven	SA Museum	ADELAIDE	SA	cooper.stev@saugov.sa.gov.au
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Donald	Jenny	Macquarie University	SYDNEY	NSW	jdonald@ma.bio.mq.edu.au
Donaldson	Felicity	UNSW	KINGSFORD	NSW	fr_donaldson@hotmail.com
Donnellan	Steve	South Australian Museum	ADELAIDE	SA	Donnellan.Steve@saugov.sa.au
Dougall	Annette	University of New England	ARMIDALE	NSW	adougall@metz.une.edu.au
Driscoll	Don	CSIRO	CANBERRA	ACT	Don.Driscoll@dwe.csiro.au
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Edwards	John	Oxford University	OXFORD		jhe@bioch.ox.ac.uk
Elphinstone	Martin	Southern Cross University	LISMORE	NSW	melphins@scu.edu.au
Fair	Jason	University of Melbourne	PARKVILLE	VIC	j.fair@genetics.unimelb.edu.au
Faith	Dan	Australian Museum	SYDNEY	NSW	danf@austmus.gov.au
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Goddard	Mike	Victorian Institute of Animal Science	ATTWOOD	VIC	Mike.Goddard@nre.vic.gov.au
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Low	David	UNSW	SYDNEY	NSW	dlow@bio.usyd.edu.au
Lucas	Daniel	Walter and Eliza Hall	PARKVILLE	VIC	dluca@wehi.edu.au
Ma	Wujun	CSIRO	CANBERRA	ACT	w.ma@pi.csiro.au
Mackie	Joshua	University of Melbourne	MALVERN	VIC	j.mackie@student.unimelb.edu.au
Magoc	Lorin	Melbourne University	PARKVILLE	NSW	l.magoc@pgrad.unimelb.edu.au
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McDonald	Maurice	CSIRO	KINGSTON	ACT	maurice.mcdonald@ffp.csiro.au
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Montgomery	Meg	UNSW	SYDNEY	NSW	m.montgomery@student.unsw.edu.au
Moran	Chris	Sydney University	SYDNEY	NSW	Chris.Moram@vetsci.usyd.edu.au
Moritz	Craig	University of Queensland	ST LUCIA	QLD	cmoritz@zoology.uq.edu.au
Nayudu	Murali	ANU	CANBERRA	ACT	Murali.Nayudu@anu.edu.au
Newcomb	Richard	Horticultural Research	AUCKLAND	NZ	newcomb@hort.cri.nz
Nicolle	Dean	Flinders University	BEDFORD	SA	Dean.Nicolle@flinders.edu.au
O'Keefe	Melanie	Walter and Eliza Hall	PARKVILLE	VIC	okeefe@wehi.edu.au
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Pase	Luke	University of Melbourne	PARKVILLE	NSW	l.pase@ugrad.unimelb.edu.au
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Perrine	Francine	ANU	CANBERRA	ACT	perrine@rsbs.anu.edu.au
Perry	Trent	Melbourne University	GLEN IRIS	VIC	trent1977@hotmail.com
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Sharma	Ish	CSIRO	CANBERRA	ACT	ish.sharma@pi.csiro.au
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Sherwin	Bill	UNSW	SYDNEY	NSW	W.Sherwin@unsw.edu.au
Siddall	Nicole	Melbourne University	PARKVILLE	VIC	n.siddall@ugrad.unimelb.edu.au
Smith	Kelly	University of Melbourne	PARKVILLE	VIC	k_smith@hotmail.com
Spielmeyer	Wolfgang	CSIRO Plant Industry	CANBERRA	ACT	w.spielmeyer@pi.csiro.au
Stace	Helen	University of Western Australia	NEDLANDS	WA	hmstace@uwa.edu.au
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Thomson	Glenys	University of California	BERKELEY	USA	glenys@allele5.biol.berkeley.edu
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