CHRIS MORAN

39TH ANNUAL CONFERENCE OF THE GENETICS SOCIETY OF AUSTRALIA

at The University of Queensland
5th - 9th July 1992

PROGRAM & ABSTRACTS

GENETICS SOCIETY OF AUSTRALIA

SUMMARY OF PROGRAM

	AM	PM	EVENING
SUN 5/7		4 - 7 **GSA Registration** (Emmanuel College)	7 - 10 **GSA Mixer** (Emmanuel College)
MON 6/7	8.30 - 10.10 **Bacterial Evolution & Genetics** (PLT3) 10.40 - 12.00 **Mutation & Transposons** (PLT3)	1.00 - 2.40 **Molecular Evolution** (PLT3) 3.10 - 4.30 **Molecular Genetics I** (PLT3) 3.10 - 4.30 **Conservation Genetics** (PLT2) 4.30 - 6.30 **Posters and Trade Displays**	FREE
TUES 7/7	8.30 - 10.10 **Sex Determination** (PLT3) 10.40 - 12.20 **Sex and Genome Organisation** (PLT3)	1.40 - 3.00 **Molecular Genetics II** (PLT3) 1.40 - 3.00 **Population & Evolutionary Genetics I** (PLT2) 3.30 - 5.30 **Molecular Genetics III** (PLT3) 3.30 - 5.30 **Population & Evolutionary Genetics II** (PLT2) 5.45 - 6.30 **Annual General Meeting GSA**	7.15 - 11.00 **Annual Dinner** (Kookaburra Queen)
WED 8/7	8.30 - 12.00 **Gene Mapping** (Joint Symposium with AGMW Workshop) (PLT1)	12.15 - 5.30 **BBQ** (at rainforest site)	FREE
THUR 9/7	8.20 - 10.20 **Ecological & Evolutionary Genetics I** (PLT2) 10.50 - 12.30 **Ecological & Evolutionary Genetics II** (PLT2)	1.30 - 3.30 **Ecological & Evolutionary Genetics III** (PLT2) 4.00 - 6.20 **Ecological & Evolutionary Genetics IV** (PLT2)	

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GENERAL INFORMATION

WELCOME

The organising committee for the Conference welcomes delegates and wishes them a happy and informative meeting. The committee will be pleased to assist with any difficulties. The committee comprises:

Craig Moritz (Local Secretary)
Georgia Chenevix-Trench (Social Functions)
Liz Jazwinska (Trade)
Ken Reed (Most Things)
Helen Bouter (Accommodation)

REGISTRATION AND HELP

The registration desk will be located at Emmanuel College from 4 pm - 10 pm on Sunday, June 5th, and then in the Physiology Foyer from 7.45 am on Monday, June 6th. We aim to have personnel at the desk at all times to provide information and assistance. If the desk is unattended, the organisers (with coloured name tags) should be approached for assistance.

VENUE, MEALS, AND SERVICES

All oral presentations will be in the main lecture theatres of the Physiology building (see map on back cover). Posters, trade displays, morning and afternoon tea, will be in the adjacent Foyer.

Lunches will be available at cafeterias on campus. All delegates will be Honorary members of the University Staff and Graduate Club, where refreshments, lunches and dinner are available. There are several good restaurants within easy walking distance at the nearby St Lucia shopping area.

A shopping arcade in The University of Queensland Union Complex includes a pharmacy, hairdresser, gift shop, clothing shop, second-hand book and stationery shop, newsagency and sweet shop, travel agency, bicycle shop and computer shop. These services are open, Monday - Friday, 0830 - 1700 hours. Banks on Campus are open, Monday - Thursday, 0930 - 1600; Friday, 0930 - 1700 hours.

ACCOMMODATION

Single rooms have been allocated in Emmanuel College to all those who had indicated their need for College accommodation. Keys are available at the College office. Those who have not paid in advance must pay for their rooms at registration (Bed and Breakfast, $38.00). Rooms should be vacated by 10.00 am on the day of departure but baggage may be left at the College until final departure.

INFORMATION FOR CONTRIBUTORS

PAPERS

Submitted papers will be 15 minutes in length, with an additional 5 minutes for questions. Chairpersons will keep strictly to time to permit switching between sessions. Invited papers of longer duration also include 5 minutes for questions. Speakers should contact the Chairperson before the start of the session. Facilities will be available for the projection of overhead transparencies and 35 mm slides (see diagram below for details of loading slides for rear projection). Slides should be taken to the projectionists in the preparation area Room 328, Biochemistry, at least 20 minutes before the session is due to start. Facilities will be available there to view slides and ensure they are loaded correctly.

POSTERS

Posters for the **GSA meeting** should be in place in the Physiology foyer by the end of morning tea on Monday the 6th. A poster session with refreshments will be from 4.30 to 6.30 pm on Monday and you are asked to be at your poster for this period. Your poster should be taken down by 9.00 am on Wednesday to make room for the AGMW contributions.

You will have a 1m x 1.5m space within which to put your poster. Stick-on velcro patches are available @ $0.20 (i.e., at cost). Your poster will be assigned a number supplied to you at the time of registration and should be posted in the location bearing that number.

PARKING ON CAMPUS

The University of Queensland has new and strict rules about parking of vehicles, and in all areas a fee is involved.

For those of you who may wish to bring a vehicle on campus we provide the information below. The areas/colour-zones referred to are indicated on the campus map enclosed with registration materials.

(i) **Day permits** - may be obtained from dispensing machines located in various areas of the campus and these provide an entitlement to park in the green zone. These permits should be placed on the dashboard of the vehicle so that they can be checked by the Traffic Wardens.

(ii) **Visitor Parking** - The following areas have been set aside for visitor parking as follows:

Mansfield Place	$1 per hour
Mill Road Car Park	40 cents per hour
Oval No. 4 Car Park	40 cents per hour

(iii) **Hours of Parking Regulation**

Monday to Friday 7 am to 6.30 pm

After 4 pm, a Green Zone or day permit will allow parking in any zone of the campus except in the "Visitor" and metered parking areas. Vehicles displaying Blue Zone permits will be permitted to park in the Red Zone.

If you have any further enquires about the campus parking plan, please contact Traffic Administration Section on (07) 365 2235.

SOCIAL FUNCTIONS

Restaurants There are a number of BYO restaurants with a wide price range on Hawken Drive - ten minutes walk from the Great Court and five minutes from Emmanuel College. The nearest 'Bottle Shop' is the Staff Club, of which all GSA registrants are honorary members during the conference. A new one may have opened on Hawken Drive by the time of the meeting.

Lunches Sandwiches, hot meals and drinks can be purchased from trolleys outside the Physiology Lecture Theatre, from the Physiology refectory or from the Staff Club.

Mixer The mixer will be held at Emmanuel College from 7.30 - 10.00 pm on Sunday 5th July. Light hors d'oeuvres will be served but we suggest that you eat first in St Lucia.

Dinner The conference dinner will be held on Tuesday 7th July on the Kookaburra Queen - a Paddlewheeler on the Brisbane River. Buses will leave from the Physiology Lecture Theatre at 6.45 pm; the cost is included in the price of the ticket. Tickets are $40 full price and $25 for students. Additional tickets can be purchased on Sunday or Monday **only**. If you have your own transport, the boat leaves from The Pier at Waterfront Place, Eagle Street in the City at 7.15 pm. Remember, this is winter in Brisbane and if you want to sit out on deck **you will need a coat**! Due to the large number of (subsidised) students tickets we will unfortunately be unable to provide drinks so please bring your wallets - and cigarettes as there is no machine on board.

Barbecue A joint barbecue will be held with the participants of the Gene Mapping Workshop at Boombana on Wednesday afternoon. Buses will leave **immediately after** the morning session from the Physiology Lecture Theatre and the cost is included in the price of the ticket. Tickets are $20 and drinks will be available at cost. Additional tickets can be purchased on Sunday, Monday or Tuesday **only**. If you have your own transport, head for The Gap and Boombana is just past Jolly's Lookout on the Mount Nebo/Mount Glorious Road - it is about 1 hour from the University. There are several short rainforest walks from Boombana, including one to Jolly's Lookout from which the bus will collect walkers at 4.05 pm. The bus leaves Boombana at 4.00 pm. It will be several degrees cooler at Boombana than at St Lucia.

Cafe Prego

Excellent Mediterranean Food at Excellent Prices

Garlic or herb bread	$1.50	Ravioli Ricotta	$8.80
Bruschetta Pomodoro	$2.80	Spaghetti Carbonara	$8.80
Bruschetta Tapenade	$3.20	Fettucine Fellini	$9.80
		Spaghetti Marinara	$12.50
Soup of the Day	$3.50	Spaghetti aux Calamars	$10.50
Prawns Thai Style	$6.50		
Calamari a la Basquaise	$5.80	Profiteroles au Chocolat	$3.80
Vegetarian terrine	$4.80	Souffle Glace aux fruits de la Passion	$3.80
Salade Nicoise	$3.80	Creme Caramel	$3.80
		Bavarois Grand Marnier	$3.80
Noisette of Veal sauce chevreuil	$12.50		
Entrecote de Boeuf	$12.50		
Chicken bonbon a la Prego	$12.50		
Fish of the Day Cajun Style	$13.50	Corkage	$1.00

BYO - 224 Hawken Drive, St Lucia Telephone 371 2308 - **BYO**

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TUESDAY — SUNDAY

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For all reservations and bookings please telephone Ric or Kerry-Lyn on 870 3322.

ACKNOWLEDGMENTS

On behalf of the Genetics Society of Australia, the organising committee would like to thank The University of Queensland for providing facilities.

We also thank the following sustaining members for supporting the conference with their Trade displays:

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We are especially grateful to **Pharmacia (Australia) Pty Ltd** for their substantial contribution to the conference costs.

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The Genetics Society of Australia Inc would like to record its appreciation to the following companies.

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PROGRAM FOR GENETICS SOCIETY OF AUSTRALIA

MONDAY, 6TH JULY

Bacterial Evolution & Genetics: PLT3

8.30 - 8.40 Welcome & Introduction

8.40 - 9.30 GSA Guest Speaker's Address: Selection-induced mutations in *E. coli* and yeast: some hints on possible mechanisms
B. Hall

9.30 - 9.50 Integrons: versatile genetic elements in disseminating antibiotic resistance genes
H. Stokes and *R. Hall*

9.50 - 10.10 Sensor-regulatory networks in bacterial pathogenesis: a two-component transcriptional regulatory system controlling expression of *Pseudomonas aeruginosa* Type 4 Fimbriae
J. Mattick, M. Hobbs, E. Collie, P. Free and S. Livingston

Mutation & Transposons: PLT3

10.40 - 11.00 Why is UV-induced mutagenesis in some enteric bacteria weak?
S. Thomas

11.00 - 11.20 Studies of spontaneous mutations using the mouse H-2 region
J. Dempsey

11.20 - 11.40 Mutational outbreak and unstable genes in natural populations of *Drosophila melanogaster*
M. Golubovsky

11.40 - 12.00 Transposable elements from *Lucilia cuprina*
H. Perkins and T. Howells

Molecular Evolution: PLT3

1.00 - 1.40 M.J.D. White Address: Populations, molecules, and honeybee mitochondrial DNA
R. Crozier (President, GSA)

1.40 - 2.00 The formation of pseudogenes in mitochondrial DNA from parthenogenetic lizards (*Heteronotia boneoi*: Gekkonidae)
E. Zevering

2.00 - 2.20 The organisation of chloroplast DNA sequences in nuclear DNA of tobacco
M. Ayliffe and J. Timmis

2.20 - 2.40 Evolution of marsupial milk protein genes
C. Collet and R. Joseph

Molecular Genetics I: PLT3 (Concurrent session)

3.10 - 3.30 DNA repair, hairless locus genotype and skin cancer susceptibility in the mouse
L. Ruiting and C. Moran

3.30 - 3.50 Clonal selection for deletions and duplications of chromosome 17 in human myeloid malignancy
G. Peters, A. Correll, A. Dobrovic, T. Han, D. Kotasek, R. Sivalingam-Stratil, J. Ford and R. Sage

3.50 - 4.10 New DNA markers for tracing alien chromatin introduced into bread wheat
U. Hohmann, B. Clarke, E. Lagudah and R. Appels

4.10 - 4.30 The development of cytological and molecular markers for cereal cyst nematode (CCN) resistance in rye
C. Taylor and I. Dundas

Conservation Genetics: PLT2 (Concurrent session)

3.10 - 3.30 Genetic tags for turtles: analysis of the geographic source of harvested green turtles
J. Norman

3.30 - 3.50 Molecular conservation genetics of the helmeted honeyeater
H. Allen, W. Sherwin and N. Murray

3.50 - 4.10 Population bottlenecks and patterns of genetic diversity in *Stylidium*
D. Coates

4.10 - 4.30 Mitochondrial DNA population differentiation and gene flow in the Coconut Crab (*Birgus latro*)
S. Lavery

POSTERS AND TRADE DISPLAYS 4.30 - 6.30

FREE EVENING

TUESDAY, 7TH JULY

Sex determination: PLT3

8.30 - 9.10 Keynote Speaker: Genes and sex and a mouse called Randy
P. Koopman

9.10 - 9.30 Triploid intersex and chimeric chickens: implications for avian sex determination
M. Thorne and B. Sheldon

9.30 - 9.50 Environmental sex determination in Crocodilians
J. Joss and C. Smith

9.50 - 10.10 Intersex Tasmanian Devils
L. Hughes

Sex and genome organisation: PLT3

10.40 - 11.00 Evolution of sex chromosomes and sex determining genes
J. Marshall Graves, J. Foster, F. Brennan and G. Hampikian

11.00 - 11.20 Sex-associated genetic linkage in the fat-tailed dunnart *Sminthopsis crassicaudatus* (Marsupialia)
R. Hope, J. Bennett, C. Chesson and D. Golding

11.20 - 11.40 Gene mapping in the Tammar wallaby
L. McKenzie, D. Cooper and C. Collet

11.40 - 12.00 Genetics and the evolution of viviparity in mammals
D. Cooper

12.00 - 12.20 Characterization of subtelomeric and centromeric sequences of the mouse
O. Miller and D. Miller

Molecular Genetics II: PLT3 (Concurrent session) Chair: Jeremy Timmis

1.40 - 2.00 Isolation of the *Notch* homologue from the Australian sheep blowfly, *Lucilia cuprina*
A. Game, T. Williams, A. Davies, M. Delbridge, J. McKenzie and P. Batterham

2.00 - 2.20 Molecular analyis of a complex family of neuropeptides in blowflies
P. East, H. Duve, E. Hines, T. Sutherland and A. Thorpe

2.20 - 2.40 *Trans*-splicing in sheep parasitic nematodes
M. Callaghan and K. Beh

2.40 - 3.00 The molecular genetics of porcine malignant hyperthermia
I. Hughes + C. MORAN

Population & Evolutionary Genetics I: PLT2 (Concurrent session)

1.40 - 2.00 Genetic variation for growth hormone in sheep
Y. Parsons, L. Piper, T. Adams and D. Cooper

2.00 - 2.20 Relationships among hybridising taxa of the varied sittella complex
I. Scott, L. Christidis and M. Westerman

2.20 - 2.40 The position of Leadbeater's possum (*Gymnobelideus leadbeateri*) within the Petauridae based on DNA-DNA hybridization data
D. Edwards and M. Westerman

2.40 - 3.00 A chromosomal banding study of euchromatin transformation
M. Mahony, S. Donnellan and M. Schmid

Molecular Genetics III: PLT3 (Concurrent session)

3.30 - 3.50 Detection of yeast proteins recognizing promoter sequences of the *Aspergillus nidulans amdS* gene
N. Bonnefoy, M. Hynes and M. Davis

3.50 - 4.10 Putting a finger on *amdS* regulation by the *amdA* gene of *Aspergillus nidulans*
R. Lints, M. Davis & M. Hynes

4.10 - 4.30 Comparison of the *gatA* genes of *A. nidulans* and *U. maydis*
M. Straffon, M. Hynes and M. Davis

4.30 - 4.50 Analysis of *facB*, an acetate regulatory gene from *Aspergillus nidulans*
R. Todd, M. Davis and M. Hynes

4.50 - 5.10 Floral meristem identity is controlled by the *L E A F Y* gene in *Arabidopsis thaliana*
J. Alvarez and D. Smyth

5.10 - 5.30 Arabinose metabolism mutants of *Arabidopsis thaliana*
S. Sherson, J. Medd and C. Cobbett

Population & Evolutionary Genetics II: PLT2 (Concurrent session)

3.30 - 3.50 The genetic load revealed by slow inbreeding in *Drosophila melanogaster*
B. Latter, J. Mulley, D. Reid and L. Pascoe

3.50 - 4.10 Fitness and asymmetry modification revisited; malathion-resistance in *Lucilia cuprina*.
J. McKenzie and K. O'Farrell

4.10 - 4.30 MHC class II genes in parasite resistant sheep
J. Maddox, S. Fabb, R. van Oorschot, C. Smole and K. Gogolin-Ewens

4.30 - 4.50 Phylogenetic relationships of the thylacine (*Thylacinus cynocephalus*) among dasyuroid marsupials: evidence from cytochrome-b
C. Krajewski, A. Driskell, P. Bavertsock and M. Braun

4.50 - 5.10 Phylogenetic analysis of the night parrot, *Geopsittacus occidentalis*
P. Leeton, L. Christidis and M. Westerman

5.10 - 5.30 Biochemical relationships among diploid and tetraploid desert burrowing frogs
S. Donnellan, M. Mahony and J. Roberts

ANNUAL GENERAL MEETING GSA: 5.45 - 6.30
Bus departs at 6-45pm

ANNUAL DINNER (Kookaburra Queen): 7.15 - 11.00

WEDNESDAY, 8TH JULY

Gene mapping (Joint Symposium with AGMW Workshop): PLT1

8.30 - 9.15 Applications of mouse linkage maps
N. Copeland (Invited speaker: joint with AGMW)

9.15 - 10.00 Linkage analysis in man - the Lodscore method
M. Farrall

10.30 - 11.00 Glycogenosis disorders in man and cattle
A. Reuser, H. Wisselaar, M. Hermans, B. Eussen, B. Oostra, W. Aspden, B. Harrison, J. Hetzel, R. Drinkwater

11.00 - 11.30 Mapping genes for neuromuscular diseases
N. Laing

11.30 - 12.00 Chromosome walking in *Arabidopsis thaliana*: Mapping *ms-1*, a gene critical for male gametophyte development
K. Blömer, L. Farrell, A. Chaudhury, R. Chapple, B. Sherman and E. Dennis

BBQ (at rainforest site) 12.15 - 5.30

THURSDAY, 9TH JULY (all sessions concurrent with AGMW)

Ecological & Evolutionary Genetics I: PLT2

8.20 - 8.40 Genetic variation for olfaction in *Drosophila buzzatii*
T. Armstrong and S. Barker

8.40 - 9.00 Genetic variation in cactophilic *Drosophila* for oviposition on natural yeast substrates
S. Barker

9.00 - 9.20 Genetic variation between founding and ancestral populations of *Drosophila buzzatii*
R. Krebs

9.20 - 9.40 The population structuring of a recently arrived pest: an allozyme study of *Lucilia cuprina* in New Zealand
D. Gleeson, D. Rowell and J. Oakeshott

9.40 - 10.00 The population genetics, reproductive biology and morphology of the Serpent's head cowry *Cypraea caputserpentis* (*Kenyonae*)
R. Standish

10.00 - 10.20 Variability in *Rattus sordidus* population densities: ecological and gene flow hypotheses
W. Ruscoe

Ecological & Evolutionary Genetics II: PLT2

10.50 - 11.10 Mating systems and genetic structure in Banksia populations
D. Coates and J. Sampson (Invited speaker)

11.10 - 11.30 Geographic variation in allele frequency in *Banksia spinulosa* in southeast Queensland
M. Arthur

11.30 - 11.50 Potential for gene flow via hybridisation in *Banksia*
S. Schibeci

11.50 - 12.10 The mating system, nectar production and pollinator foraging behaviour in subspecies of *Eucalyptus caesia*
S. Hopper (Invited speaker)

12.10 - 12.30 Interclonal variation in aggressive behaviour in sea anemones
D. Ayre and R. Grosberg

Ecological & Evolutionary Genetics III: PLT2

1.30 - 1.50 Gene flow and dispersal in stream invertebrates
J. Hughes, S. Bunn, D. Hurwood and M. Kingston

1.50 - 2.10 The genetic component of total catchment management - an examination of catchment position and the population genetic structure of a running water decapod
M. Kingston

2.10 - 2.30 Genetic diversity and evolutionary history of rainforest vertebrates
C. Moritz

2.30 - 2.50 Genetic consequences of relentless habitat fragmentation in eastern Australian rainforests
L. Joseph and D. Rowe

2.50 - 3.10 Regional genetic divergence in a vagile Australian moth, *Helicoverpa punctigera*
S. McKechnie and A. Hoffmann (Invited speaker)

3.10 - 3.30 Family and population structure in the platypus
N. Gemmell, T. Grant, P. Western, J. Watson, N. Murray and J. Marshall Graves

Ecological & Evolutionary Genetics IV: PLT2

4.00 - 4.20 Phospho-Glycerate Kinase polymorphism in the scallop *Pecten fumatus*: selection in natural and experimental populations
L. Woodburn and N. Murray

4.20 - 4.40 Philopatry and the genetic structure of populations
A. Hunt

4.40 - 5.00 The maintenance of genetic diversity by unidirectional gene flow in the mosquitofish *Gambusia holbrooki*
B. Congdon

5.00 - 5.20 Effects of pollution at enzyme loci in three species of marine invertebrates
H. Chapman

5.20 - 5.40 The effects of heavy metal pollution on the genetic structure of populations of the hairy mussel, *Trichomya hirsuta*
N. Phillips

5.40 - 6.00 The relationship between temperature and PGI allelic variation in the isopod, *Porcellio laevis*
P. Mather and S. McCluskey

POSTERS

1. Modelling of an endangered population of hairy nosed wombats. *D. Alpers, W. Sherwin*
2. The search for *rec-2*: a gene that modulates the level of meiotic recombination in *Neurospora crassa*. *F. Bowring and D. Catcheside*
3. Evidence for hybrid formation in natural populations of the bean rust fungus. *K. Braithwaite, J. Manners, D. Maclean and J. Irwin*
4. Chloroplast restriction map and maternal inheritance in *Eucalyptus nitens*. *M. Byrne and G. Moran*
5. Genetic and molecular analysis of the *Notch* homologue in the Australian sheep blowfly, *Lucilia cuprina*. *A. Davies, A. Game, T. Williams, M. Delbridge, J. McKenzie and P. Batterham*
6. Flow sorting marsupial chromosomes. *D. Francis, G. Chojnowski, I. Bertonocello and J. Marshall Graves*
7. Developmental expression of the *topaz* and *white* eyecolour genes of *Lucilia cuprina*. *R. Garcia and T. Howells*
8. Microhabitat selection and colour morph frequencies in a highly polymorphic intertidal snail (*Clithon oualaniensis*). *M. Gardner*
8. Determination of familial relationships in a platypus population. *N. Gemmell, T. Grant, P. Western, J. Watson, N. Murray and J. Marshall Graves*
10. Abnormal pachytene pairing behaviour in hybrid combinations of water buffalo. *C. Gillies and K. Dai.*
11. Characterisation of MHC genes in koalas. *W. Greville, B. Houlden and B. Sherwin*
12. Genetic analysis of transgenic *Caenorhabditis elegans* transformed with a parasite tubulin gene. *W. Grant*
13. Isozymes - a useful tool in forest tree genetics and improvement. *A. House and C. Harwood*
14. Characterisation of the cadmium-sensitive mutant, *cad1*, of *Arabidopsis thaliana*. *R. Howden and C. Cobbett*
15. Molecular variation within and among host races of root knot nematodes (*Meloidogyne*). *A. Hugall, J. Stanton and C. Moritz.*
16. Genetic analysis of variability in flight activity in the monarch butterfly *Danaus plexippus*. *J. Hughes, M. Zalucki, J. Chaseling and C. Lange*
17. A genetic analysis of extracellular protease production in *Aspergillus nidulans*. *M. Katz, B. Cheetham and P. Flynn*
18. Isolation by distance of barramundi (*Lates calcarifer*) populations. *C. Keenan*
19. Germplasm evaluation and preliminary genetic data from Sturt's Desert Pea (*Swainsona formosa*). *G. Kirby*
20. Identification of *Hordeum vulgare* cv."Galleon" mutants deficient in va-mycorrhiza formation. *P. Murphy.*
21. The ß globin gene cluster of *Sminthopsis crassicaudata*. *R. Murphy, S. Cooper and R. Hope*
22. Characterisation and sequence analysis of a small cryptic plasmid from *Selenomonas ruminantium*. *Zhang Ning and J. Brooker*
23. DNA fingerprinting in citrus. *S. Orford, N. Steele Scott and J. Timmis*
24. Sex-specific differential methylation in the body of the kangaroo X-linked hypoxanthine phosphoribosyltransferase gene. *A. Piper, A. Bennett, L. Noyce and S-Y Chong*
25. Allozyme variation within the *Euasticus hystricosus* (Parastacidae) population, and its relevance for conservation management. *M. Ponniah*
26. A race-specific mini-chromosome in the fungal phytopathogen *Colletotrichum gloeosporioides*. *A. Poplawski, A. Masel, J. Irwin and J. Manners*
27. Do sequences in Xq27.3 play a role in X inactivation? *M. Schmidt, J. Clarke and J. Hopwood*
28. Gene flow among conspecific population of *Baetis* sp. within and between subcatchments in the Conondale Range, Qld. *S. Schmidt*
29. Using PCR to detect nuclear gene variation across diverse species. *R. Slade, A. Heideman, P. Hale and C. Moritz*
30. A molecular population genetics approach to understanding the evolution of organophosphate resistance in *Lucilia cuprina*. *K-A Smyth, T. Boyce, R. Newcomb, R. Russell and J. Oakeshott*
31. Analysis of spontaneous mutation at the *scarlet* locus of *Drosophila melanogaster*. *J. ten Have and T. Howells*
32. Hexose transport mutants of *Arabidopsis thaliana*. *P. VanKuyk, S. Davidson and C. Cobbett*
33. The effects of estuaries and islands on the genetic structure of four species of marine inshore fishes. *R. Watts*
34. C-banding and electron microscopic study of the holocentric chromosomes of the earwig *Labidura truncata*. *G. Webb.*
35. *In situ* hybridization using probes derived from laser microdissected chromosomes. *G. Webber, R. Hope, B. Wainwright, Shinji Hadano and Joh-E Ikeda*
36. *supernova*: A gene involved in the cleavage division of the *Drosophila* embryo. *M. Webster and N. Brink.*
37. Fluctuating asymmetry and genetic variation in relation to pollution impacts on *Uca coarctata*. M. Wiggins
38. The mouse X-Y shared gene *ube1* detects homologues on the marsupial X and Y chromosomes. *S. Wilcox and J. Marshall Graves*
39. *cog* and *sor*: two elements involved in the control of genetic recombination in the *histidine*-3 region of *Neurospora crassa*. *P. Yeadon and D. Catcheside*

Molecular Conservation Genetics of the Helmeted Honeyeater.

Heidi B.Allen[1], W.B.Sherwin[2], N.D.Murray[1]

[1] Dept. of Genetics and Human Variation and Centre for Conservation Genetics, La Trobe University

[2] School of Biological Sciences, University of N.S.W.

Conservation genetic research on an endangered taxon can be applied to three main management objectives:(i) taxon identification (and hence priority); (ii) close-order management of wild populations; and (iii) management of captive populations. Here, we describe our work on the Helmeted Honeyeater, which is being applied to all three of these objectives.

The Helmeted Honeyeater, originally described as *Meliphaga cassidix*,is currently classified as *Lichenostomus melanops cassidix*, a sub-species of the Yellow-tufted Honeyeater. Its original geographic distribution in West and South Gippsland has contracted to a single natural population of less than twenty breeding pairs plus thirty to forty non-reproductive individuals. Because of its endangered status, the wild population is being actively managed by the Victorian Dept. of Conservation and Environment, and a captive breeding programme has been established at Healesville Zoo.

Our research has been designed to help guide these programmes. By analysing DNA extracted from small blood samples, we have aimed to detect variation in nuclear and mitochondrial genes. Here we describe the detection of VNTR variation with the pSP.2.5.E1 *per* probe. A single major locus appears to account for much of the variation seen in Southern blot "fingerprints". So far ten alleles have been detected in the natural population of *L.m.cassidix*.

This marker has been used to clarify the number of breeding pairs in the natural population, and to identify cases of infidelity amongst wild breeding pairs. These results lead to a greater understanding of social relationships. They also have implications for the estimating the effective size of the natural population and for the management of inbreeding in the captive colony.

Comparisons of *L.m.cassidix* with other subspecies of *L.melanops* are also being undertaken and will be presented. The results will have implications for evaluating the Helmeted Honeyeater's priority for conservation action but only when a sound theoretical framework and a comparative data-base have been established for a range of species.

MODELLING OF AN ENDANGERED POPULATION OF HAIRY-NOSED WOMBATS.

D.ALPERS, W.SHERWIN.
SCHOOL OF BIOLOGICAL SCIENCE,
UNIVERSITY OF NEW SOUTH WALES.

The northern hairy-nosed wombat, *Lasiorhinus krefftii*, is extremely endangered. Less than 100 individuals remain and these exist as a single population at Epping Forest National Park. Small populations are inherently at risk of losing genetic variability through inbreeding. Adverse consequences of reduced genetic diversity include lower fertility, survivability and disease resistance. The extent to which this species is being affected by such problems is being investigated via both genetic and demographic analyses. The establishment of a second colony, to reduce the risk of single catastrophe effects, is currently under investigation. Computer modelling programs are being used to ascertain the potential genetic and demographic problems involved in this proposal. Using what we know of the biology of this species, simulated populations are modelled and tested under different conditions. Various rates of harvesting are used in an attempt to establish the optimal transfer rate of animals from the original to the second colony, as both the first and second colonies will need to retain the maximum genetic diversity possible. Computer modelling is an effective management tool, both in preliminary planning stages and for on-going monitoring of populations.

Floral meristem identity is controlled by the *LEAFY* gene in *Arabidopsis thaliana*

John Alvarez & David R Smyth
Department of Genetics & Developmental Biology, Monash University, Clayton, Melbourne, Vic 3168, Australia

As the main apical meristem of wild type *Arabidopsis* grows meristems arise on its flanks. At first these develop into rosette leaves with short internodes. Once flowering has been initiated the main apex produces a limited number of small cauline leaves which are separated by long internodes as the stem bolts. Flowers then arise successively on the flanks of the main stem for as long as it continues to grow. Further flowering stems usually arise in the axils of the cauline leaves to produce second order branches. Higher order branches may also appear.

A series of mutants has been isolated at the *leafy* locus which disrupt this pattern. In strong mutants the same number of rosette leaves is produced as in wild type but the main apical meristem produces more cauline leaves. Flower-like structures then arise but they differ from wildtype flowers in that they have inflorescence properties. Firstly they have leaf-like sepals which arise in larger numbers than usual with a leaf-like phyllotaxy. Secondly, additional abnormal flowers often arise within the axils of these "sepals", especially on the first formed flowers. Thirdly *leafy* flowers do not have petals or stamens (although they do eventually produce a terminal gynoecium). Finally mutant flowers may often be associated with a small subtending cauline leaf (bract) on the main stem.

The wild type *LEAFY* gene product may specify that meristems arising on the flanks of the apex become flowers. In *leafy* mutants, this control may be weakened such that flanking meristems initially develop with inflorescence characteristics although flower-like structures do arise progressively. Interestingly all floral characteristics are lost in *leafy apetala1* double mutants suggesting that the *LEAFY* and *APETALA1* wild type genes normally interact to control floral meristem identity.

The *LEAFY* gene has recently been cloned (Weigel, D. *et al.* (1992) Cell **69**, in press). In wild type plants its expression is first detected in cells which will soon become flower meristems on the flanks of the main apex, and it remains active throughout the young flower meristems until floral organs start to develop. This expression pattern is consistent with a primary role for *LEAFY* in determining floral meristem identity.

Genetic Variation for Olfaction in *Drosophila buzzatii*

Tim P. Armstrong and J.S.F. Barker
Department of Animal Science, University of New England

Theory suggests that genotype specific habitat selection may maintain genetic variation in a heterogeneous environment, but there is little evidence demonstrating genetic variation for habitat selection in natural populations. However, results from experimental studies with the cactophilic *Drosophila buzzatii*, indicate a considerable potential for habitat selection (see also Barker this meeting).

Olfactory responses are an important behaviour in resource selection by female *D. buzzatii*. Significant genetic variation for long and short distance olfactory response to volatiles known to be produced by yeasts found in necrotic cacti, was found among isofemale lines of *D. buzzatii*. Further, volatiles that were attractive over long distances were not necessarily attractive over short distances, and *vice versa*, indicating that different chemical cues are important at different stages of resource selection.

In nature flies respond to a `cocktail' of chemical cues produced by cactophilic yeasts. Different yeasts produce different chemical profiles. Significant genetic variation for short and long distance olfactory response to four naturally occurring yeast species was found among four isofemale lines of *D. buzzatii* . Two isofemale lines that were highly attracted to ethanol in the volatile response experiment, were also highly attracted to the yeasts *Candida sonorensis* and *Clavispora opuntiae*. Both these yeasts produce higher levels of ethanol than do the other two yeast species tested. Thus, ethanol appears to be an important volatile in the attraction of female *D. buzzatii* to a potential feeding and breeding resource, and there exists genetic variation for olfactory response to this chemical.

THE ORGANISATION OF CHLOROPLAST DNA SEQUENCES IN NUCLEAR DNA OF TOBACCO.

M. A. AYLIFFE and J. N. TIMMIS
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Three hundred clones containing DNA with high sequence homology to chloroplast (cp)DNA were isolated from nuclear genomic libraries of *Nicotiana tabacum*. One lambda EMBL4 clone containing a long tract of homology to cpDNA, representative of a frequent type of clone, will be described in detail. This clone is homologous in its entirety to cpDNA but it was unequivocally assigned to the nuclear genome.

The majority of the 15.5 kb sequence is >99% homologous with its chloroplast DNA counterpart but a single base deletion causes premature termination of the reading frame of the *psa*A gene. One section of the clone contains a concentration of deleted regions and these were used to identify and quantify the sequence in native nuclear DNA by PCR methods. An estimated 15 copies of this specific region are present in a 1c tobacco nucleus.

Interclonal Variation in Aggressive Behaviour in Sea Anemones

by D.J. Ayre[1] and R.K. Grosberg[2]. 1- Department of Biology University of Wollongong and 2- Department of Zoology, University of California at Davis.

Adults of the fissiparous sea anemone *Anthopleura elegantissima* can use the nematocysts of their fighting tentacles (acrorhagi) to attack and kill non-clonemates. This behaviour is thought to be especially important in maintaining spatially and temporally stable interclonal borders between apparently evenly matched clones. It has also been claimed that anemones which occupy positions at interclonal borders are physically specialised as warriors.

Laboratory trials revealed substantial interclonal variation in both the nature and time course of aggressive responses to inter-clonal contact. These data provide the first demonstration of both genotype specific enhancement (memory) and genotype specific habituation (tolerance) in sea anemones. Initial aggressiveness and the time course of the development of memory and tolerance varied among a set of six clones. This temporal variation allows the exchange of aggressive dominance between initially aggressive and intially passive clones following repeated contact. Nevertheless, a complete matrix of pairings of each of seven clones revealed that clones have consistent and predictable dominance rankings in naive encounters. Further pairings of neighbouring clones revealed that neighbours need not be evenly matched and that 'warriors' are not always able to defeat the larger anemones from the centre of clones.

Our data imply that the outcome of aggressive interactions between any two genotypes of *A. elegantissima* will be dependent upon their histocompatibility, innate aggressiveness, ability to respond to repeated stimulation and prior history of inter-clonal contact.

Genetic variation in cactophilic Drosophila for oviposition on natural yeast substrates

J.S.F. Barker, Department of Animal Science, University of New England

Theory predicts that environmental heterogeneity in space or in time can maintain genetic polymorphism. Stable polymorphisms are expected to be more readily maintained if there are genotype specific habitat preferences. Genotype specific preferences for oviposition sites in Drosophila could be a major factor promoting habitat selection, and thus the maintenance of genetic variation. This hypothesis is being tested using the cactophilic species, D. buzzatii and D. aldrichi, where available evidence indicates a potential for such habitat selection, the habitats (oviposition sites) being yeast species found in the natural environment of these flies (cactus rots). Genetic variation for oviposition preferences was tested using isofemale lines - for D. buzzatii, a total of 60 lines from seven localities widely distributed through the species range in Australia, and for D. aldrichi, 21 lines from three of these localities. Females were given a choice of five yeast species as oviposition sites. Genetic variation for oviposition preferences on these natural substrates was demonstrated. There was significant variation among isofemale lines within populations in their patterns of preferences for oviposition on the five yeast species. However, analyses of preferences for each yeast species separately showed that the genetic variation for preferences relates to only three of the five species. Heritabilities of individual female preferences for these three species were low, ranging up to 9%. Little geographic differentiation was apparent among populations, most likely due to similar selection regimes within each population. Within populations, this kind of habitat selection could act to maintain polymorphisms, both at loci determining the habitat preferences and at other loci in linkage disequilibrium with them.

DETECTION OF YEAST PROTEINS RECOGNIZING PROMOTER SEQUENCES OF THE *ASPERGILLUS NIDULANS AMDS* GENE

Nathalie Bonnefoy, Michael J. Hynes and Meryl A. Davis

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

The *Aspergillus nidulans amdS* gene, encoding an acetamidase enzyme, is regulated by an array of well characterized *trans*-acting factors, most of which posses an homolog protein in the yeast *Saccharomyces cerevisiae*. A new regulator, the *Aspergillus nidulans* 'CCAAT-Box' Factor or AnCF, has been detected by its binding to a 30 bp oligonucleotide containing the *amdS* 'CCAAT-Box' sequence. In yeast, a 'CCAAT-Box' binding complex consisting of 3 proteins encoded by the *HAP2*, *HAP3* and *HAP4* genes is known to activate the transcription of several genes in the absence of glucose.

The existence of yeast proteins recognizing the *amdS* promoter sequences has been investigated *in vivo*, using a *lacZ* reporter gene and *in vitro*, by mobility shift assay.

ß-galactosidase activities are determined in yeast cells transformed with a multicopy vector carrying a *CYC-lacZ* fusion, in front of which *amdS* promoter sequences have been cloned. In wild-type cells, the whole *amdS* promoter, compared to the promoter-less construct, yields to a 5000 fold increase of ß-galactosidase activity under limiting glucose conditions. Under the same conditions, several copies of the 30 bp oligonucleotide containing the 'CCAAT-Box' sequence also shows an induction. However, overall levels are reduced. In a *hap2* mutant, the whole *amdS* promoter, but not the 30 bp oligonucleotides, still mediates induction under low glucose conditions. The effect of a *cis*-acting mutation in the *amdS* 'CCAAT-Box' is currently being examined.

In vitro binding studies are being performed using the 30 bp oligonucleotide which binds AnCF, and crude proteins extracts of either wild-type or mutant hap yeast cells, grown in different carbon sources. Several binding activities are detected, one of these appearing only if cells have been cultivated without glucose.

These *in vivo* and *in vitro* data show that some yeast proteins can recognize the 'CAATT-Box' of the *amdS* promoter, suggesting the existence of yeast factor functionally homologous to AnCF.

The search for *rec-2*: a gene that modulates the level of meiotic recombination in *Neurospora crassa*.

F.J. Bowring and D.E.A. Catcheside.

School of Biological Sciences, Flinders University, Bedford Park, SA.

In *Neurospora crassa*, a number of genes that alter the frequency of meiotic recombination in various genomic regions (*rec* genes) have been identified. Three *Neurospora rec* genes: *rec-1*, *rec-2* and *rec-3* are known. Each of these genes alter the frequency of recombination at two or more separate and specific loci suggesting that they code for a diffusible product[1]. *rec-2* influences both the frequency of recombination between alleles at *his-3*, and crossing over at three other loci[2]. Two naturally occurring alleles of *rec-2* have been identified: *rec-2* and *rec-2*$^+$, the latter being a completely dominant suppressor of recombination at the target loci. The presence of *rec-2*$^+$ in a cross leads to a thirty three fold reduction in recombination between *his-3* alleles when compared to that observed in crosses homozygous *rec-2*[3]. The *rec-2* locus has been mapped to linkage group V between the *am* and *spray* loci. We are currently attempting to walk along linkage group V in a *rec-2*$^+$ genomic library using the cloned *am* gene as a starting point.

It has recently been demonstrated that duplicated DNA in *N. crassa* is subjected to hyper-mutation after the commitment to meiosis but prior to nuclear fusion[4]. This phenomenon, termed repeat-induced point mutation (RIP), complicates the assay for *rec-2*. The introduction of cloned DNA containing *rec-2*$^+$ into a *rec-2* strain may not lead to the expected reduction of recombination at *his-3* since the most probable transformation events lead to the formation of a duplication and the concomitant likelihood that this will be RIPed. Thus, we are also looking for relaxed recombination as a result of RIP in a *rec-2*$^+$ strain transformed with putative *rec-2*$^+$ clones. We have assayed 20kb of DNA so far and have not observed any effect on recombination at *his-3*.

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2 Catcheside, D.G. (1975) Regulation of genetic recombination in *Neurospora crassa*. In The eukaryote chromosome. W.J. Peacock & R.D. Brock (eds.). Canberra, Australian National University Press.

3 Angel, T., Austin, B. & Catcheside, D.G. (1970) Regulation of recombination at the *his-3* locus in *Neurospora*. Aust. J. Biol Sci., **23**: 1229-1240.

4 Cambareri, E.B., Jensen, B.C., Schabtach, E. and Selker, E.U. (1989) Repeat-induced G-C to A-T mutations in *Neurospora*. Science, **244**: 1571-1575.

EVIDENCE FOR HYBRID FORMATION IN NATURAL POPULATIONS OF THE BEAN RUST FUNGUS.

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The bean rust fungus (*Uromyces appendiculatus*) is an important pathogen of French bean (*Phaseolus vulgaris*) and other *Phaseolus* species. The fungus is highly variable and a large number of pathogenic races have been identified in the USA, Latin America, and Australia. The last race survey in Australia identified 25 races and Queensland is believed to be a local centre of origin of the pathogen. A collection of isolates from Queensland, NSW and Victoria has been assembled. This includes field isolates collected between 1990 and 1992 and isolates collected during the 1970s which were kept in storage. The aim of this work was to use molecular markers to assess the extent of genetic variation within the current Australian bean rust population and the extent of population change over the last 20 years.

Based on the banding patterns detected by restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPDs), the Australian bean rust population can be divided into three groups. The first group is generally characterised by the isolates collected during the 1970s. This group is very distinct from a group of recent field isolates from the 1990s. This result suggests that either considerable changes have occurred in the population over the last 20 years or that there have been two separate introductions of the pathogen into Australia, thus forming two distinct subpopulations. The third group of isolates reveal diagnostic polymorphisms from both of the other groups. It is possible that these isolates are natural sexual recombinants and the first two groups represent parental types.

CHLOROPLAST RESTRICTION MAP AND MATERNAL INHERITANCE IN *EUCALYPTUS NITENS*.

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A restriction map of the chloroplast genome in *Eucalyptus nitens* was constructed. Heterologous probes from the petunia and tobacco chloroplast genomes were used to hybridize to single and double digests of DNA from two *E. nitens* individuals. Sequential mapping with five enzymes enabled a restriction map to be constructed. The genome was determined to be 151 kb in size and possesses the inverted repeat characteristic of the majority of the angiosperms. The chloroplast genome in *E. nitens* is essentially colinear with the petunia chloroplast genome and hence with the majority of the angiosperms analysed. This similarity enables the use of heterologous probes from petunia to investigate polymorphism in the chloroplast DNA in this species. Analysis of restriction fragment length polymorphism in the chloroplast DNA of six interpopulation crosses of *E. nitens* was carried out for five probe-enzyme combinations. The chloroplast genome was shown to be maternally inherited in all progeny of these six crosses.

Trans-SPLICING IN SHEEP PARASITIC NEMATODES

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The recent discovery of the presence of a common 39 nucleotide sequence at the 5' ends of mRNA transcripts in the protozoan parasite Trypanosoma brucei introduced the prospect of an unconventional type of RNA splicing in these parasites. This process has been termed trans-splicing and has since been found to occur not only in all known trypanosomes but also in several free-living and parasitic nematode species. In the nematodes so far studied, however, a 22nt sequence, conserved across species, is added only to a portion of mRNA transcripts, which differs from the case in trypanosomes where all transcripts appear to be modified. The role of trans-splicing and the reason why only a subset of the mRNA transcripts in nematodes undergoes this process are still undetermined.

We are utilising a synthetic oligodeoxynucleotide complementary to the 22nt "spliced leader" (SL) RNA to determine the occurrence of the spliced leader gene in three parasitic nematodes of sheep and its possible role in gene expression in these parasites.

TITLE Effects of Pollution at Enzyme Loci in Three Species of Marine Invertebrates

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ABSTRACT

Work carried out by a team of investigators in Israel have shown that heavy metals cause differential mortality in animals of different genotypes. There is potential for this effect to be used as a biological indicator of pollution. That is sites with a chronic input of pollution reflect a different genetic structure and that these changes could used to indicate where changes are occurring that may not otherwise be detectable by methods such as direct chemical monitoring.

The current study investigates the feasability of such a monitor. Gene frequencies in three species of estuarine invertebrates, oysters (*Saccrostrea commercialis*), mudwhelks (*Pyrazus ebeninus*) and the marine burrowing shrimp (*Calianassa australiensis*) have been measured from a number of estuaries in Moreton Bay with different types of land use and therefore presumably different levels of pollution.

Significant differences were found for some of the loci and follow up work has involved looking for a link between the pollution levels and the gene frequencies. This involved measuring the pollutant levels within the animals themselves. Other followup work has involved determining the susceptibility of *Calianassa* to a heavy metal (copper) and an organophosphate pesticide (Temephos) both at the larvae and adult stage.

Population bottlenecks and patterns of genetic diversity in *Stylidium*.

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

The genetic consequences of a bottleneck, and spatial and temporal changes in genetic diversity, were studied in populations of the rare and geographically restricted species *Stylidium coroniforme*. Thirteen out of 15 allozyme loci analysed were polymorphic. The level of polymorphism was relatively high for an insect pollinated outcrossing species. In one population , which was subjected to a prolonged decline in numbers resulting in a bottleneck, probably of three plants, there was no measurable reduction in either allelic diversity or average heterozygosity. Maintenance of heterozygosity in this population could be attributed to rapid recovery after the bottleneck, the progressive elimination of selfed or otherwise inbred products during seed development and selection favouring heterozygotes as plants mature. Evidence for the latter was a significant decrease in the fixation index from younger ($F = 0.28$) to older plants ($F = 0.14$). Founder flush cycles are considered to be a major factor contributing to the relatively high level of differentiation observed between the three Wongan Hills populations, which suggests that the bottleneck observed was not atypical for these populations. The allozyme divergence between the two population systems within this taxon ($D = 0.29$) may be indicative of two different but morphologically cryptic species. Similar levels of genetic divergence were observed between populations of other *Stylidium* species which all show comparable demographic patterns within populations. It was concluded that high levels of genetic diversity in small populations is typical of perennial *Stylidium* species which are relatively short lived, disturbance adapted and have population systems which probably undergo frequent founder flush cycles.

Mating systems and genetic structure in banksia populations.

David J. Coates and Jane F. Sampson. Western Australian Herbarium, Department of Conservation and Land Management, PO Box 104, Como, WA 6152.

Mating systems and population genetic structure were studied in two rare and geographically species *Banksia cuneata and Banksia brownii.* Estimates of the mating system in natural stands of both species were made using polymorphic allozyme loci. Analyses indicated that both species have a mixed mating system with significantly lower levels of outcrossing found in two populations of *B cuneata* (t = 0.67,0.76) and one population of *B. brownii* (t = 0.68) than reported for other Banksia species. In *B. cuneata* reduced outcrossing was attributed to increased biparental inbreeding associated with high plant density and reduced or altered pollinator activity. In *B. brownii* significant levels of self pollination, both within and between inflorescences on the same plant, appeared to be the major cause of reduced outcrossing. Although both species are largely bird pollinated the foraging behaviour of these pollinators combined with spatial genetic structure has resulted in significant levels of inbreeding in some populations of these species.

Given its restricted geographic range one might expect a bird pollinated species such as *B. cuneata* to show little differentiation between populations. However, the six populations of this species were clearly differentiated into two population groups with significant gene flow estimated between populations within groups but not between groups.

EVOLUTION OF MARSUPIAL MILK PROTEIN GENES

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Studies on the molecular evolution of mammalian genes are almost entirely restricted to the eutherians. Comparison of a eutherian gene to the marsupial or monotreme homologues is rare. However, the recent cloning of the five major milk protein genes from the tammar wallaby *(Macropus eugenii)* provides an opportunity to examine the evolution of some rapidly evolving proteins in a group of mammals which diverged from eutherians some 150Mya.

Facets of marsupial lactation have directed our interests towards the evolution of the regulatory regions of the tammar milk protein genes as well as the evolution of the genes proper. Firstly, the macropodid marsupials have adopted a different lactational strategy than the eutherians in that two types of milk are produced, which differ in protein concentration and constitution, to cater for the changing requirements of the developing pouch young. Secondly, there are differences in the hormonal-responsiveness of the tammar whey proteins from those of the eutherians.

Over the last few years we have amassed a considerable amount of data regarding gene structure and have gone some way towards addressing the nature of the sequences involved in milk protein gene expression in the tammar. Characterisation of the cDNA sequences of the whey proteins, ß-lactoglobulin and α-lactalbumin, and the caseins (α- and B-) has been completed and some sequence of the genomic clones has been obtained.

Both α-lactalbumin and ß-lactglobulin show considerable sequence divergence from their eutherian homologues at all non-essential amino acid residues although tertiary structure appears to be conserved. The intron-exon structure of these genes is also conserved. Another tammar whey protein, late-lactation protein, or LLP, has also been characterised and the available evidence suggests this protein may be an ancient duplication of ß-lactoglobulin. This evidence is discussed in more detail in the talk of Des Cooper at this meeting.

Tammar α- and ß-casein reveal extensive sequence divergence, including major insertion/deletion events, when compared with their eutherian homologues. Regions of sequence similarity are restricted to the major phosphorylation sites and the signal peptides reflecting the constraints acting upon these functionally important regions. It would appear that the duplication of the α-casein gene from the ancestral (ß?) casein gene occurred prior to the divergence of eutherians and marsupials 150 Mya. The duplication which gave rise to the two types of α-casein genes may have postdated the therian split.

GENETICS AND THE EVOLUTION OF VIVIPARITY IN MAMMALS

D.W. Cooper

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The palaeontological record for ancient mammals is poor. Attempts to reconstruct the course of mammalian evolution must depend largely upon inferences drawn from the characteristics of extant species. The availability of protein and nucleotide sequences is especially important. Viviparity has evolved many times in diverse groups. Both eutherian ("placental") and marsupial mammals are viviparous, but whether viviparity evolved in their common ancestral lineage or separately in each lineage is not yet clear.

A resolution of this question might be possible by considering three specialisations.

(1) During the last phase of lactation in some marsupials a protein called late lactation protein (LLP) appears in the milk. This protein is a marsupial specialisation, and is a concomitant of the birth of very immature young. It probably evolved from another milk protein, β-lactoglobulin, about 150-160 MYR BP, a little before the time usually given for the marsupial-eutherian divergence, 130 MYR BP. This suggests that the marsupial mode of reproduction might pre-date the divergence of the two groups.

(2) The pouch is also a marsupial specialisation. Pouch development is under the control of the X chromosome. Whether this control is a dosage phenomenon or whether it is due to imprinting is unresolved. An identification of the genes involved and of the role played by their eutherian homologues (if any) might help define the time at which the pouch first evolved, and thus give a minimum time for the evolution of viviparity in marsupials.

(3) Both eutherians and marsupials possess placentae. Eutherians are distinguished from marsupials by their use of this organ in an endocrine role. This is the fundamental specialisation which allows the long gestation periods, and hence the particular kind of viviparity seen in eutherians. Both protein and steroid hormones are produced from the eutherian placenta. A consideration of the nucleotide sequences for these protein, hormones, and of the nucleotide sequences for the proteins from which they have evolved (presumably through gene duplication) can be used to define when the eutherian use of the placenta as an endocrine organ began.

Imprinting – Paternal X inactivation in marsupials

XXY Pouch No scrotum {X^{Pat} necessary for pouch}

XO No pouch, Scrotum.

The Maintenance of Genetic Diversity by Unidirectional Gene Flow in the Mosquitofish *Gambusia holbrooki*

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Spatial and temporal variation in Pgi allozyme frequencies were examined relative to distance, flow velocity, and barriers to dispersal, for *Gambusia holbrooki* populations inhabiting an intermittent stream in South-eastern Queensland.

Isolation by distance explained only a small amount of the genetic variation between sites. Similarly, levels of differentiation could not be explained by a simple relationship between distance and flow velocity common to both catchments examined.

Barriers to dispersal allowed movement only in the downstream direction during peak flooding. Different alleles occurred in high frequencies in the headwaters of each catchment. The dominance of a single allele in headwater populations is attributed to the lack of upstream movement, and the ephemeral nature of these populations. This same allele was maintained as the most common within each catchment by down stream emigration. Inter-catchment differences were therefore attributed to the random fixation of different alleles in the headwaters of each catchment, and the dispersal of these alleles downstream during peak flood events.

Significant frequency differences between populations in the lower western catchment occurred despite substantial immigration. Differentiation fluctuated over a yearly cycle and was dependent on the level of immigration entering the lower site from alternative upstream sources. Immigration from source populations was in turn related to flow velocities in the tributaries which converge above this site.

All allele types were maintained in the lower reaches of both catchments despite the input of a single allele from headwater populations. Given the mechanism by which frequency differences are established and maintained in the lower western catchment, and the severe restrictions on upstream movement throughout both catchments, it is hypothesized that the less common alleles are maintained in the lower reaches of each catchment by immigration originating in the headwaters of tributaries where alternative alleles have drifted to relatively high frequencies.

Development and Applications of a Molecular Genetic Linkage Map of the Mouse Genome

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Interspecific backcrosses provide almost limitless genetic variation for gene mapping. We have used an interspecific backcross to develop the first comprehensive molecular genetic linkage map of the mouse genome. More than 1,000 loci have been positioned on the map; the current average map resolution is 1.6cM (- 3 Mb). We have primarily mapped known genes rather than anonymous fragments of genomic DNA as they provide the maximum amount of biological information. Since all loci were mapped using a single backcross panel, gene order can be determined unambiguously. Given the high density of markers on the map it is now possible to position any new locus on the map with virtual 100% certainty. In my talk I will review how our interspecific map was constructed, describe some of the salient features of the current map, and discuss some of the many recent applications of the map including those pertaining to comparative gene mapping. Research sponsored by the National Cancer Institute under contract no. NO1-CO-74101 with ABL.

Populations, molecules, and honeybee mitochondrial DNA. R. H. Crozier. La Trobe University. The complete sequence of honeybee (*Apis mellifera*) mitochondrial DNA is reported, being 16343 bp long in the strain sequenced. Relative to their positions in the *Drosophila* map, eleven of the tRNA genes are in altered positions, but the other genes and regions are in the same relative positions. Comparisons of the predicted protein sequence indicate that the honeybee mitochondrial genetic code is the same as that for *Drosophila*. The base composition shows extreme bias, being 84.9% AT (cf 78.6% in *Drosophila yakuba*). In protein-encoding genes, the AT bias is strongest at the third codon positions (which in some cases lack guanines altogether), and least in second codon positions. Multiple stepwise regression analysis of the predicted products of the protein-encoding genes shows a significant association with abundance for %T, but not for the number of codons per codon family or other parameters associated with codon family base composition. Differences in amino acid abundances are apparent between the predicted *Apis* and *Drosophila* proteins, with a relative abundance in the *Apis* proteins of lysine and a relative deficiency of alanine. The differences in abundances between *Drosophila* and *Apis* are associated with %AT in the codon families. Considering all protein-encoding genes, there are statistically significant associations between neighboring bases for all three combinations within and between codons. These data support an important role for DNA-level processes in determining protein makeup, but an effect on protein function is not indicated. Overall, transversions are about three times as abundant as transitions when comparing *Drosophila* and *Apis* protein-encoding genes, but this ratio varies between codon positions. Some population data indicate that, unlike in other animals, transitions do not markedly outnumber transversions in the evolution of honeybee mtDNA. Comparisons between genes for similarity between *Apis* and *Drosophila* confirm the extreme divergence of the bee genome from the other animal genomes known so far. It is tempting to ascribe this divergence to AT mutation pressure, but the similarly great divergence of an ant cytochrome b with relatively high GC content castes doubt on this explanation.

Genetic and molecular analysis of the *Notch* homologue in the Australian sheep blowfly, *Lucilia cuprina*.

A.G. Davies, A.Y. Game, T.J. Williams, M.L. Delbridge, J.A. McKenzie & P. Batterham
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The aim of this study was to confirm the homology between the *Scalloped wings* (*Scl*) gene of *Lucilia cuprina* and the *Notch* gene of *Drosophila melanogaster* by genetic and molecular methods. Our interest in *Scl* relates to the development of diazinon resistance in the field and subsequent evolution of a fitness/asymmetry modifier. It is hypothesised that the modifier is an allele of *Scl*.

The map position of the two genes, *Scl* and *Notch*, places them in the same conserved linkage group. Adult *Scl* heterozygotes show notching of the wing margin and thickening of wing vein junctions, a phenotype which closely resembles the adult *Notch* phenotype.

Homozygous *Notch* embryos display the so-called 'neurogenic' phenotype with expansion of nervous tissue at the expense of epidermal structures. The phenotype of homozygous *Scl* embryos were examined by immunohistochemistry and were found to have an equivalent phenotype to the *Notch* homozygotes.

Finally a *Notch*-homologous *Lucilia* genomic clone was hybridised *in situ* to *Lucilia* polytene chromosomes and was found to hybridise to the cytological location of *Scl*. Further confirmation of the *Notch-Scalloped wings* homology is provided by molecular characterisation of the genomic clone which will be discussed in the presentation "Isolation of the *Notch* homologue from the Australian sheep blowfly, *Lucilia cuprina*".

Biochemical Relationships among diploid and tetraploid desert burrowing frogs.

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Abstract.– Myobatrachid frogs of the genus *Neobatrachus*, currently thought to comprise ten species, are found in sub–humid, semi–arid and arid areas of Australia. Karyotypes are known from all ten species, six being diploid (2n=24) and four being tetraploid (4n=48). The evolutionary origin of the four tetraploid species of *Neobatrachus* is not resolved by the available karyotype data. In the present study, allozyme electrophoresis of 27 loci was used to characterise genetic variation among 29 populations of six diploid species of the myobatrachid frog genus *Neobatrachus*. All six species are well differentiated genetically with percentage fixed differences between species ranging from 11% to 59%. The genetic data are in agreement with the currently accepted species boundaries. The four tetraploid species were examined for 25 of the 27 loci assayed in the diploid species. In contrast to the diploid species, the tetraploid species shared electromorphs at all the loci examined. The tetraploid species were examined for the presence of electromorphs specific to individual diploid species. The majority of these electromorphs were observed in the tetraploid species. Where the range of a tetraploid species contacts that of a diploid species and the diploid population can be characterised by unique electromorphs, then evidence of current gene flow was found in the direction of the tetraploid populations. Thus the overall picture is one of past episodes of gene flow involving all of the diploid species and geographically limited ongoing episodes.

Molecular Analysis of a Complex Family of Neuropeptides in Blowflies

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Antisera specific for the carboxyl terminal sequence Arg-Phe-amide (RFamide) have been widely used for immunocytochemical studies of invertebrate nervous systems. In relatively few cases, primarily in coelenterates and molluscs, are the amino acid sequences of the endogenous peptides known. Among insects, three unrelated families of RFamide peptides have been described; those ending in FLRFamide (from cockroach, locust and moth), the sulfakinins, a widespread and highly conserved group structurally related to the vertebrate gastrin/CCK peptides, ending in HMRFamide, and the FMRFamide related peptides, currently known only from dipterans. Although the RFamides are apparently a large and diverse group of insect neuropeptides, their *in vivo* physiological functions remain poorly characterised.

As part of our studies on the RFamide peptides of the Australian sheep blowfly *Lucilia cuprina*, we have used immunocytochemistry and confocal laser scanning microscopy to locate sites of synthesis and release in the central nervous system, and to identify potential peripheral sites of action. The gene encoding the FMRFamide prohormone has been cloned and sequenced to provide information on the peptide sequences.

The FMRFamide prohormone gene of *Drosophila melanogaster* has been analysed in detail and the pattern of RFamide immunoreactivity in the CNS has been described by several groups. The patterns of expression in *D. melanogaster* and *D. virilis* are almost identical to each other and very similar to that in *L. cuprina*, suggesting conservation of peptide function among these species. It was therefore surprising to find that only one peptide in this family was identical in all three species. Because physiological sudies are not technically practical in *Drosophila* we have cloned and sequenced the FMRFamide prohormone from another blowfly, *Calliphora vomitoria*, to facilitate comparative studies. Despite a high level of nucleotide sequence conservation between the *Lucilia* and *Calliphora* genes (80% identity), there has been a remarkable divergence of peptide structure, only five out of fifteen peptides being the same in the two species. These results will be discussed in the context of some known physiological functions of the blowfly peptides.

The position of Leadbeater's possum (*Gymnobelideus leadbeateri*) within the Petauridae based on DNA-DNA hybridisation data.

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The Petauridae is comprised of two distinct subfamilies, the Petaurinae and the Dactylopsilinae. On the basis of morphological characteristics, including teeth, *Gymnobelideus leadbeateri* has generally been placed in the subfamily Petaurinae. Using microcompliment fixation, Baverstock *et al.* (1991) suggested that *G. leadbeateri* may be more closely related to members of the other subfamily. In order to resolve this conflict we have applied the DNA-DNA hybridisation technique, which has been successfully applied to other marsupial groups. Using a member of the Pseudocheiridae as an outgroup, the two subfamilies were differentiated. *G. leadbeateri* was found to be more closely related to members of the Dactylopsilinae. Two statistical tests were applied to investigate the stability of the observed relationships. No instabilities were observed using the jackknife technique (Lanyon 1985). The relationship was less convincing however when bootstrapping (Krajewski and Dickerman 1990) was applied, although a majority (66%) of the trees generated by the analysis did show the *Gymnobelideus*-Dactylopsilinae relationship.

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Lanyon, S.M., 1985. *Syst. Zool.* **34**:397-403.

Flow Sorting Marsupial Chromosomes

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Marsupials to date have proven to be very useful in studying the function and evolution of the mammalian genome. The majority of such studies have been centred on mapping groups of genes using the technique of radioactive *in situ* hybridisation. Normally somatic cell hybrid analysis would be used to confirm such gene localisations, however in marsupials the production and stability of hybrid cell lines has proven to be nearly impossible, and consequently few hybrid panels exist.

To circumvent this problem, in addition to opening up new areas of genome research, the alternative approach of flow sorting marsupial chromosomes was taken. Chromosomes from the marsupial species *Dasykaluta rosamondae* were isolated using the hypertonic potassium chloride - propidium-iodide method (1). The chromosome suspension was sorted into individual chromosome types (except for chromosomes 5 and 6) at high purity using a `Facsstar Plus Cell Sorter' equipped with a 5W water cooled argon-ion laser with an excitation of 488nm. Due to the small amount of DNA purified from each sort (1-2 ng's) the adaptor ligation system of PCR (2) was used to amplify each sorted chromosome type. This method of universal PCR produced enough DNA to enable Southern analysis, chromosomal suppression *in situ* hybridisation, or the production of whole chromosome libraries to be performed.

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2. Akowitz,A., and Manuelidis,L.(1989) *Gene* **81**, 295-306.

Isolation of the *Notch* homologue from the Australian sheep blowfly, *Lucilia cuprina*

A.Y. Game, T.J. Williams, A.G. Davies, M.L. Delbridge, J.A. McKenzie and P. Batterham

Department of Genetics, University of Melbourne, Parkville 3052

Map location and mutant adult phenotype suggest that the *Scalloped wings* gene of *Lucilia cuprina* is the homologue of the *Notch* gene of *Drosophila melanogaster*. Staining of the embryonic nervous system in *Scl* homozygotes shows extensive hypertrophy as found in mutant *Notch* embryos. See also poster entitled "Genetic and molecular analysis of the *Notch* homologue in the Australian sheep blowfly".

Using a probe containing the cdc-10/SWI6 repeat region of *Notch* we have isolated a 10 kbp clone from a *L. cuprina* genomic library. This clone *in situ* hybridises to the same polytene band region (23A) to which deficiencies of *Scl* have been localised. DNA sequencing has revealed cdc-10/SWI6 and epidermal growth factor-like repeats characteristic of *Notch*. Sequence homologies and codon usage between *Notch* and *Scl* will be discussed.

DEVELOPMENTAL EXPRESSION OF THE *topaz* and *white* EYE COLOR GENES OF *Lucilia cuprina*

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Division of Biochemistry and Molecular Biology
Faculty of Science
Australian National University

The products of the *topaz* and *white* genes of *Lucilia cuprina* belong to a group of proteins called the ATP-binding cassette (ABC) family of transmembrane transporters. They form part of a permease involved specifically in the transport of precursors of the pigment xanthommatin. *Topaz*- and *white*-dependent pigmentation is found in several body structures of both larvae and adults, most notably in the adult eyes.

Preliminary Northern blot analysis of poly A^+ RNA from mid-pupae showed that the *topaz* transcript is approximately 2.4 kb in size while that of *white* is around 2.5 kb. Detailed developmental profiles of the transcription of the two genes were carried out covering the period from late third instar larvae through to adult emergence (in 24-hour windows). The profiles show that the peak of transcription in both *topaz* and *white* coincides with the period of rapid deposition of xanthommatin (days 3 and 4 of the pupal life). It is also evident that the two genes are turned on before the onset of pigment formation, as early as the first day after pupariation. The transcription of both genes then increases steadily until the peak is reached at mid-pupae. Continued transcription of the genes at peak levels does not appear to be necessary over the late pupal period of pigment accumulation, probably because the permease complexes are relatively stable in the pigment cell membranes. A sudden rise again in the levels of transcripts of the two genes immediately after adult emergence possibly reflects a renewed need for rapid precursor transport over the final stages of pigment deposition.

MICHAEL GARDNER

TITLE:

'Microhabitat selection and colour morph frequencies in a highly polymorphic intertidal snail (Clithon oualaniensis)'

OUTLINE:

The genus Clithon has a very broad geographic distribution and can be found in the intertidal zone in much of the tropical and subtropical waters of the Indian and Pacific Oceans. The species is characterised by having a large number of colour morphs as the snail varies for both background colour and shell pattern. Large amounts of allozyme variation also exists. Preliminary studies have shown that colour morph frequencies vary between different microhabitats in the same localities. An explanation of the maintenance of this variation is the focus of the honours year project. Methods used to achieve this include: electrophoretic studies, relation of colour morph and allelomorph distributions in relation to microhabitat, and an examination of basic life history structure of the different populations.

DETERMINATION OF FAMILIAL RELATIONSHIPS IN A PLATYPUS POPULATION.

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In the summer of 1990-91 the first captive breeding of Platypuses (*Ornithorhynchus anatinus*) in 50 years, and only the second ever, occurred in a small resident population at Warrawong Sanctuary, South Australia.

We have used molecular techniques, including DNA fingerprinting and mitochondrial DNA haplotype analysis, to determine family relationships within this population. Using hypervariable DNA sequences cloned from other species to probe blots of DNA from the Warrawong platypuses, we have observed banding patterns which seem to be individual specific and which allow the identification of family relationships within the population.

The implications of this case study to current and future investigations of wild platypus populations, in particular our study of platypus from the Shoalhaven River, N.S.W will be discussed.

FAMILY AND POPULATION STRUCTURE IN THE PLATYPUS.

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The mating system of the platypus is entirely unknown. However, mark-release-recapture studies undertaken in the upper Shoalhaven River in New South Wales since 1973 suggests that there are two categories of platypuses occupying the area. There are those which are often recaptured (residents) and others which are seldom, or never, recaptured after initial marking (transients). Data from lactation show that not all females in the area breed each year, and that those which do breed in most years are resident individuals. Numbers of males breeding each year are unknown, but it is hypothesised that it is predominantly the resident animals of both sexes which make a disproportionate contribution to the gene pool of the next generation.

We are using DNA fingerprinting and mitochondrial DNA haplotype analyses to test this hypothesis and too ultimately identify familial groups in the population thereby elucidating the mating system of the platypus. This study is also providing estimates of genetic variability within the Shoalhaven population and of the degree of sequence divergence between animals from the Shoalhaven River and those from elsewhere in the platypus' geographic range.

ABNORMAL PACHYTENE PAIRING BEHAVIOUR IN HYBRID COMBINATIONS OF WATER BUFFALO

Gillies, C.B. and Dai, K.
School of Biological Sciences A.12, University of Sydney, NSW 2006.

The water buffalo of Asia (*Bubalus bubalus* L.) has been classified into two types, the larger Murrah or River breed from India and Pakistan (2n = 50) which is used mainly for milk, and the smaller Swamp breed from Southeast Asia (2n = 48) which is used for draught and meat. Attempts are being made in a number of Asian countries to combine by hybridization the characteristics of the two types. The resultant 2n = 49 hybrids are relatively fertile, and backcross and F2 progeny are readily obtained, although some reduction has been observed in male reproductive performance. The chromosome number difference between the two breeds is the consequence of a tandem fusion of chromosomes 4 (submetacentric) and 9 (acrocentric) of the River type to form the large metacentric found in the Swamp type. We will illustrate by means of synaptonemal complex spreads the formation of trivalents at pachytene in the F1 spermatocytes, and clarify the nature of the chromosomal rearrangement involved. We will also illustrate abnormalities of prophase I chromosome pairing behaviour which may have significant effects on fertility in F1, backcross and F2 bulls. Abnormalities which are more frequent in hybrid progeny include partial pairing failures, and pairing interactions between the trivalent, the XY pair and other autosomes.

We acknowledge the assistance of M. Hilmi, Faculty of Veterinary Medicine & Animal Science, Universiti Pertanian Malaysia, and ACIAR.

The population structuring of a recently arrived pest: an allozyme study of *Lucilia cuprina* in New Zealand

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Lucilia cuprina was first described in New Zealand in 1988. Since that time, this species has rapidly become the most common cause of fly-strike in sheep. This poses a serious threat to the New Zealand sheep industry which relies heavily on the production of both quality hide as well as wool. Due to the paucity of information of *L.cuprina* in New Zealand, it is therefore difficult to evaluate potential control measures without first determining the population structure of *L.cuprina* .

An isozyme study has been carried out from a range of collection sites throughout New Zealand and some sites within Australia for comparison. Results have shown higher levels of polymorphism than would be expected from a recent colonization event. Further analysis has revealed a distinct structuring of the New Zealand populations which has important implications for future control strategies.

MUTATIONAL OUTBREAK AND UNSTABLE GENES IN NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

M.D.GOLUBOVSKY

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The long-term studies of mutation process in natural populations of Drosophila melanogaster confirmed the old idea of Hugo de Vries about mutability fluctuations in nature. Now it is possible to indicate the principal features of mutations outbreaks. (1) Two types of outbreaks were investigated: global and local ones. During the global ones mutant alleles of the same gene(s) appear practically simultaneously in geographically distant populations. The phenomenon was named "mutation fashion" or mode on the mutations. (2) The duration of outbreaks were 7-11 years. (3) The mutations occuring during outbursts are mostly unstable and insertional. (4) During an outbreak activation of different movable elements was found even in one natural population. (5) repeat of mutation fashion is possible (Eg. yellow gene late 1930's and 1980's).

The detailed genetic analysis of unstable mutations of singed genes (sn-1,21,0) isolated from distant natural populations during the global outbreak in 1973 included: analysis of the rate and direction of mutation of different alleles and their derivatives in germ and somatic cells; *in situ* hybridization test which showed that >50% of unstable alleles from nature are connected with P-DNA insertion; the investigation of a case of natural genetic engineering when two neighboring genes sn and clw appeared to be under control of one definite movable element and so were simultaneously expressed and mutated; the analysis of peculiarities of homologous recombination in insertional homo and heterozygous interalleleic combinations; the interaction of different movable elements in the system of genotype.

Factors inducing a synchronous outbreak of mutability of the same gene(s) in remote populations should have the following properties: (1) they are universally prevalent in natural populations; (2) they have specificity for certain target chromosomal regions and induce an unstable mutations; (3) they change the intensity and direction of the effect in a specific way with time. It is suggested that a biocenotic interaction of "parasite-host" type can lead to mutation outbreak.

Viruses are universal and each eukaryotic organism represents a cenosis for viruses. They fulfill a triple role in biocenoses: as powerful selective agent, as an intensifiers of the host mutation process, activators of host intrinsic pool of movable elements and, finally, as donors, stores or transmitters of movable elements.

Genotype in a broad sense consists of a population of obligatory and facultative genetic elements. Interaction between environmental factors and these two kinds of elements is the main source of hereditary changes in nature.

Genetic Analysis of Transgenic *Caenorhabditis elegans* Transformed with a Parasite Tubulin Gene.

Warwick Grant

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The free-living nematode *Caenorhabditis elegans* can be transformed with genes from the animal parasitic nematode *Trichostrongylus colubriformis*. Transformation is achieved by direct microinjection of cloned DNA into the ovaries of *C. elegans* hermaphrodites and results in transgenic worms in which the introduced DNA appears to be inherited as an extrachromosomal array. This array is lost in approximately 50% of germline cells and presumably also in a similar proportion of somatic cells, giving rise to animals in which the copy number and sites of expression of the transgene may vary. Wild-type *C. elegans* transformed with a tubulin gene from *T. colubriformis* do not show any alteration in sensitivity to mebendazole (MBZ, a benzimidazole which inhibits tubulin polymerisation) but do show a significant increase in generation time. When the same transgene is introduced into crosses between the wild-type and *ben-1* (benzimidazole resistant) strains of *C. elegans* the ratio of resistant *vs* susceptible progeny observed in the F2 is significantly distorted. The degree of distortion is strongly temperature dependent, reflecting (i) the temperature dependence of dominance in the alleles of *ben-1* tested and (ii) the difference in the temperature optimum for drug binding to tubulin in the parasite and *C. elegans*. The degree of variability in the resistant/susceptible ratio in the progeny between individual worms carrying the transgene is much greater than the variation between individuals in the non-transgenic controls, reflecting perhaps the variability in the copy number and expression of the extrachromosomal array in the transgenic strains.

Characterisation of MHC Genes in Koalas

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With human help, koalas have recovered from a severe decline in distribution which took place last century. Results of allozyme surveys suggest that some current management practices are altering the distribution of genetic variants in koalas. This may be correlated with management problems such as lowered fertility or disease tolerance. To further investigate this question, we have chosen to study the MHC loci, as they are highly variable, contain regions subject to different (known) selective pressures, and may be directly related to management problems.

We have designed primers based on conserved regions for MHC class I and II, and have successfully amplified and cloned exons 2 and 3 of a class I-related sequence from RNA. When sequenceing is complete, we will use RFLPs or other methods to survey populations for their relative levels of variation at this locus.

SELECTION-INDUCED MUTATIONS IN *E. COLI* AND YEAST: SOME HINTS ON POSSIBLE MECHANISMS

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Beginning in 1988 evidence began to accumulate that some spontaneous mutations are induced by selection, i.e. that they do not occur randomly with respect to their usefulness to the cell. Evidence that these mutations arise as specific responses to environmental challenges will be presented, and examples of selection-induced mutations in *E. coli* and yeast will be discussed. Selection-induced mutations are time, not replication, dependent, and they have been observed primarily in populations of cells subjected to intense, long-term selective conditions. Evidence will be presented to show that during long-term selection mutations occur in genes that are directly under selection, but do not occur in other genes. Possible mechanisms for selection-induced mutations will be discussed. Mutations in a variety of genes involved in recombination, and in DNA repair are known to influence mutation rates in growing cells. Data concerning the influence of several such genes on selection-induced mutations will be presented.

New DNA markers for tracing alien chromatin introduced into bread wheat

U. Hohmann, B.C.Clarke, E.S.Lagudah, R.Appels

A group of grasses in the genus *Agropyron* sensu lato, provide genes for bread wheat that can confer BYDV and rust resistance as well as salt tolerance. Repetitive DNA families which are highly amplified in *Agropyron* relative to wheat have been found to provide useful probes for chromatin segments introduced into wheat during the course of a breeding program. An extensive analysis of one family of sequences, named Acc2, has been carried out using PCR and DNA sequencing in order to refine the specificity of this family as a probe for *Agropyron* chromatin. The 600-family defined as a result of these studies, as well as the Acc2-family, can be used to estimate approximate amounts of *Agropyron* chromatin present in wheat. In combination with RFLP markers, the repetitive sequence families provide useful "fingerprints" for the *Agropyron* chromatin carrying useful agronomic traits utilized in wheat breeding.

Sex-associated genetic linkage in the fat-tailed dunnart *Sminthopsis crassicaudata* (Marsupialia).

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Two linkage groups have been described previously in the fat-tailed dunnart[1]. Linkage group I includes the gene loci *GPI, ADA* and *PI*, and Linkage group II includes *TRF*, *SOD1* and *PGD*. The data on linkage obtained from crosses involving pairs of these loci reveal large differences between the sexes in recombination frequencies, linkage being much closer in females. We have recently derived a DNA panel from 57 two-generation family groups, each group comprising two parents and 5 or more of their offspring. Using Southern analysis and molecular hybridization, members of the panel were typed for variation at three polymorphic EcoRI sites, one site (detected by the probe pSG2-H) being about 3 kb upstream of the embryonic ß-globin gene *HBE*[2]. Some members of the panel were also typed for variation at the *GPI, ADA, SOD1* and *PGD*, loci. Two-point linkage analysis using lod scores confirmed our previous results for sex-associated linkage between *ADA* and *GPI* (Linkage group I) and between *PGD* and *SOD1* (Linkage group II)[1]. Two of the RFLP loci show no linkage with any of the other loci. Of particular interest, however, was the finding that whilst *PGD* and the locus detected by pSG-2H show independent assortment in males, these loci are closely linked in females suggesting that the ß-globin gene cluster in the dunnart is a member of linkage group II. As the adult ß-globin gene has been mapped to chromosome 4 in the dasyurid marsupial *Dasyurus viverrinus*[3], and since the G-banded karyotype of this species is very similar to that of the dunnart, we might expect Linkage group II in the dunnart to correspond with chromosome 4. However, in the closely related species *Sminthopsis macroura,* whose G-banded karyotype is nearly identical with that of *S. crassicaudata*, *SOD1* has been ascribed by *in situ* hybridization to chromosome 1[4]. Resolutions to these possible inconsistencies will be discussed, as will our general approach to constructing linkage maps in *S. crassicaudata.*

1. Bennett, J.H. et al., *Nature* **323**: 59-60 (1986). 2. Hope, R.M. et al., *Biochem Genetic* **30**, 7/8 (1992 - in press). 3. Wainwright B.J. and Hope, R.M. *Proc. Natl. Acad. Sci. 82*: 8105-8 (1985). 4. Maccarone, P. et al., *Genomics 13*, (1992 - in press).

♀ - telomeric chiasmata, ♂ scattered chiasmata.

THE MATING SYSTEM, NECTAR PRODUCTION AND POLLINATOR FORAGING BEHAVIOUR IN SUBSPECIES OF *EUCALYPTUS CAESIA*.

Stephen D. Hopper (Kings Park & Botanic Garden, West Perth, W.A. 6005), Robert Wyatt (Dept of Botany, University of Georgia, Athens, GA 30602 USA), and G.F. Moran (CSIRO, Forest Research, PO Box 4008, Canberra, ACT 2601). *E. caesia* is a rare mallee endemic to large isolated granite outcrops in south-western Australia. Only *ca.* 2100 wild plants are known, distributed among 17 populations ranging in size from 1 to 580 individuals. There are two subspecies, *caesia* and *magna*, differing in habit, in floral, fruit and leaf size and density, and in diurnal patterns of nectar production (*magna* secretes day and night, *caesia* only at night). Both subspecies are preferentially outcrossing, with 58-87% of seeds derived from outcrosses using isozyme estimation procedures. Our study has revealed an unusually dynamic and divergent pollination system within the one eucalypt species. *E. caesia* is pollinated predominantly by birds. Over eleven years, the array and number of honeyeaters present in populations dramatically varied seasonally, across years, and geographically. In *caesia* stands, birds increase their observed feeding activity and aggressive interactions throughout the day, with larger honeyeaters chasing away smaller species from dense floral resources. Territorial behavior is evident. In *magna* populations, feeding rates are consistent throughout the day, aggressive interactions are far fewer, and those that occur do not increase in frequency towards late afternoon. No clear evidence for territorial behaviour in *magna* populations exists. The divergent pollination systems of *magna* and *caesia* may result in divergent patterns of gene flow. The small isolated populations of *E. caesia* appear to undergo genetic drift and higher levels of inbreeding than occurs in forest eucalypts. Given the strong selection for outcrossed heterozygous individuals evident through the life cycle in eucalypts, the insular habitat and resultant population structure of *E. caesia* undoubtedly places a selective premium on pollinators that maximise outcrossing.

ISOZYMES - A USEFUL TOOL IN FOREST TREE GENETICS AND IMPROVEMENT

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Since their discovery in the late 1950s, isozymes have played an important role in studies of organismal and population genetics. The advent of more powerful and precise methods of understanding gene function and inheritance has not made isozymes redundant in the field of tree population genetics. In this poster we present a general view of the use of isozymes in forest tree genetics and tree improvement, with two recent case studies:

(1) *Grevillea robusta* (Proteaceae: silky oak) is an important agroforestry species eastern Africa. Genetic variation was examined in natural Australian provenances and in land races from five African countries. Estimates of the breeding system in natural populations indicate that the species is predominantly outcrossing with a small proportion of self-fertilsation. Mean expected heterozygosity and other measures of genetic diversity were lower in the land races than in the natural populations. Seven rare alleles, present in at least one natural population, were absent from all African populations, and there is strong evidence of genetic drift and very small founder populations in some land races: substantial levels of inbreeding are likely in these.

(2) *Eucalyptus urophylla* (Myrtaceae: Timor mountain gum) is one of only two species in the genus that do not occur in Australia. The species has great promise for plantation forestry in the seasonally dry lowland tropics. It has a remarkably wide altitudinal range and shows marked morphological variation in some characters. Preliminary isozyme analysis using 25 natural populations reveals moderate levels of genetic diversity compared to other widespread species in the genus. Most genetic diversity is within populations rather than between. The mean number of alleles per locus and the percentage of polymorphic loci are high, but the mean observed heterozygosity is lower than the expected, suggesting some inbreeding. There are no clear geographic patterns of rare alleles or reversals in common allele frequencies between populations.

The implications of these results for the use of these species is briefly discussed.

CHARACTERISATION OF THE CADMIUM-SENSITIVE MUTANT, *cad1*, OF *ARABIDOPSIS THALIANA*

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A genetic approach using the small crucifer *Arabidopsis thaliana*, was adopted to investigate the mechanisms by which plants respond to heavy metals. A Cd-sensitive mutant has been isolated and genetic analysis has shown that the sensitive phenotype is recessive to the wild type and segregates as a single Mendelian locus. Crosses of the mutant to marker strains showed the mutation is closely linked to the *tt3* locus on chromosome five.

In addition to Cd, the mutants are also significantly more sensitive to mercuric ions, only slightly more sensitive to Cu and Zn while being no more sensitive than the wild type to Mn, thus indicating a degree of specificity in the mechanism affected by the mutation. Undifferentiated callus tissue is also Cd-sensitive suggesting the mutant phenotype is expressed at the cellular level. Both wild type and mutant plants show increased sensitivity to Cd in the presence of buthionine sulfoximine, an inhibitor of the biosynthesis of the cadmium-binding $(\gamma EC)_nG$ peptides, suggesting that the mutant is still able to synthesize these peptides. However, the effects of a *cad1* mutation and BSO together on cadmium-sensitivity are essentially non-additive indicating they may affect different aspects of the same detoxification mechanism. Assays of Cd uptake by intact plants indicate the mutant is deficient in its ability to sequester Cd.

Molecular genetic contribution to *Meloidogyne* taxonomy

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Worms of the plant parasitic root-knot nematode genus *Meloidogyne* are a significant agricultural pest, especially in the tropics. Within the genus there are many recognized species, but a small group of parthenogenetic types have spread globally accounting for >80% of crop losses. Increasingly, control of *M.* depends on the rapid identification of the species and strains present. We are identifying genetic groups of *M.* isolated from crops throughout Queensland in order to develop DNA-based diagnostic tests.

Original taxonomy of *M.* was based on a loose combination of morphology and differential infection of specific hosts, the 'standard host-range test'. Subsequent work on various allozymes indicated a very good correlation of host-range groups to esterase phenotypes so this has also become a standard test. There is considerable interest in the development of DNA-based diagnostics, but all studies so far have been restricted to a small range of samples. We have developed a collection of ~70 isolates to which standard tests have been applied, as well as RFLP analysis of mtDNA. For selected strains, we are also investigating sequences of rDNA internal transcribed spacer (ITS), and intend to do the same for IGS.

The mtDNAs fall in two major groups: those with little sequence divergence (~1%) and a high degree of length heteroplasmy, due in most part to 129bp tandem repeats; and the other of two isolates related to each other (≤95%) but very different from the rest, with higher %GC and unusually large size (~27kb). There are but two ITS sequences which differ by 19% of sequence, one for each of the two main mtDNA groups. In general mtDNA type correlates well to esterase (although some mtDNA types are composite), but poorly to host range. The principle diagnostic morphological test, perineal pattern, cannot be used to predict any other trait. On the basis of the available evidence some species boundaries may be inaccurate. The two strains with the very distinctive mtDNA and ITS are diagnosed as *M. hapla* and are a genetic type not previously reported.

THE MOLECULAR GENETICS OF PORCINE STRESS SYNDROME.

Ian Hughes and Chris Moran

Department of Animal Science, University of Sydney. NSW 2006

Abstract. Porcine stress syndrome (PSS) is an economically important recessive genetic disease of pigs that is precipitated by exposure to stress or inhalational anaesthetics such as halothane. Recently the primary defect in PSS has been localized, through biochemical studies, to a calcium release channel, known as the ryanodine receptor (*ryr1*), in the sarcoplasmic reticulum of skeletal muscle.

Subsequent to the cloning of the *ryr1* gene (of rabbits and humans), linkage analyses and *in situ* hybridization studies further implicated a defect in *ryr1* as the cause of both PSS and a related condition, malignant hyperthermia, in humans.

The *ryr1* gene of pigs has now been cloned and sequenced by a group in Canada. Significantly, only one mutation causing an amino acid substitution was identified between a homozygote normal and susceptible pig. This C to T transition at position 1843 was subsequently shown to be associated with PSS in five Canadian and one British pig breed.

We have developed a diagnostic test for this mutation based on the Canadian work. PCR is used to amplify a 67bp region at the centre of which is located nucleotide 1843. In the wild type, 1843-C forms part of an Hha restriction site which is destroyed by the T mutation. Thus, following Hha digestion of the PCR product, the normal homozygote yields a 33-bp and a 34-bp fragment which migrate as a single band on a polyacrylamide gel, where as the PCR product of the recessive homozygote will be refractory to cleavage resulting in a 67-bp band. The heterozygote is identified by a 67 and 33/34-bp band. An error rate for genotyping of 2.5% was realized for the test although this figure was reduced as the test was optimized.

Our results, using Australian Large White, Australian Landrace and Belgian Landrace, are in complete agreement with those of the Canadians which strengthens the conclusion that PSS is due to a single mutation in the *ryr1* gene. Further work by both laboratories suggests there to be a common ancestry for all PSS pigs arising from a single founder animal.

Gene Flow and Dispersal in Stream Invertebrates

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Many stream invertebrates have both aerial and aquatic phases in their life-histories. The relative importance of aerial to aquatic dispersal has seldom been questioned, even though some species have extremely short adult lives. Other species are more likely to disperse only via the stream as they have no aerial or terrestrial phase in their life history. This project uses the idea that the degree of differentiation among populations can indicate the degree of dispersal between them.

In the Conondale Ranges, in SE Queensland a group of streams have been chosen which should be able to answer these questions for a range of species. The study area contains two catchments, one either side of the range, each of which consists of two subcatchments. The distance between two subcatchments within a single catchment is greater than the distance from one subcatchment to its adjacent subcatchment across the range. This design should allow us to separate the effects of aerial relative to aquatic dispersal.

The shrimp *Paratya australiensis* was chosen as the first species to investigate because dispersal by stream was assumed and the results could be used for comparison with other species with unknown dispersal mechanisms. Sampling was carried out on two occasions, in 1990 and 1992. Almost no temporal variation in gene frequencies was observed. The amount of spatial variation within and between subcatchments was extremely large, with Fst values greater than 0.5 in some cases. The use of this species as a model for later comparisons is discussed.

A REVIEW OF MARSUPIAL SEX DETERMINATION

R. Leon Hughes. *Anatomical Sciences, The University of Queensland.*

The embryonic origin as well as the sexual determination of both the marsupial mammary gland and the scrotum exhibit unique characteristics not represented in either monotremes or eutherian mammals. The phenotypic expression of a scrotum in XO intersexual marsupials challenges the long held Jost hypothesis that default embryonic sexual morphogenesis results in the expression of female phenotype.

The embryogenesis of the basic anatomical primordia of sex determination in both marsupial and eutherian mammals first exhibit genetic determination with subsequent secondary endocrine programming. The basic dichotomy between eutherian mammals and marsupials in sex determination derives from fundamental phylogenetic diversity in sexual morphogenesis rather than a dichotomy based on genetic programming versus endocrine programming.

The relevance of karyotyped intersexual marsupials will be reviewed in relation to the unique morphogenetic features of marsupial sex determination

Mammary gland in marsupials
- hair follicle
- sweat gland → mammary gland.

Gubernacular ligament
- suspends scrotum
  or mammary gland.

XO marsupials - challenge to Jost hypothesis of ♀ being default sex.
- scrotum + spermatic chord
- neutral indeterminate gonad - i.e not female.

Genetic analysis of variability in flight activity in the monarch butterfly *Danaus plexippus*

J.M. Hughes, M.P. Zalucki, J. Chaseling and C. Lange, Griffith University, Nathan, Qld.

Individual monarch butterflies vary in the time that they first become active in the morning and this variation is consistent among individuals, so that particular individuals are consistently active before others.

This project looks at heritabilities of a number of variables associated with flight behaviour. These include differences in the time and temperature at which individuals begin warming up behaviour as well as their ability to fly at temperatures below the mean flight threshold. Mated females were collected from the field, eggs collected from them and their offspring reared through to adult. The resulting adults were tested for differences in warming up behaviour and ability to fly at low temperatures. They were then mated amongst each other and the resulting eggs divided into two temperatures. Offspring were reared through to adults and all flight variables measured.

Here the results from the first generation are presented, where the effect of dam only can be examined. The variable showing the greatest effect of dam was the time at which individuals initiated warming-up, although significaant dam effects where also seen in some other variables.

These differences in flight behaviour among individuals have implications for sampling, both of genetic data, but also ecological data, such as mark/recapture methods.

Philopatry and the genetic structure of populations

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Recent studies have demonstrated the overall homogeneising effects of gene flow due to broadcast spawning of marine larvae. However, the consequence for the genetic structure of populations with restricted dispersal (philopatry), and hence restricted gene flow, have rarely been examined. This study contrasts the genetic structure of populations of the philopatric intertidal starfish, *Patiriella exigua*, with the closely related and co-occuring broadcast spawner, *Patiriella calcar*. Allozyme data obtained using electrophoretic techniques were used to estimate the within and between population variance in allelic frequencies (F_{IS}, F_{ST}) for populations along the south east coast of Australia. These data indicate the strong genetic connectedness of populations of *P. calcar* over the sampling range. In contrast the restricted dispersal of *P. exigua* results in marked genetic differentiation between populations separated by less than 10 km but suggest little genetic differentiation within populations.

Genetic systems and the management of the genetic load.

S.H. James

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The genetic system of a species may be viewed as the system by which it manages its recessive deleterious and lethal genes, that is, its genetic load. The management system may be post-zygotic or pre-zygotic in its operation, and these two alternatives lead to quite different consequences.

Pre-zygotic management of the genetic load is based on high levels of outbreeding, including systems which preclude self-fertilization, it leads to population genetic architecture approximating the panmictic model of classical population genetic theory, and it promotes the expectations arising from that theory. In particular, recessive lethal genes tend to be confined to very low population frequencies, and their impact upon the population genetics and evolutionary biology of such species can be effectively ignored.

On the other hand, systems exhibiting a post-zygotic management of the genetic load are characteristically inbreeding and capable of self-fertilization, but they accomodate and adjust their very high levels of post-zygotic abortions in a variety of often quite spectacular ways. Although inbreeding, their post-zygotic selection systems cause their surviving progeny to be (secondarily) outbreeding. They exhibit high levels of genetic heterozygosity and often permanent hybridity. They exhibit genomic coalescence, especially dysploidy, or polyploidy. The extent of their genomic coalescence is determined by the level of inbreeding in their population systems and by their capacity to accomodate high levels of post-zygotic abortion. These concepts are illustrated by the chromosomal evolution observed in *Isotoma petraea*, *Stylidium*, *Drosera* and the Myrtaceae.

GENETIC CONSEQUENCES OF RELENTLESS HABITAT FRAGMENTATION IN EASTERN AUSTRALIAN RAINFORESTS

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The distribution of genetic diversity in bird species within and between rainforest isolates in eastern Australia has been addressed through restriction site mapping of mtDNA. In north-east Queensland within-species sequence divergence values range from 0.2 % to 2 %. Phylogenies derived from the mapped sites and other sources, and the varying degrees of rainforest specialization shown by the birds allow historical and ecological interpretations of these values. Restriction digests of PCR amplifications from nuclear and mitochondrial loci (introns, control region and cytochrome b) are also being used to measure genetic diversity within and between naturally and artificially fragmented populations. Early results of these analyses in several species will be shown and coupled to the restriction site data.

Environmental Sex Determination in Crocodilians

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In contrast to mammals and birds which are both endothermic vertebrates, many reptiles do not appear to have sex chromosomes, rather the sex of the hatchling is determined by the incubation temperature of the eggs. This phenomenon of environmental sex determination (ESD) has been recognised for over a decade. Among the reptiles, a few squamates, many chelonians and all the crocodilians display ESD. The mechanism by which temperature determines sex is not understood but there are several hypotheses as to how it may do so. This presentation will describe ESD in crocodilians and discuss some of these hypotheses.

The essential pattern of ESD in crocodilians is that high temperatures (32-34° C) of incubation produce male hatchlings and lower temperatures (29-30°C) produce females. Some studies suggest that where temperature above that which produces 100%males is not lethal, it can produce stunted females. Intermediate temperatures produce a mixture of sexes, with no intersexes having been observed and sex being absolutely determined some time prior to hatching. Early studies on ESD in reptiles concentrated on determining the temperature sensitive period (TSP) when temperature is acting on the embryo to determine its sex irreversibly. TSPs have been determined by temperature-shift experiments. Our own study attempts to more precisely detect the earliest differences in development of the indifferent gonad, ie. the differentiation of Sertoli cells. In the alligator, at male-producing temperatures, primordial Sertoli cells can be recognised ultrastructurally at around 32 days of incubation, whereas the onset of ovarian differentiation is somewhat later at around 40 days.

Hypotheses for environmental sex detemination consider (i) that the enzymes controlling steroidogenesis are particularly temperature-sensitive, such that sufficient estrogen to direct ovarian differentiation is only formed at female-producing temperatures; (ii) that temperature controls the expression of a testis-determining gene which necessarily must be present in all embryos; and (iii) that asynchronous development of the gonads and some other sex-inducing organ results in the expression of males or females at different temperatures. These hypotheses are not mutually exclusive. There is mounting evidence that all three may be true at least in part.

Natural habitat
-skewed sex ratio
in favour of
females

A GENETIC ANALYSIS OF EXTRACELLULAR PROTEASE PRODUCTION IN *Aspergillus nidulans*

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The production of extracellular enzymes such as proteases is a complex biological process which undoubtably requires the participation of many gene products. The genes involved in the process can be subdivided into at least two categories-genes required for the synthesis of the enzymes (the structural genes and regulatory genes whose products control the expression of the structural genes) and the genes required for secretion of the enzymes. We have begun an analysis of extracellular protease production in *Aspergillus nidulans*. The expression of extracellular proteases in *A. nidulans* is regulated in response to at least 3 environmental signals-carbon, nitrogen and sulphur metabolite repression. When preferred sources of these nutrients are low in the culture medium, enzymes required for the metabolism of alternative C,N and S sources, such as protein, are produced. We have isolated a number of mutant strains of *A. nidulans* which have altered levels of extracellular proteases. The characterization of the strains is in progress. One mutant strain has been shown to carry a mutation in a single gene which we have designated the *xprE* gene. The *xprE1* mutation results in low levels of extracellular proteases even under conditions of nitrogen starvation. However, derepression of protease production, in response to sulphur and carbon limitation, appears to be unaffected. The *xprE* gene has been mapped to a region of chromosome VI of *A. nidulans*, approximately 17 map units from the *sB* gene. No other genes in this region are known to be involved in nitrogen metabolism. We are currently seeking revertants of the strain carrying the *xprE1* mutation in an effort to identify other genes involved in the regulation of the protease genes.

Isolation by distance of barramundi (*Lates calcarifer*) populations.

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Nearly 6,000 barramundi from 50 samples taken throughout northern Australia were analysed for evidence of population isolation and stock subdivision. A total of 16 genetically discrete stocks were identified. These differed somewhat from those described by Shaklee *et al.* (1990) because of the use of the *G*-test, rather than established χ^2 tests. Only when the *G* statistic (summed over all loci) was significant were populations considered to be different. A comparison of two independent methods of analysis used by this study confirmed the strength of this approach. Statistically isolated groups of populations were consistently found in separate UPGMA clusters (based on Roger's Genetic Distance) with one exception. The effectiveness of these tests in evaluating population structure was substantiated by genetic models of gene flow between these stocks.

These stocks represent a minimum number of identifiable stocks either (i) because of the unavailability of samples from some areas, or (ii) because of insufficient sample sizes. The identification of genetically discrete stocks implies that these are essentially reproductively isolated, by geography and/or behaviour, and hence are self-sustaining populations that require independent management (in the short term). However, the appearance of reproductive isolation does not necessarily imply that significant genetic exchange does not occur in the longer term. Using the 1-dimensional stepping stone model of gene flow, the observed F_{ST} value of 0.062 could be maintained even if 1% of the population migrated to adjacent stocks every generation.

Shaklee, J.B., Phelps, S.R. and Salini, J.S. (1990). Analysis of fish stock structure and mixed-stock fisheries by the electrophoretic characterization of allelic isozymes. Chapter 8 *in* "Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management" (Whitmore D.H. ed.) CRC Press, Boca Raton.

The Genetic Component of Total Catchment Management - An Examination of Catchment Position and the Population Genetic Structure of a Running Water Decapod.

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The concept of Total Catchment Management implies the integration of the well known inter-relationships among land, water and biological resources. Preliminary analysis of the relationship between catchment position and the spatial variation in gene frequencies, of a widespread and abundant atyid shrimp *Paratya australiensis* supports the view that the distribution of genetic resources may, for some taxa, also be catchment-related.

Estimates of genetic variation, based on three enzyme loci (Pgi, Pgm, Mpi), among 20 sub-populations within a single sub-catchment in south east Queensland suggest that headwater populations display a greater propensity for allelic fixation than downstream populations. One possible explanation is that headwater populations are more susceptible to genetic drift due to the absence of significant upstream gene flow, while downstream populations maintain within-population diversity through gene flow from adjacent populations. This hypothesis is also supported by: 1) correlations among genetic and environmental parameters, such as altitude and average gradient; and 2) the observation that for those alleles present in significant proportions, any given population contains alleles present in populations further upstream, but not necessarily those futher downstream. The consequences for sampling populations within catchments and managing stream-dwelling species are discussed.

Germplasm evaluation and preliminary genetic data from Sturt's Desert Pea (Swainsona formosa)

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There is considerable interest in developing cultivars of Sturt's Desert Pea for different horticultural purposes: cut flowers, pot plants, hanging baskets and cut runners. A germplasm survey of plants grown from seed collected from S.A. and W.A. populations has revealed considerable phenotypic variation for eight quantitative characters thought to be important in breeding programmes. Good correlations have been observed between relatives for these characters.

Sturt Pea plants frequently die of wilt disease, however susceptibility to wilt varies between families and the prevalence of the condition has markedly declined in the latest generation after intense selection for wilt resistance in previous generations.

A number of flower colour and pattern variants have been collected. Limited data are available on the inheritance of a few of these phenotypes.

GENES AND SEX AND A MOUSE CALLED RANDY

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Sex determination in mammals hinges on the presence and activity of a dominant Y-linked testis-determining gene, *TDF* in humans or *Tdy* in mice. This gene directs testis formation from the bipotential embryonic gonad (the genital ridge), resulting in further male differentiation. In the absence of *Tdy* activity, ovaries form and females develop.

Analysis of Y chromosome DNA present in a number of XX men led to the identification of a gene dubbed *SRY* (sex-determining region Y gene). Studies on the homologous gene in mice, *Sry*, have clarified the role of *Sry* and illuminated the biology of mammalian sex determination. First, *Sry* is present on the minimum portion of the mouse Y chromosome known to be male-determining. This region is referred to as the Sxr' region. No gene functions other than *Tdy* have yet been mapped to Sxr'. Secondly, *Sry* is deleted in a line of sex-reversed XY female mice known to be mutant in *Tdy* . The deleted region is only 10 kilobases (kb) in length and does not appear to affect any gene or gene function other than *Sry*. Point mutations in the *SRY* gene also correlate with loss of ability to direct maleness in XY women. Thirdly, *Sry* is expressed in the genital ridge at the time of differentiation into testes. Fetal expression of *Sry* is confined to this period only, and is not seen in any tissue other than the genital ridge. *Sry* expression does not depend on the presence of germ cells, as had been predicted from the biology of testis determination. The protein product of *SRY* contains an HMG box able to bind to specific DNA sequences and may act as a regulator of gene transcription.

The biological role of *Sry* was tested directly in transgenic mouse experiments: if *Sry* is *Tdy*, it should be capable of directing testis formation and hence male development in chromosomally female (XX) embryos. Injection of *Sry* as a 14kb fragment of genomic DNA into fertilized mouse eggs produced an adult XX transgenic mouse which was fully sex-reversed. These experiments demonstrate that only one Y-linked gene is needed for male sex determination, and *Sry* is that gene. The existence of this mouse lays to rest the search for the the testis determining factor. The challenge is now to find out how *Sry* acts, what other genes are involved in the sex determination pathway, and how these genes interact to bring about the sexual phenotype.

Just experiments on embryos
ovaries removed → ♀
testis " → ♀

i.e femaleness is the ground state.

Sox genes
- Sry gene family
- 79 aa. conserved region of homology only.

Phylogenetic relationships of the thylacine (*Thylacinus cynocephalus*) among dasyuroid marsupials: evidence from cytochrome-b.

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DNA sequences from the mitochondrial cytochrome-b gene were obtained from a museum skin of the presumed extinct thylacine and compared to homologous sequences from representatives of the marsupial family Dasyuridae. The relationship of the thylacine to dasyruids has been suggested by previous morphological and molecular studies. The thylacine's position within the dasyuroid radiation, however, has not been addressed with genetic data. Phylogenetic analysis of 574 bp suggests that the thylacine is a sister group to Dasyuridae and lends support to the hypothesis that *Thylacinus* represents an ancient marsupial lineage. Relationships within Dasyuridae support the results of other recent molecular studies, particularly in showing the affinity of endemic New Guinean subfamilies to larger Australian clades.

Genetic Variation between Founding and Ancestral Populations of *Drosophila buzzatii*

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Drosophila buzzatii colonised the cactus environment of Queensland and New South Wales from the Chaco region of Argentina about 60 years ago. Secondary colonisations of isolated cactus stands in Victoria occurred later. These three regions differ climatically, for the cactus species present, and for the presence of a competitor species, *D. aldrichi*, which occurs only in the northern part of the cactus distribution in Australia. Differences among populations, one from each of these three localities, were compared for performance on simulated rots of *Opuntia stricta* and *O. tomentosa*, for their responses to and effects on *D. aldrichi*, and for mating preferences among populations. Performance was measured as an index composed of larvae to adult viability, developmental time and body weight of emerging adults. The *D. buzzatii* from Dixalea, Queensland and El Chanar, Argentina were more similar than were either to Bulla, Victoria, primarily for performance on the two cacti, and for their response to and effect on *D. aldrichi* on *O. tomentosa*. However, significant mating isolation was found between *D. buzzatii* from the Dixalea and Argentina populations, and it was nearly significant between Bulla and Argentina, while the Dixalea and Bulla flies showed little isolation. As the climate in central Queensland and in the Chaco region of Argentina is also similar, with the climate of Victoria much cooler, natural selection may have led to the greater differences for larval traits in the Bulla population, while differences between the original founder population that reached Australia and its ancestral population may have contributed to the apparent mating isolation between Dixalea and Bulla *D. buzzatii* and those from Argentina.

The genetic load revealed by slow inbreeding in *Drosophila melanogaster*

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The loss in reproductive fitness and competitive ability caused by slow inbreeding is of interest to conservation biologists, to the breeders of large domestic animals, and to population geneticists studying the maintenance of genetic variability in natural populations.

A set of 60 subpopulations derived from two wild and one cage population were maintained without reserves in ½ pint bottles by synchronous mass transfer for 210 generations, followed by full-sib mating with reserves for 50 generations. The rate of inbreeding during mass tranfer was determined from the drift variance for electrophoretic markers, and the loss of genetic variance in quantitative traits, averaging 0.54±.07% per generation.

Of the 60 subpopulations, 45 survived to reach virtual homozygosity at the end of the 260 generation period. The reproductive fitness of the surviving populations, by comparison with a panmictic population formed by intercrossing the inbred lines followed by a period of random mating, was 73±2% under non-competitive conditions, and 17±3% under intense competition.

Comparisons with recently sampled wild populations after approximately 160 generations of slow inbreeding showed the wild populations to have a competitive ability of only 49±4% relative to the corresponding panmictic population.

These observations can be explained by the elimination of deleterious recessive alleles of large effect and adaptation of the inbred populations to the laboratory regime during the period of slow inbreeding, together with the chance fixation of deleterious recessive alleles of very small effect. The homozygous autosomal genetic load due to such minor genes is estimated to be of the order of 0.6% per map unit under competitive conditions.

Mitochondrial DNA Population Differentiation and Gene Flow in the Coconut Crab (*Birgus latro*).

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The coconut crab is the largest terrestrial arthropod and is found on isolated tropical islands throughout the Indo-West Pacific region. In recent years population numbers of this species have dropped dramatically. In order to examine the population structure of the species for management purposes, mtDNA variation has been analysed in 160 individuals from nine locations throughout the species' range. Composite haplotypes were obtained for each individual using five restriction enzymes with four or five base-pair recognition sites. Considerable mtDNA variation was found, with a haplotype diversity of 0.962. There was a clear phylogenetic distinction between the mtDNA of Indian and Pacific Ocean individuals. Among Pacific islands, some haplotypes were shared between locations, but analysis of haplotype frequencies and nucleotide divergence revealed significant differentiation between the populations of some islands. The levels of gene flow between islands were estimated using a cladistic approach. The data also allows examination of the phylogeographic history of the species.

Phylogenetic analysis of the Night Parrot, *Geopsittacus occidentalis*.

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The Night Parrot is a small, yellow-green bird inhabiting the arid interior of Australia. The species was first described from a specimen collected in 1845; the last live night parrot to be collected was in 1912 from Western Australia. In the intervening period, a total of twenty-two specimens were deposited in museums throughout the world. Sixteen of these specimens were collected for the South Australian Museum in the 1870s, indicating a local irruption of the species at this time due to favourable conditions. Such evidence leads to the speculation that this bird may be an uncommon nomadic species, rather than one actually on the verge of extinction. The current work on the Night Parrot (presented here) was prompted by the discovery in 1990 of a recently dead specimen, near Boulia in Queensland; the first confirmed sighting for seventy-eight years (this specimen being held at the Australian Museum).

Understandably, very little is known of such an elusive bird. The affinities of the Night Parrot with the coastal Ground Parrot (*Pezoporus wallicus*) were first recognized by John Gould in 1849. Indeed, recent studies on their osteology and anatomy suggest that the differences between *Geopsittacus* and *Pezoporus* are insufficient to warrant the recognition of seperate genera. In addition, this bird may also share a close relationship with the New Zealand Kakapo (*Strigops habroptilis*), although biochemical evidence suggests that the Ground Parrot (and the Night Parrot?) is allied to the Budgerigar (*Melopsittacus undulatus*) and, more distantly, to the remaining broad-tailed, or platycercine parrots.

Given that no adequate tissue samples of this bird are available, the development of the polymerase chain-reaction amplification of DNA has enabled a further investigation of the Night Parrot's taxonomic status. The National Museum of Victoria holds three Night Parrots, one of these being a ninety-eight year old specimen from the Gawler Range in South Australia. A region of the mitochondrial cytochrome b gene was amplified and sequenced from DNA obtained from a feather tip of this bird. This sequence was aligned with those from other parrots, including the Ground Parrot, Kakapo, Budgerigar and Western Rosella (a platycercine parrot), in an attempt to resolve a phylogenetic relationship of the Night Parrot with other Australasian genera.

Putting a finger on *amdS* regulation by the *amdA* gene of *Aspergillus nidulans*

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The *amdA* gene mediates actetate induction of *aciA*, a structural gene of unknown function. In the presence of the semi-dominant mutation, *amdA7*, a high constitutive level of *aciA* expression is observed (1). This mutation also results in increased expression of another gene, *amdS*, which encodes an acetamidase (2). Two *cis* -dominant mutations, *amdI66* and *amdI666*, which map to the 5' control region of *amdS*, affect *amdS* expression in an *amdA* dependent manner. Sequence analysis of the *amdS* 5' region has revealed that *amdI66* results from the tandem duplication of an 18 bp GA-rich sequence while *amdI666* is a tandem triplication of the same 18 bp sequence (3). Furthermore, the *amdA* loss of function mutation, *amdA7R2*, is epistatic to these *cis* mutations. These observations, together with *in vivo* titration studies suggest that the *cis* mutations may define the AmdA binding site in the *amdS* 5' control region (4).

The *amdA* gene has been mapped to Linkage Group VII of the *A. nidulans* genome between the genes *gatA* and *alcC* (5). A chromosome walk from both of these flanking markers has been carried out in a lambda Gem11 *amdA*+ library. The location of *amdA*+ within this series of overlapping clones was determined by transformation and selection for complementation of the *amdA* loss of function mutant. Sequence analysis of *amdA*+ genomic and cDNA clones reveals that the gene encodes a C2H2-type zinc finger motif. This finger region has significant homology to CreA, an *Aspergillus nidulans* regulatory protein which binds GC-rich sequences (Nicole Chamalaun, pers. comm.). Identification of this potential DNA binding domain in AmdA suggests that it may activate *amdS* expression by directly binding those sequences defined by the *amdI66* and *amdI666* mutations. Current research is now directed towards understanding the mode of *amdA* action by examining the effect of multiple copies of *amdA* and by assessing the abitity of AmdA protein extracts to bind *amdI66* and *amdI666* sequences.

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MHC Class II Genes in Parasite Resistant Sheep

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Major histocompatibility complex (MHC) class II genes encode a family of dimeric cell surface glycoproteins. These cell surface molecules play a crucial role in the generation of immune responses to foreign organisms by presenting antigenic peptides to T lymphocytes. A high degree of polymorphism has been found in the antigen-binding domains of these molecules (encoded by exon 2) which results in the differing abilities of these molecules to stimulate immune responses to different antigens. Studies in mice have shown that the level of immune response to some nematode infestations depends on which MHC class II alleles they possess.

We are investigating the effects of polymorphism at ovine MHC class II loci (*Ovar-D*) in relation to the resistance or susceptibility of sheep to gastro-intestinal nematodes. To date we have cloned and sequenced representatives of seven ovine MHC class II genes (DQA1, DQA2, DQB1, DRA, DRB1, DRB2, DRB3). In addition, the existence of at least two other MHC class II genes (DPB1, DQB2) has been demonstrated by restriction fragment length polymorphism (RFLP) studies.

RFLP studies have been used to define a number of alleles for each of the *Ovar-DQA1*, *Ovar-DQA2*, *Ovar-DQB1*, *Ovar-DRA* and *Ovar-DRB* genes. Several alleles have also been characterised by sequencing the second exons of *Ovar-DQA1*, *Ovar-DQA2* and *Ovar-DQB1* genes following amplification of this region by the polymerase chain reaction.

The distribution of alleles of the *Ovar-DQA1*, *Ovar-DQA2*, *Ovar-DQB1* and *Ovar-DRB* genes are being studied in five flocks of sheep which have been selected for resistance or susceptibility to the gastro-intestinal nematodes *Trichostrongylus colubriformis* or *Ostertagia circumcincta*. Preliminary data suggests that no associations exist between any of these genes and resistance to either of these parasites.

A CHROMOSOMAL BANDING STUDY OF EUCHROMATIN TRANSFORMATION

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One of the primary features which has emerged from the comparative analysis of many of the families of advanced anurans is that when closely related species are examined, they are found to be markedly uniform in respect of their chromosome number, relative chromosome size and centromere position. Yet studies on the chromosomal location of the ribosomal RNA genes (NORs), and distribution of C-band heterochromatin, reveal considerable variation in several polytypic genera. It has been difficult to explain the change in NOR location and C-band position between species where there is no evidence that major karyotypic rearrangements have occurred. One hypothesis is that regions of non C-band euchromatin are transformed into C-band heterochromatin by a process that has been termed euchromatin transformation. The major assumption inherent in this hypothesis, that homologous chromosome regions were correctly identified from standard stained and C-banded preparations, remains untested. Using banding techniques, we investigated the means by which chromosomal location of the NOR and C-band regions differ in two genetically closely related groups of the Australian hylid frog *Litoria lesueuri*. The structural rearrangements observed involve a minimum of two break points and the translocation of the NOR and associated heterochromatin between nonhomologous chromosome pairs. The large differences in the location and size of the C-band heterochromatin in this example is not the result of euchromatin transformation and can be explained by amplification of the C-band material.

EVOLUTION OF SEX CHROMOSOMES AND SEX DETERMINING GENES

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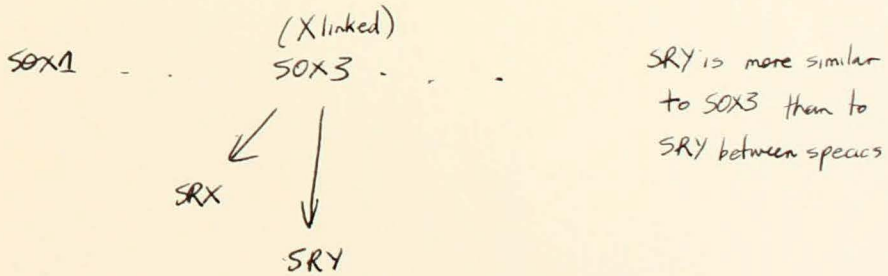
In order to deduce the course of evolution of sex chromosome organization and function, my group has compared the genes of the X and Y chromosomes in distantly related mammal groups, the eutherians, marsupials and monotremes, which diverged 150 and 170 million years ago. We have found that a large region is conserved across all three groups, and was presumably part of an ancestral X. However, the region represented by the short arm of the human X is autosomal in marsupials and monotremes; thus it was not part of the ancestral X and must have been acquired by the X quite recently during the eutherian radiation.

The sex-determining gene TDF must lie on the Y in all mammals since the Y is testis determining. The autosomal location of a candidate gene ZFY in marsupials and monotremes eliminates it from consideration. In contrast, the SRY gene, recently cloned from the sex determining region of the human Y and shown by mutational analysis and transgenesis to be testis determining in man and mouse, detects male-specific sequences in marsupials. We have cloned an SRY-homologous sequence from the marsupial Y chromosome and shown that it is conserved within an 80 amino acid motif, as would be expected if this gene plays the same sex determining role in marsupials and eutherians. Unexpectedly, a sequence homologous to the marsupial SRY has been detected on the X chromosome, raising the question of whether sex determination is a function of dosage of an XY shared gene.

SRY sequence is not highly conserved between eutherians & marsupials

Hypothesis

? SRY is repressor of ovary determining factor rather than a testis determining factor ?



P.B. Mather and S. McCluskey

Abstract: 'The relationship between temperature and PGI allelic variation in the isopod, *Porcellio laevis*.'

While genetic polymorphisms at individual enzyme loci have been widely studied, the relevance of the variation, either physiological or evolutionary still often remains unexplained. Phospoglucose isomerase (PGI) is one enzyme which has been widely studied in this regard and relationships between environmental variables (particularly temperature) and PGI variation are common in the literature. This study examines PGI variation in a common species of isopod in Australia (*Porcellio laevis*) and relates it to individual responses in imposed (stressful) and 'natural' (choice) temperature conditions.

SENSOR-REGULATORY NETWORKS IN BACTERIAL PATHOGENESIS: A TWO-COMPONENT TRANSCRIPTIONAL REGULATORY SYSTEM CONTROLLING EXPRESSION OF *PSEUDOMONAS AERUGINOSA* TYPE 4 FIMBRIAE

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Pseudomonas aeruginosa is an opportunistic pathogen. This involves expression of a number of factors, including toxins, degradative enzymes, and fimbriae, the latter of which are involved in the attachment of cells to host tissue. Similar fimbriae occur in a variety of bacterial species.

The gene encoding the fimbrial subunit (*pilA*) is transcribed from a σ^{54}-dependent promoter, a type of promoter which utilises a specific initiation factor (the product of the *rpoN* gene) and which is characteristically dependent on a cis-acting regulator bound nearby. This regulator is itself normally activated (by phosphorylation) in response to some environmental signal.

We used transposon (Tn) mutagenesis to generate a library of some 12,700 mutants, of which 147 appeared to be impaired in fimbrial structure or expression, as judged by altered colony morphology. A subset of these mutants were found to have occurred in the three loci that had been previously characterised, including the subunit gene *pilA* and the initiation factor gene *rpoN*. However there are clearly a number of new loci involved in fimbrial biosynthesis or function.

We were interested to isolate the gene encoding the regulator which governs the transcription of *pilA*. Regulators of this type share conserved sequences from which degenerate oligonucleotide probes were derived. Southern blots with these probes revealed a number of hybridising bands, indicating that there are a set of such regulators in *P. aeruginosa*. When the mutants were similarly screened, one group (R1) was identified in which one of these bands had shifted due to transposon insertion. These mutants had no detectable transcription of *pilA*.

Inverse PCR was used to amplify genomic DNA sequences flanking the transposon in an R1 mutant, which was then used to screen a wild-type cosmid reference library. The Tn mutation sites in R1 mutants were localised within the cloned DNA and two genes affected, *pilS* and *pilR*, were sequenced. These genes were physically mapped on the *P. aeruginosa* chromosome by pulsed-field gel electrophoresis, and shown to be capable of complementing the corresponding R1 mutations, both at the level of restoring the phenotypes associated with functional fimbriae and by the restoration of *pilA* transcription. *pilS* and *pilR* clearly belong to the family of two-component regulatory systems which have been described in many bacterial species. By analogy with these systems, PilS is a sensory protein which when stimulated by the appropriate environmental signals activates PilR through kinase activity. Similarly, PilR controls *pilA* transcription by binding to sites upstream of *pilA* and interacting with the RpoN initiation factor.

The identification of *pilS* and *pilR* makes possible a more thorough examination of the signal transduction systems controlling expression of virulence factors in *P. aeruginosa*. We would like now to define the environmental cues to which PilS responds, and to identify other genes under the control of PilR. There are clearly several such systems in *P. aeruginosa* (and other bacteria), with parallels in eukaryotic cells. These systems allow regulation of a range of genes in response to environmental stimuli, with a full dynamic range by differential affinity for target DNA binding sites, and the integration of such responses by cross-talk at the protein or DNA level.

REGIONAL GENETIC DIVERGENCE IN A VAGILE AUSTRALIAN MOTH, *HELICOVERPA PUNCTIGERA*

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Significant inter-regional moth movement in this species is thought to be responsible for high levels of gene flow and the widespread homogeneity in allozyme frequencies. We established, in duplicate, five mass-reared laboratory populations of *H.punctigera* from widespread Australian collection sites. Under uniform rearing conditions the populations were sampled for several quantitative traits and for mitochondrial DNA (mtDNA) variation. Several traits differed significantly among populations, including adult weight, development time and hind-wing size. The laboratory population differences are not due to genetic drift in culture because they are evident in duplicate lines from the same location that were independently maintained. Differences are also unlikely to be associated with laboratory adaptation because stocks from all locations were initiated at approximately the same time. Using polymerase chain reaction (PCR) we performed direct DNA sequencing of a short (750 bp) PCR product from the 'AT rich' region of the mtDNA. This is a known hypervariable region of the mtDNA and is an informative region for detecting population differentiation. In the few moths analysed to date we detected 5 polymorphic sites in 250bp of sequence and found marked differences in haplotypes frequencies from the different populations. We are now working on larger samples of field-collected moths. These data indicate that despite the high vagility of this species heritable regional differentiation occurs for specific genetic traits.

Fitness and asymmetry modification revisited; malathion-resistance in *Lucilia cuprina*

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The evolution of resistance by *Lucilia cuprina* to the insecticide diazinon initially resulted in increased bilateral asymmetry of resistant phenotypes relative to values observed in susceptibles. At that time, resistant phenotypes were at a selective disadvantage relative to susceptibles in the absence of diazinon. The disruptive influences of the resistant (*Rop-1*) allele were dominant, however continued use of diazinon after resistance was common in natural populations of *L. cuprina* selected a dominant modifier to ameliorate these effects (McKenzie and Game, 1987 Heredity 59: 371-381; McKenzie and Clarke, 1988 Genetics 120: 213-220).

The present study shows the modifier also acts on phenotypes resistant to malathion. Resistant phenotypes at the R_{mal} locus display increased levels of fluctuating asymmetry and lower relative fitness than susceptibles. The effects are partially dominant and correlated. The modifier acts as a dominant to generate asymmetry and fitness values of resistant phenotypes comparable to those of susceptibles.

The results will be discussed in terms of the debate of the relationship between changes in fitness and asymmetry under conditions of genetic and/or environmental stress.

GENE MAPPING IN THE TAMMAR WALLABY

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There is considerable current activity and interest in comparative gene mapping. Amongst vertebrates, most of the data are from eutherian mammals. Marsupials are the most nearest group to eutherians. Marsupials have low chromosome numbers, with two modes of 2n= 14 and 16, together with evidence of considerable conservation of karyotypes over wide evolutionary distances. A linkage map of a marsupial is desirable. We have therefore carried out a breeding program which should enable a linkage map to be drawn up with a similar level of efficiency as is possible in the *Mus musculus/Mus spretus* system.

We have crossed two geographically distant races of *Macropus eugenii* (the tammar wallaby) which has a 2n = 16 chromosome number and an XX/XY female/male sex determination system. The male genome length is 1150cMs. Tammars can be bred in captivity in colonies of several hundred and their reproduction manipulated so that each female can produce 4-5 offspring per year. Two races, one from Kangaroo Island in South Australia and the other from Garden Island in Western Australia, readily hybridise and the F1s of both sexes are fertile. Over 100 backcross progeny have been produced from matings between F1 males and Kangaroo Island females, so allowing the genetic diversity of these two races to be exploited.

To date, backcross progeny have been typed for 22 autosomal markers which show large gene frequency or fixed differences between the two populations. A further 20-25 homologous probes are available and we are examining the use of heterologous probes for conserved genes.

Here we report detection of the first two genetic linkage groups in a marsupial with known chromosomal assignments. Linkage group 1 consists of the gene for a novel marsupial milk protein, late lactation protein, which is known to be located on chromosome 3 in the tammar, and two anonymous tammar cDNA clones, pB3 and pB65. Linkage group 2 consists of β-globin, which is also located on chromosome 3 and α-lactalbumin. Thus, including the previously reported linkage between GPI and Pi, there are now three known linkage groups in the tammar wallaby. With the available data we can generate male recombination frequencies. Information regarding female recombination frequencies will become available late 1992.

A discussion of strategies for comparative gene mapping will be given.

~23 chiasmata per meiotic cell in ♂ , 50 cM/chiasma

23
50
1150 cM

Characterization of subtelomeric and centromeric sequences of the mouse

Jack

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The chromosomes of *Mus musculus* are telocentric, and have large concentrations of repetitive DNA at the centromeric end consisting of major and minor satellite sequences. These are not interspersed and we have shown that the minor satellite, sat II, is closer to the end of the chromosome than is the more abundant major satellite, sat I. Centromeric activity remains associated with sat II, rather than sat I, in the T199H translocation. The end of each chromosome is capped by telomeric hexamer repeat sequences, (TTAGGG)n. We have now investigated the region between the telomeric and sat II arrays. Using mouse DNA as template and the telomeric hexamer repeat $(CCCTAA)_4$ as primer, we obtained a series of amplified PCR products 300-1200 bp in size. These were cloned into pBluescript. One of the clones , ST1, contained a 718 bp insert in which inverted $(CCCTAA)_4$ repeats flanked a 670 bp sequence. Dot blot analysis showed that ST1 is present 50-200 times in the mouse genome and in situ hybridization showed it is present at every telomere in female cells. ST1 sequences are also present in *Rattus norvegicus*, where they are present in both telomeric and centromeric locations. In the mouse, some subtelomeric ST1 sequences are so close to minor satellite sequences that a single primer from each, acting together, generate a range of PCR products 80-1300 bp in size.

GENETIC DIVERSITY AND EVOLUTIONARY HISTORY OF RAINFOREST VERTEBRATES

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The tropical rainforests of Australia have a long history of expansion and contraction. Currently, this system is confined to scattered montane and mesic regions on the central to northern east coast of Queensland. During the driest periods of the last glacial cycle (18,000 ybp), the rainforest was considerably reduced, with some suggestions that it survived only in small remnant patches on mountain tops and in some coastal locations. The tight climatic definition of this ecosystem, along with these long-term cycles in climate, provide a fascinating backdrop against which to study genetic variation.

We have commenced a long-term study of patterns of intra- and inter-specific variation in rainforest restricted animals with particular focus on those distributed across the major rainforest blocks within the Wet Tropics. The goal is to identify common patterns of interpopulation variation and phylogeny, and thus to infer rainforest history. At an even more basic level, these studies are providing the first direct evidence on amounts of genetic diversity within animal species endemic to these forests.

Our first analysis was of the crepuscular skink *Tropidophorus queenslandiae*. The unusual looking skink is undoubtedly restricted to rainforest and is restricted to the area between Cooktown and Ingham. Comparisons of mtDNA cleavage sites revealed strong differentiation (10%) between populations on the Atherton Tablelands in comparison to those from the more northern Windsor, Carbine, and Thornton uplands. This is not accounted for by isolation by distance. An analysis of allozyme differences (with M. Adams) revealed a similar pattern. The major genetic break occurs at the Black Mountain Break - an relatively dry area proposed to have been the center of a major rainforest disjunction in the past. This break had also been identified as significant for rainforest mammals.

The phylogenetic affinities of *T. queenslandiae* are very uncertain. A preliminary analysis of mtDNA sequences indicates that they are most closely related to *Eulamprus tenuis*, a habitat generalist, and also to *E. amplus*, a rainforest specialist. This and the broader phylogeny of *Eulamprus* indicates several shift in habitat specialisation through time.

FREQUENT ALLELES OF THE HUMAN VITAMIN D RECEPTOR GENE ARE FUNCTIONALLY DISTINCT.

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The vitamin D receptor is the central regulator of the vitamin D endocrine system. Mutations which have a profound effect on the receptor result in clinical disorders of calcium homeostasis and bone formation. Little is known of more subtle effects of receptor genetic variation. Osteocalcin is a bone protein regulated by the vitamin D receptor and osteocalcin which appears in serum is monitored as an index of bone formation rate. Twin studies have shown a strong component of genetic control on serum osteocalcin levels as well as bone mass. However, paradoxically, osteocalcin levels are negatively correlated with bone mass.

We hypothesized that if functionally significant genetic variation exists in a trans-acting factor gene (such as that of the vitamin D receptor), this should be detected by a correlation of alleles of the gene with the product of a target gene regulated by the factor in question. The vitamin D receptor is the most powerful known regulator of the osteocalcin gene, acting directly through a vitamin D responsive enhancer in the osteocalcin promoter. This model was applied to the relationship between the osteocalcin gene and the vitamin D receptor by correlating genetic variation in the vitamin D receptor gene with osteocalcin serum concentrations.

We describe here that frequent RFLPs which define human vitamin D receptor alleles fulfil the requirement as markers for functionally distinct receptor genes in that they are highly correlated with either high or low serum concentrations of osteocalcin in normal subjects. This genotypic classification essentially splits the normal range: the mean (±SD) osteocalcin values of genotype subgroups are homozygote A 17.3±7.2 ng/ml, heterozygote 9.0±4.6 ng/ml and homozygote B 7.7±4.7 ng/ml. Since the osteocalcin gene is on chromosome 1 and the vitamin D receptor gene is on chromosome 12 the only mechanism by which increased levels of serum osteocalcin can be correlated with vitamin D receptor gene RFLPs is through different functionality of the vitamin D receptor alleles. This example of functionally different naturally occurring alleles of a trans-acting factor provides the paradigm for other genes of the steroid receptor superfamily and their relationship to physiological variation and homeostasis as well as diseases of multifactorial etiology, such as osteoporosis.

The ß Globin Gene Cluster of *Sminthopsis crassicaudata*.

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A *Sminthopsis crassicaudata* genomic DNA library was screened with a marsupial ß globin probe. A clone was isolated which contains a complete copy of the putative adult ß globin gene. This clone was analysed using Southern blotting and PCR techniques.

Molecular linkage has been demonstrated between the previously cloned embryonic ß globin gene and the putative adult ß globin gene. Conclusions have been drawn concerning the number and arrangement of ß globin genes in an ancestor to all modern-day eutherians and marsupials.

CHARACTERISATION AND SEQUENCE ANALYSIS OF A SMALL CRYPTIC PLASMID FROM *SELENOMONAS RUMINANTIUM*.

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A medium copy number 2.5 kb cryptic plasmid, pJDB21, from the Gram negative ruminal anaerobe, *Selenomonas ruminantium* , subspecies *lactilytica* was mapped and sequenced. Five open reading frames (ORFs) were predicted and expression of ORF 1 was demonstrated in *S. ruminantium* by Northern blot. Analysis of the predictive amino acid sequence of ORF 1 protein indicated more than 30% homology with the replication protein (rep) common to many Gram positive plasmids, and a highly conserved sequence representing the origin of replication in these plasmids, was located upstream of ORF 1. This is consistent with pJDB21 using a rolling circle form of replication. Predictive RNA secondary structure suggested the presence of a control motif upstream of the translation start of ORF 1. Transformation of *Escherichia coli* K-12 UB1636*pol* A $_{ts}$ with pJDB21 showed the plasmid could replicate independently of DNA polymerase I and produced a single stranded DNA intermediate. Deletion analyses localised the *E. coli* replication function to a 1.4 kb sequence that was mapped to the predicted rep protein.

Genetic Tags for Turtles: Analysis of the Geographic Source of Harvested Green Turtles. Janette Norman, Centre for Conservation Biology, University of Queensland.

There are only seven species of marine turtle worldwide - all are classified as endangered or threatened. The primary causes of the decline of marine turtle populations are over-exploitation in commercial harvests, incidental capture in trawls, habitat destruction and pollution. Effective conservation management of these species is hindered by an absence of fundamental information on population biology and ecology, particularly population structure and the spatial and temporal distribution of populations. This is primarily a consequence of the difficulties involved in using traditional mark-recapture methods to study a marine animal that is both migratory and long-lived (an estimated 35-50 years to maturtity).

Recent population genetic studies have shown that mtDNA RFLPs provide an effective means of describing population structure in marine turtles and for identifying discrete breeding units (demes) for management. This paper is concerned with the practical application of population-specific mtDNA RFLPs as "genetic tags" for diagnosing the geographic source of turtles at feeding grounds, in harvests and in trawls.

Using the green turtle (*Chelonia mydas*) as a model I have developed a PCR based assay for site polymorphisms within the control region of the mitochondrial genome of marine turtles. PCR-sequencing was used to design primers within Cyt-b and 12sRNA to amplify the entire control region, and internal primers allowing independent amplification of the 5' and 3' portions of the control region. Population-specific RFLPs within the control region of marine turtles can now be rapidly identified by PCR amplification and digestion with appropriate restriction endonucleases. The practical application of this PCR-based assay for diagnosing the geographic source of harvested green turtles will be demonstrated.

DNA FINGERPRINTING IN CITRUS

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DNA fingerprints have been demonstrated in a variety of organisms, most notably in humans. Production of cultivar-specific DNA fingerprints in citrus species would allow precise varietal identification and the method has the capacity to become a powerful tool in the breeding of improved citrus varieties.

Possible DNA fingerprinting probes were isolated from the genome of Eureka lemon, *Citrus limon*. One clone, S5j, detected hypervariable restriction fragment patterns in a variety of citrus species, as demonstrated by Southern analysis. The genomic sequences that hybridize to S5j appear to be somatically stable in comparisons of DNA fingerprints between individual Navel orange trees, but evidence of somatic instability was observed within a single old Navel orange tree. The basis of the variation detected remains to be elucidated. The S5j probe was used to distinguish between nucellar and zygotic offspring of a citrus cross, an important breeding application.

A partial sequence of the informative clone was obtained. Portions of the sequence were AT-rich, with partial homology to mitochondrial sequences from a diverse range of eukaryotes. A number of short, direct repeats were present within the clone. The biological significance of these non-random patterns of DNA is unknown.

GENETIC VARIATION FOR GROWTH HORMONE IN SHEEP

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We are involved in a collaborative project to map the sheep genome through linkage studies. The CSIRO Division of Animal Production at Armidale maintains two pedigreed merino flocks and is collecting production character data. The project is funded by the Australian Wool Research Development Corporation. Using a bank of ovine probes we have detected RFLPs at a number of loci, among them the growth hormone locus. The growth hormone gene has been used extensively in genetic engineering experiments in laboratory animals as well as in livestock. Hormonal control of growth is complex and not well understood. We are investigating the possibility that variation at the growth hormone locus is related to growth rate variation in sheep. RFLP variation at the growth hormone loci in sheep is considerable and has been detected with two restriction enzymes, TaqI and PvuII. Both RFLPs are controlled by three co-dominant and one recessive null allele as determined from data on a large number of sire-dam-offspring combinations from both merino flocks. At present we are carrying out statistical analysis to determine if any relationship exists between the genetic variation observed and growth rate of the animals.

TRANSPOSABLE ELEMENTS FROM *LUCILIA CUPRINA*

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Various transposable elements from *D. melanogaster* have been used to identify similar sequences in the Australian sheep blowfly. Subclones from retrotransposons 297, B104, mdg3, gypsy and copia, from the I-element, and from the transposons P and hobo have been used to probe Southern blots of blowfly total genomic DNA at low stringency. All probes gave positive bands but in some cases these correspond to satellites visible on the gels and represent non-specific binding. The 297, B104, and mdg-3 derived probes gave the strongest specific signals after one week exposures. A *Lucilia cuprina* genomic library was screened with 297, B104, I element, P element and hobo derived probes. Positively hybridizing plaques were obtained in all cases, the numbers of which indicated a copy number of approximately 150-200 hybridizable sequences per genome for 297 and B104, and about 30 per genome for the I element, the P element and hobo probes. Subsequent work has been concentrated on the 297- and P-like clones.

Almost complete sequence information from one of the 297-like clones (Elsie-13) has been obtained which indicates that it contains a retrotransposon-like element similar to 297. Homology has been found to protease, reverse transcriptase, RNAse H, and integrase coding domains which are characteristic of various retro-elements. A number of deletions, insertions, and duplications disrupting the potential ORFs indicate that this particular element is inactive. The organization of the ends of this element suggests that recombinational events have occurred and that this element may be one of a group of tandemly repeated heterochromatic elements. Partial sequencing of three other clones also indicates homology to reverse transcriptase genes, however, differences between them indicate that they belong to related but distinct retrotransposon families.

Sequence has also been obtained from two of the P-hybridizing clones which reveals the presence of two elements (Lu-P1 and Lu-P2) which are similar to the P element in both structure and sequence but have diverged from it and from each other considerably (≈50% amino acid identity between the Lu-P elements and the P element, 60% identity between Lu-P1 and Lu-P2). Neither appears to be mobile and no inverted terminal repeats have been identified, however the internal 'coding' regions appear for the most part to be well conserved. The presence of a third related but distinct element in another as yet unsequenced clone is suggested by hybridization studies.

These results indicate that transposable elements and their derivatives are a significant component of the blowfly genome, but that the identification of active elements will not be easy and may be best accomplished by genetic means.

CLONAL SELECTION FOR DELETIONS AND DUPLICATIONS OF CHROMOSOME 17 IN HUMAN MYELOID MALIGNANCY.

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The wild-type alleles at human anti-oncogene loci are dominant suppressors of malignancy. Functional loss of both alleles is generally necessary before malignancy will result. Knudson's two-step model describes the sequential accumulation of two recessive alleles at such loci (1985). This model has been very successful in explaining early childhood cancers, but has an inherent difficulty in accounting for adult tumors of the sporadic (ie non-familial) type. The model requires that a cell with a single mutation form a clone of sufficient size (ie $>> 1/u$, where u = mutation rate) to ensure the second mutation in at least one cell. With recessive mutations, there is no reason to expect such proliferation of the heterozygous clone. This difficulty has led to the suggestion that some anti-oncogene mutations are co-dominant (Fearon and Vogelstein, 1990).

This theoretical difficulty is particularly apparent in the myeloid malignancies, where 25% of cases of the disease Chronic Myeloid Leukemia (CML) accumulate a clone homozygously mutant for the anti-oncogene p53, on chromosome 17p13. 4/5 of these cases possess an isochromosome 17q, with gross deletion of one p53 allele. The curious predominance of the isochromosome has led to proposal of a proliferative advantage through triple-dosage of allele(s) on 17q (Becher, 1990). Through detailed examination of three unusual cases, we here find evidence of independent selection for trisomy 17q and monosomy 17p. Thus isochromosome formation provides a route by which a truly recessive p53 mutant can accumulate when heterozygous. Isochromosomes come about because eukaryote chromosomes are bi-armed structures whose discrete centromeres can undergo intra-chromosomal exchange without loss of function. Evolution to frank malignancy may rely on these "chromosomal", rather than "genic", attributes. With molecular analysis alone, a mechanism of this type would be difficult to comprehend.

THE EFFECTS OF HEAVY METAL POLLUTION ON THE GENETIC STRUCTURE OF POPULATIONS OF THE HAIRY MUSSEL, *TRICHOMYA HIRSUTA*. Ngaire Phillips, Division of Environmental Science, Griffith University, Brisbane, AUSTRALIA.

The effects of heavy metals on populations of the hairy mussel *Trichomya hirsuta* in Lake Macquarie, New South Wales, were examined using a population genetics approach. Both field and laboratory based studies were undertaken to determine whether particular genetic variants were more or less tolerant to heavy metals than other variants. The concentration of metal in animals from sites close to a zinc sulphide smelter situated near the lake, and those distant from the smelter, were correlated with the frequency of genotypes and alleles, and also with the percentage heterozygosity, at a number of loci. Toxicity tests were then undertaken to establish a direct cause-effect relationship between the heavy metal and particular genetic variants. Finally, an attempt was made to elucidate the mechanism of action of the metal at particular loci. The enzyme activity of enzyme extracts from individuals of known genotype was measured in the absence and presence of metal. The results indicated differential survival of individuals with particular genotypes and alleles under conditions of heavy metal pollution. The potential use of this approach to pollution monitoring is discussed.

Sex-specific differential methylation in the body of the kangaroo X-linked hypoxanthine phosphoribosyltransferase gene.

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X chromosome dosage compensation in eutherian and metatherian mammals is achieved by inactivating one X-chromosome in cells of female embryos. In eutherians, the X to be inactivated is chosen randomly however in metatherians the paternally-derived X is inactivated. Inactivity, at least for constitutive genes, is more complete in eutherians than metatherians, in which some genes are only partially inactivated in certain tissues. The greater stability of random versus paternal X-inactivation appears to be due to promoter-associated hypermethylation of inactive-X-linked eutherian, but not metatherian constitutive genes. This promoter hypermethylation may be a maintenance mechanism specific to constitutive genes, rather than an initiating mechanism of inactivation, since no such hypermethylation has been seen for non-constitutive eutherian inactive-X-linked genes and it is not clear whether this hypermethylation occurs concurrently with or shortly following X-inactivation. Thus the underlying inactivation mechanism may be similar in both eutherian and metatherian mammals. We have previously characterised a genomic clone partially encoding the *Macropus robustus* X-linked hypoxanthine phosphoribosyltransferase (HPRT) gene. The methylation state of this gene has been examined by digestion of genomic DNA from blood, muscle, brain and liver of male and female animals with the methylation sensitive endonucleases Hpa II and Hha I and hybridisation with probes from the *Macropus* HPRT clone. The majority of Hpa and Hha sites examined were found to be largely or completely methylated on the active X of males. In contrast, these sites were present in females in both methylated and unmethylated forms, with, at some sites, approx equal amounts of each form present. Assuming the methylation state of the active X is similar in males and females these results indicate that the inactive X is hypomethylated relative to the active X in the body of the HPRT gene. The finding that sites that were unmethylated on the paternal inactive X were methylated in sperm, indicates that demethylation of paternal-X-linked sites occurs during embryogenesis. Inactivity usually correlates with hyper- rather than hypomethylation, however most such studies refer to methylation of 5' regulatory regions, where methylation is thought to interfere with the action of transcription factors. However active-X specific methylation has also been observed in the body of eutherian X-linked genes and thus may be implicated in an aspect of X-inactivation common to eutherians and metatherians. For example specific 3' methylation could prevent binding of "inactivating proteins" to the active X. We are currently investigating possible protein-DNA interactions at 3' differential methylation sites by *in vitro* DNase footprinting.

Allozyme variation within the *Euasticus hystricosus* (Parastacidae) population, and its relevance for conservation management. Mark Ponniah, Division of ENS, Griffith University.

E. hystricosus is an endemic freshwater crayfish which is distributed in streams in the Conondale and Blackall ranges. Its limited geographical range indicates that effective management of the population will be essential for the future viability of the species.

To effectively manage a population, it is essential to know the extent of genetic differentiation and gene flow that is present within and between conspecific populations.

Allozyme electrophoresis is being used to measure the amount of genetic variation and gene flow that is present within and between conspecific populations of *E. hystricosus*. Samples have been collected from four different streams in the southern part of the Conondale Range. In addition, samples will be collected from a site in the Blackall Range and from two sites in the northern Conondale Range. Preliminary investigations indicate that there is: very little heterozygosity present within conspecific populations, and little allelic variation present between conspecific populations. This could suggest that the lack of allelic variation between conspecific populations is due to extensive gene flow, or more likely to there being very low levels of overall allelic variation in the population. Streams in this area are characterised by extreme fluctuations in stream flow. Therefore, the lack of heterozygosity which has been observed could be the result of the population going through a series of bottlenecks during periods of extended drought.

The implications of these findings, for managing the population of *E. hystricosus*, will be discussed.

A race-specific mini-chromosome in the fungal phytopathogen *Colletotrichum gloeosporioides*.

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The important legume *Stylosanthes* is used to improve cattle pastures in tropical and subtropical regions of the world, including Northern Australia. The widespread use of this genus is severely limited by its susceptibility to anthracnose. This disease is caused by the fungus *Colletotrichum gloeosporioides*. In Australia there are two distinct diseases (Types A & B) caused by two fungal pathotypes; the Type A pathogen infects most species of *Stylosanthes* causing defined necrotic lesions, whilst the Type B pathogen is specific to *S. guianensis* with a blight symptom. These pathotypes can be easily distinguished by dsRNA components and RFLP analysis and are genetically distinct. Within each type there is further pathogenic specialisation with regard to the virulence of fungal races on specific host cultivars of *S. scabra* for Type A and *S.guianensis* for Type B.

Pulse field electrophoresis has revealed that extensive variation exists in the molecular karyotype of *C. gloeosporioides* isolates. This variation is mainly restricted to mini-chromosomes (300-1200 Kb) which vary in size and number between isolates. A 1.2 Mb mini-chromosome is present in duplicate race 3 isolates of the Type B pathogen and absent from duplicate isolates of the other 3 races. 10 distinct DNA fragments specific to this mini-chromosome of the Type B Race 3 were cloned. Hybridization analysis has shown that these DNA fragments are completely absent from other Type B races. Random Amplified Polymorphic DNAs (RAPDs) have been used to isolate Type B Race 3 specific DNA probes. Hybridization analysis with these probes also showed them to be specific to the 1.2 Mb mini-chromosome. These results indicate that this mini-chromosome is strain and perhaps race specific and that chromosome variation does not arise from simple rearrangement of the existing genome. Either whole chromosome deletion or addition from an unknown source are alternative mechanisms. The 1.2 Mb mini-chromosome lacks a repeat sequence present and monomorphic on all other chromosomes of the Type B pathogen. This is consistent with recent introgression of this chromosome into the Type B genome.

The chromosome-specific probes have been used to identify possible donors of this chromosome. For example all Type A's carry a homologous 2 Mb mini-chromosome. Nitrate reductase mutants have been produced and paired to simulate parasexual exchange of chromosomes. Parasexual crossing has been obtained between some of the Type B isolates and one Philippine isolate but not to other pathotypes of *C. gloeosporioides*.

DNA REPAIR, HAIRLESS LOCUS GENOTYPE AND SKIN CANCER SUSCEPTIBILITY IN THE MOUSE.

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Abstract. Hairless (*hr/hr*) mice are qualitatively more sensitive to the induction of skin cancer by UV irradiation than congenic wildtype mice. A particular dose of simulated sunlight can be delivered which will cause all hairless mice to develop multiple skin tumours, while no shaved wildtype controls develop any tumours at all.

Although the *hairless* gene is partly cloned, it was considered worthwhile to determine whether there is any obvious difference in the rate of induction or repair of pyrimidine dimers in the skin of hairless and wildtype mice. A repair defect is an obvious candidate for the enhanced tumour susceptibility of the mutant mice and pyrimidine dimers are the most common genotoxic by-product of UV exposure.

Mice were exposed to simulated sunlight (5.4 joules/cm^2 of UV in the 290 to 400 nm wavelength range), equivalent to two minimally erythemal doses. High molecular weight DNA was prepared from epidermis of dorsal skin at various intervals after irradiation. Samples of DNA were digested with UV-DNA endonuclease from *Micrococcus luteus*, which cleaves at pyrimidine dimer sites, and electrophoresed in alkaline agarose gels to allow determination of the number of endonuclease sensitive sites per 100kb and thus to determine rate of repair.

There was no evidence of any difference in the rate of induction or repair of pyrimidine dimers between hairless and wildtype genotypes, whether at the level of the whole genome or in the active H-*ras* gene. The explanation for skin cancer resistance in wildtype mice compared to hairless mutants must be sought in a mechanism other than repair of pyrimidine dimers.

Variability in *Rattus sordidus* population densities: ecological and gene flow hypotheses. W A Ruscoe Centre for Biological Population Management Queensland University of Technology GPO Box 2434 Brisbane Q4001.

Ecological studies in two geographically separate regions (Ingham and Mackay) have supported an hypothesis that the relative density of *Rattus sordidus*, a significant pest species of sugarcane, is correlated with the availibility of non-cane refuge sites. This has led to a number of suggestions for control strategies based on limiting available refuge areas.

An alternate explanation for the differences the relative population densities also exists. That is, populations in different geographic localities vary in genetic structure such that the presence of particular allelic forms of genes favour one population in one habitat type and not in others. If this is the case then we would expect significant differentiation in gene frequencies on a microgeographic scale in rodent populations of both Ingham and Mackay which is related to habiatat types. This paper examines data collected to assess the two hypotheses.

Potential for Gene Flow via Hybridization in *Banksia*

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Banksia robur and *B. oblongifolia* are commonly found in the Metropolitan Water Board Catchment, north of Wollongong. These two species are different morphologically and are found in fairly different environments, which overlap in a environmental ecotone. In this ecotone, individuals of intermediate morphology are common, and have been previously assumed to be hybrids. The two putative parental species have been shown to be genetically distinct (exhibiting fixed or near fixed differences), with intermediate genotypes associated with the individuals of intermediate morphology. However, the stability of the hybrid zone can not be determined through simple observation of the appearance and disappearance of hybrid individuals, as they, like their parents, are potentially long-lived (as they resprout after fire), and the rate of recruitment is generally slow. An estimate of the current production of hybrids was estimated by observing flowering times of the species and the hybrid, and the pattern of foraging of potential pollinators and by conducting controlled pollination experiments. It was hoped that this approach may give an insight into the potential gene flow between the species, and thus the stability of the hybrid zone.

DO SEQUENCES IN Xq27.3 PLAY A ROLE IN X INACTIVATION ?

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We have been studying two female patients with interstitial deletions of Xq27.3 involving the fragile X region and the flanking sequences between DXS 297 and IDS. Interestingly, in both cases the deleted X chromosome is preferentially active in fibroblasts and in lymphocytes, as determined by cytogenetic methods, by the analysis of DNA methylation with the probe M27β (DXS 255), and by the analysis of transcription at the IDS locus using reverse transcriptase - PCR protocol. Phenotypically both patients are mentally retarded, and one is affected with Hunter syndrome, while the other is not due to a small fraction of cells (5-10%) in which the normal X chromosome is active.

The reverse inactivation pattern observed in both patients suggests that the deleted sequences play a role in X inactivation. When they are missing the cell line bearing the deleted X as inactive, and the normal X as active is under strong negative selection, which indicates that it is functionally abnormal. The first possibility is that the deleted X cannot be normally inactivated which leads to a functional disomy for the distal Xq. Alternatively, the intact active X may be locally inactivated, similarly to the situation in the fragile X syndrome. Experiments are under way to determine which one of the two possibilities is the case.

Gene flow among conspecific populations of *Baetis* sp. within and between subcatchments in the Conondale Range, Qld. Sonja Schmidt, Griffith University.

Few studies have investigated the dispersal capability of the adult stage in the life history of stream insects. It is generally assumed that the aerial adult stage represents a dispersal phase for these species, in which they could be expected to move between conspecific populations. However, it has been suggested that the movement of adults is predominantly upstream to compensate for the gradual downstream displacement of larvae, and as a result gene flow between subcatchments and even between adjacent streams would be limited.

The extent of gene flow among conspecific populations of *Baetis* sp. is examined between four small subcatchments in the Conondale Range, Qld. Two subcatchments were sampled on either side of the range. The location of the subcatchments is such that a subcatchment on one side is closer to a subcatchment on the other side than it is to the other on the same side. Two streams have been sampled within each subcatchment. Allozyme electrophoresis will be used to measure differences in allelic frequencies between streams within subcatchments and between subcatchments. It is hypothesised that if there is gene flow between subcatchments, and if it is maintained by dispersing adults, allele frequencies will differ more between distant subcatchments on one side of the range than between adjacent subcatchments on opposite sides of the range.

RELATIONSHIPS AMONG HYBRIDISING TAXA OF THE VARIED SITTELLA COMPLEX.

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The Varied Sittella *Daphoenositta chrysoptera* occurs throughout mainland Australia and Papua New Guinea. Five morphologically distinct forms are recognised in Australia and hybridise with each other where their ranges abut. This complex comprises dihybrid, trihybrid and tetrahybrid zones, and a pentahybrid zone, a unique situation in the animal kingdom.

Two competing hypotheses have been used to explain this unusual distribution pattern. One invokes a single vicariance event, or a rapid series of such events, leading to the simultaneous divergence of all five forms in isolation prior to their range expansion and hybridisation[1,2]. The other proposes a very specific order of vicariance events and complex history of differentiation[3].

Morphological and protein electrophoretic data were analysed to examine the history of the complex and relationships among the parental taxa. These data suggest a biogeographic history intermediate to the competing hypotheses. Furthermore, mitochondrial DNA (cytochrome b) sequence data are being used to further resolve the phylogenetic relationships among the hybridising taxa. The results of these studies will be reported here.

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ARABINOSE METABOLISM MUTANTS OF *ARABIDOPSIS THALIANA*

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Arabinose is a significant component of polysaccharides in many plants. UDP-arabinose is presumed to be the active arabinose donor in polysaccharide biosynthesis and is synthesized via the "de novo" pathway from UDP-glucose. However, free arabinose, such as that released during the degradation or turn-over of polysaccharides, can also be metabolised along the "salvage" pathway via arabinose-1-phosphate to UDP-arabinose.

An arabinose-sensitive mutant of *Arabidopsis thaliana* has been isolated fortuitously. The sensitivity is specific to L-arabinose and has not been observed with any other sugar. The mutant phenotype is semi-dominant and segregates as a single Mendelian locus. The mutant shows reduced ability to incorporate exogenous labelled L-arabinose into ethanol-insoluble polysaccharide material and has only 10% of the wildtype level of arabinose kinase activity. Exogenous arabinose appears to accumulate at inhibitory levels in the mutant.

Phenotypic revertants of this mutant which are resistant to arabinose have been selected. In the most extreme of these essentially no exogenous labelled arabinose is incorporated into polysaccharide material and no arabinose kinase activity is detected. One explanation for this is that the "suppressor" mutation prevents transport of of the exogenous sugar into the cell thereby preventing its accumulation at inhibitory levels. Sugar transport studies with the various mutants support this conclusion. The "suppressor" mutation is closely linked to the original arabinose-sensitive mutation suggesting this may be a single gene encoding both a transport and kinase activity.

This locus has been mapped to chromosome 4 of the *Arabidopsis* linkage map. RFLP mapping studies have shown close linkage to a particular RFLP marker for which a number of Yeast Artificial Chromosome clones of *Arabidopsis* have been identified. We are currently attempting to clone the locus from within this region.

Using PCR to Detect Nuclear Gene Variation Across Diverse Species

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At the molecular level, evolutionary and population genetics studies are often hampered by technical difficulties. These may include the following: the need for fresh or freshly frozen tissue, non-detectable polymorphism, difficulty in accessing nuclear loci at the DNA level, expense in terms of both reagents and time, technical demands, and use of potentially dangerous reagents. We suggest that PCR can be used to amplify a potentially variable fragment of nuclear DNA from diverse species using primers to evolutionarily conserved flanking regions. Primers are designed from existing sequences in a database. Variation is then detected by gel electrophoresis before and after restriction enzyme digestion, and/or denaturing gradient gels, and/or sequencing. At its simplest and most common level this involves amplifying an intronic fragment with primers that hybridise to the flanking exons. The template DNA used in the reaction is obtained rapidly and safely with Chelex extraction. An aliquot of the reaction is then digested with a restriction enzyme and electrophoresed through a polyacrylamide gel. The resultant fragments are visualised directly after ethidium bromide staining. The discrete size of the amplimer allows length variations and site gains and losses to be determined easily. The utility of the approach will depend on the capacity to amplify a specific fragment from diverse species, and on whether there is detectable variation. We demonstrate the approach with examples from the histone H2AF, aldolase, myoglobin, and MHC DQA genes.

A Molecular Population Genetics Approach to Understanding the Evolution of Organophosphate Resistance in *Lucilia cuprina*.

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The phenomenon of pesticide resistance is a touchstone example of natural selection acting in the wild. The Australian sheep blowfly, *Lucilia cuprina*, has a long history of developing resistance to chemicals used as control agents, such as dieldrin and subsequently the organophosphate insecticides, malathion and diazinon. Resistance to malathion and diazinon is attributable to the action of two esterases, malathion carboxyl esterase (MCE) and esterase three (E3), produced by closely linked loci on chromosome four.

Recently, an intriguing association between resistant and susceptible allelic forms of these proteins has been found. Chromosomes carrying the E3 allele associated with resistance always carry an allele conferring low levels of MCE activity. The converse is also true, with no recombinants found amongst 20 lines homozygous for the fourth chromosome.

We will describe progress in cloning these genes which will enable the determination of the sequence of susceptible and resistant alleles at both loci. The sequence data will be anaylsed using recently developed tests for divergence from neutrality to reveal the effect selection has had on shaping the nature and levels of genetic variation occurring at these loci. Both the observed disequilibrium of malathion and diazinon resistance and the more distal effects at other loci ('hitch-hiking') will be examined. We believe this approach, together with what is already known of the use of insecticides in Australia, will make a powerful test of how populational forces mould the genome of an organism over time.

The Population genetics, reproductive biology and morphology of the Serpent's head cowry *Cypraea caputserpentis(kenyonae)*.

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The Serpent's head cowry *Cypraea caputserpentis* lays benthic egg masses which are brooded until a late veliger stage larvae emerges. Encapsulation reduces the development time spent in the plankton which decreases the probability of widespread larval dispersal. This study was undertaken to determine the extent of larval dispersal for West Australian populations.

Larvae were reared in the laboratory to ascertain the length of the planktonic phase. Patterns of larval recruitment at Rottnest Island were related to water movement both locally and along the coast of Western Australia. The behaviour of the larvae and the hydrodynamics of the environment suggest there is potential for limited dispersal of larvae.

Morphological and genetic variation provided apparently conflicting evidence of limited larval dispersal. Morphological variation revealed a pattern of shell shape over latitude, probably due to differences in water temperature, where as genetic variation among populations of *C. caputerpentis* was random. The standardised variation in allelic frequencies among the West Australian coastal populations of *C. caputsepentis* (F_{ST} = 0.0381) was comparable to values obtained for other species with limited larval dispersal and an order of magnitude greater than that for species with widespread larval dispersal.

Integrons: versatile genetic elements in disseminating antibiotic resistance genes.

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Integrons are novel DNA elements which are able to incorporate genes, mostly antibiotic resistance genes, by site-specific recombination and are widely distributed in Gram-negative bacteria. The most common form of the integron is two conserved segments of DNA which flank one or more antibiotic resistance genes.

Integrons can disseminate antibiotic resistance and other genes in three ways:
1) Integrons as a whole are likely to be mobile elements as they are found inserted into a number of different and independent locations.
2) Integrons possess a sophisticated mechanism for acquiring genes by site-specific recombination. Antibiotic resistance and other genes are inserted individually via a circular gene cassette, where a cassette consists of a coding sequence and a 59-base element at its 3'- end. 59-base elements are imperfect inverted repeats which are recognised by an integron encoded DNA integrase.
3) Since the flanking segments of integrons are conserved between elements and some gene cassettes can be duplicated, the potential exists for the exchange and rearrangement of integron sequences by *recA* dependent recombination.

Integrons therefore are versatile elements in disseminating antibiotic resistance genes and their role in the evolution of plasmids and transposons in Gram-negative bacteria will be discussed.

Comparison of the *gatA* genes of *A. nidulans* and *U. maydis*.

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The basidiomycete smut pathogen of maize *Ustilago maydis* is being used as a model system to study gene regulation in fungi. A gene encoding a putative GABA transaminase has been isolated from an *U. maydis* genomic library via DNA homology to a portion of the analogous *Aspergillus nidulans gatA* gene. The *U. maydis UgatA* gene has been sequenced. Comparisons of the deduced amino acid sequence of the GABA transaminase genes of *A. nidulans* (*gatA*), yeast (*UGA1*) and *U. maydis* (*UgatA*) show regions of considerable homology over most of the protein.

Regulation of *UgatA* is being examined using Northern analysis. The *UgatA* transcript is induced by GABA, and, as seen in *A. nidulans*, is not under nitrogen metabolite repression control (ie. not repressed in the presence of ammonium). Other approaches being used to examine the regulation in *U. maydis* include sequence comparisons of the 5' region of *UgatA* and *gatA*, and isolation and characterization of mutants defective in their ability to use GABA as a nitrogen source in an attempt to identify trans-acting factors that regulate *UgatA*.

These studies are designed to further expand our understanding of gene regulation in eukaryotes and the conservation of regulatory proteins and/or systems.

Application of molecular markers (RFLP and RAPD) in sorghum improvement

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Osmotic adjustment is a plant character which helps sorghum withstand drought. It has been shown recently that this character is controlled by a single gene. Populations of F2 and backcross progenies segregating for osmotic adjustment from a cross between a high osmotic adjustment line TAM422 and a low osmotic adjustment line QL27 have been developed, and these segregating progenies have been screened for RFLPs and RAPDs. So far, about 30 maize genomic DNA clones have been tested for RFLP and 120 random single primers and 330 double primer combinations for RAPD between the two parental lines of this segregating population. Twenty-nine F2 individuals and 23 backcross individuals were analysed for 11 loci (3 for RFLP and 8 for RAPD). About 90 loci have been screened between two bulked samples which were pooled from homozygous high osmotic adjustment F2 individuals and low osmotic adjustment individuals respectively. No linkage has yet been found between the additive gene for osmotic adjustment and these molecular markers. Further screening is ongoing.

Thirty-eight genotypes representing a wide range of sorghum cultivars were surveyed for RFLPs with 10 maize genomic DNA clones. Single digests of each of three enzymes (*BamH 1, EcoR 1 and Hind III*) were screened for each genotype. DNA amplification fingerprints of all of these 38 genotypes were also produced using 30 single arbitrarily chosen primers. Cluster analysis of polymorphisms based on 262 DNA fragments generated by these primers has been carried out using the SAS computer program. The frequency of DNA polymorphism will be presented. The data will permit the assessment of the suitability of parental lines for novel facilitated breeding and may also permit assessment of phylogenetic relationships within *S. bicolor*.

THE DEVELOPMENT OF CYTOLOGICAL AND MOLECULAR MARKERS FOR CEREAL CYST NEMATODE (CCN) RESISTANCE IN RYE.

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Cereal cyst nematode (*Heterodera avenae*) is a serious pest of cereal crops world wide. In Australia alone it is estimated to cause yield losses valued at $70 million annually. At present, resistance relies on 2 wheat varieties. However, an additional strong resistance gene has been identified on rye chromosome 6 (6R) present in the triticale line T701-4-6. A set of four deficiency (deletion) mutants of the long arm of 6R have been isolated and used to localise this gene to an interstitial segment of the long arm of 6R. These deficiency mutants are also proving useful for developing both cytological and molecular markers for this region of chromosome 6R.

The rye-specific, repetitive DNA sequence, R173 has been used to develop cytological and molecular markers for this chromosome. Non-radioactive *in situ* hybridisation with a clone representing an almost entire R173 element has allowed the detection and characterisation of rye chromatin in alien backgrounds. It has been possible to use this technique to characterise material coming from breeding programmes involving all rye chromosomes but particularly 6R and 1R. Other cloned members of the R173 family have been used to develop PCR markers for the long arm of chromosome 6. RFLP probes already mapped to the related wheat and barley chromosomes have been obtained from other laboratories and along with the PCR markers, have been mapped to specific regions along the long arm of 6R utilising the deficiency mutants.

A backcross population involving chromosome 6 from the CCN susceptible rye cultivar "Imperial" and chromosome 6 from the resistant triticale T701-4-6 is currently being screened with the PCR and RFLP markers to develop a genetic map of this chromosome and in particular to locate the CCN locus.

Analysis of Spontaneous Mutation at the *scarlet* Locus of *Drosophila melanogaster*

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In recent years it has become apparent that transposable elements are a major source of spontaneous mutation in *Drosophila melanogaster*. The spontaneous mutant w^a, one of the first spontaneous mutants characterized in detail, was shown to contain an insertion of a *copia* element. Since then a number of spontaneous mutants have been characterized and shown to be associated with insertions of transposable elements. Most of the data available refers to the occurrence of such mutations in laboratory populations, while very little is known about the effects of transposable elements on natural populations. Other than studies of unstable spontaneous mutations at the *singed* locus and analysis of a number of natural variants at for example the *heat shock* and *alcohol dehydrogenase* loci, there appears to be no direct evidence that transposable elements are a major source of mutation in natural populations of *D. melanogaster*.

We have studied six spontaneous mutations at the *scarlet* (*st*) locus at the molecular level. Two of the mutants, st^1 and st^{sp} were isolated from laboratory populations,while the other four, st^{cob}, st^{ct89}, $st\ dct$, st^{dv} came from natural populations.

Five of these contain DNA insertions within the *st* region, but the sixth, st^{cob} shows no detectable change, at the level of Southern analysis. Regions from the *st* genes containing the insertions were amplified using inverse PCR. This gave fragments containing some *st* sequence including the insertion site, plus some sequences from the insert. These fragments were then sequenced and in four cases the insertion was identified as a being a transposable element; these include the elements *412* and *B104/roo* both members of the retrotransposon family, and the *jockey* element a member of the LINE element family. In the fifth case, the insertion appears to be the result of a partial duplication of *st* sequences. Two of the insertions (both involving *412*) occur at the same site in exon 5. The others two insertions involving transposable elements are found in intron sequences. Studies of the effects of these mutations on the transcription of *st* are currently in progress.

The results obtained show that elements involved in mutation in laboratory populations, are also important as a source of mutation in wild populations of *Drosophila melanogaster*.

WHY IS UV-INDUCED MUTAGENESIS IN SOME ENTERIC BACTERIA WEAK?

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Closely related species of enteric bacteria have markedly different mutation frequencies following irradiation with ultraviolet light. *E.coli* shows high mutation rates after irradiation, whilst *S.typhimurium* shows much lower levels of mutagenesis, and yet in other aspects of DNA repair, the two species are virtually identical. Such phenotypic differences presumably reflect alterations within genes specifically involved in mediating the UV response. The aim of this study is to determine factors promoting inaccurate repair of UV-induced DNA damage which lead to high mutation rates. In *E.coli*, protein products of the *umuC* and *umuD* genes are required for UV-induced mutagenesis. Cells of strains carrying a mutation in either gene are only marginally more sensitive to the lethal effects of UV than wild-type cells, but are non-mutable. *S.typhimurium* also contains two *umu* genes which encode proteins of similar molecular weight and high seqeunce homology to their *E.coli* counterparts, but they do not appear to be as active, for reasons unknown. Another set of *umu*-like genes (*samAB*) has recently been found on a cryptic plasmid in *S.typhimurium*. This discovery poses the question of whether UV-mutagenesis in *S.typhimurium* is weak not because of any inherent defect within its *umu* genes but because of cross-regulation between the *umuDC* and *samAB* operons. Results will be presented to show that at least some aspects of *umu* activity remain unaffected by the deletion of the *samAB* genes.

SOS
↓
Error-prone repair.
↓
Mutations.

Triploid Intersex and Chimeric Chickens: Implications for Avian Sex Determination

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Major developments in the understanding of sex determination have frequently resulted from the study of individuals with aberrant sex chromosomes. In the domestic chicken, such individuals have been identified largely due to an associated intersex phenotype. Most are ZZW triploids, or more rarely are mosaics for ZZ and ZW diploid cells. At CSIRO, the availability of a high frequency of triploid intersex chickens in a selected line of poultry, as well as a low frequency of chimeras, offers a valuable source of material for the study of avian sex determination. The CSIRO triploidy line regularly produces 8-14% hatched triploid chickens in each generation. Three triploid genotypes are observed, ZZZ, ZZW and ZWW; some of the first two genotypes are viable, while ZWW triploids die during early embryogenesis. The ZZZ triploid chickens have a normal male phenotype but are sterile, and the ZZW triploids are sterile intersexes that display a female phenotype until sexual maturity and then undergo a male sex-reversal. This includes the development of male secondary sex characteristics such as enlarged comb and wattles, spur growth, male plumage and crowing. Viable chimeric chickens including haploid-diploids and diploid-triploids, are detected in the triploidy line at a frequency of 0.5-1.0%. Haploid-diploid chickens exhibit normal female (Z/ZW) or male (Z/ZZ) phenotypes and are fertile, but the phenotype and fertility of the diploid-triploid chimeras appears to be determined by their sex chromosome complements and the ratio of diploid to triploid cells. Details of the reproductive morphology of these unusual birds are presented. All evidence from the triploid and chimeric chickens indicates that the W sex chromosome has a primary female-determining role in birds, but there is also evidence of an effect of dosage of the Z chromosome.

Analysis of *facB*, an acetate regulatory gene from *Aspergillus nidulans*.

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The *facB* gene of *Aspergillus nidulans* is involved in regulation of the genes for acetate metabolism and the acetamidase encoding *amdS* gene. Sequence analysis of the cloned *facB* gene reveals a $Zn(II)_2Cys_6$ cluster DNA binding motif and potential activation domains. *facB* loss of function mutants are of two classes. One class show reduced activation of genes under *facB* control and are thus affected in the regulatory function of *facB*. The second class are affected in stability of the enzymes of acetate utilization and thus indicate a structural function for *facB* in acetate metabolism. Therefore it appears that the FacB protein has both a regulatory and structural role.

Investigation of the various functions of *facB* is being undertaken. A *facB* null mutant has been constructed by gene replacement with a construct containing the *facB* gene disrupted by a bleomycin resistance marker. This mutant provides an appropriate recipient for analysis of transformation constructs in a $facB^-$ background. To assign functions to particular regions of the FacB protein the sequence changes in *facB* mutants are being determined. The PCR amplified *facB* genes from two *facB* loss of function mutants, two temperature sensitive *facB* regulatory mutants, and two temperature sensitive *facB* structural mutants have been cloned.

SEQUENCES OF NEW OVINE DQB ALLELES USING PCR

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Major Histocompatibility Complex (MHC) class II genes encode membrane glycoproteins that present antigens and thereby stimulate immune responses. Class II molecules are composed of an α and a β chain encoded by separate, closely linked genes. The first domains of these molecules, which contain the antigen recognition sites, are the product of exon 2 of the encoding genes. Variation in this region is of importance in host immunological defense mechanisms.

Several new nucleotide and peptide sequences of exon 2 of the ovine MHC class II gene DQB were identified using the following three oligo primers:

JM05: TCCCCGCAGAGGATTTCGTG 5' sense
JM06: TCCGCCGCTGCCAGGTGAAG 3' anti-sense
JM07: TCCGCTGCAAGGAGGTGATG 3' anti-sense

Exon 2 of the ovine DQB gene is highly polymorphic with at least 20% of the nucleotide sites and 40% of the amino acid sites exhibiting variation. The amount of variation detectable from sequence data exceeds that identified by RFLPs.

Comparison of ovine DQB exon 2 sequences with those of bovine and human show that the location of several of the variable sites, and the type of variation found at these sites, are similar.

HEXOSE TRANSPORT MUTANTS OF *ARABIDOPSIS THALIANA*

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Root growth of *Arabidopsis thaliana* is inhibited on medium containing the glucose analogues, glucosamine (GlcN), 2-deoxyglucose (2DG) and mannose (Man) and also the sugar D-galactose (Gal). We have selected four independent mutants of *Arabidopsis* which are resistant to the inhibitory effects of 1 mM GlcN. These mutants also show increased resistance to 2DG, Man and Gal. No other phenotype has been observed.

Genetic studies have shown the resistant phenotype in each mutant is semi-dominant. Because of this complementation studies have not allowed the identification of the number of genes involved however genetic mapping has shown the four mutations map to two different chromosomal loci. Sugar transport assays using the sugar, 3-O-methyl-glucose, a transportable but non-metabolizable analogue of glucose, have shown that at least three of these mutants exhibit reduced glucose uptake into root tissue.

The effects of estuaries and islands on the genetic structure of four species of marine inshore fishes

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The genetic population structures of *Mugil cephalus*, *Leptatherina presbyteroides*, *Craterocephalus capreoli* and *Apogon rueppellii* were examined using protein electrophoresis. Samples of each of these fishes were collected from marine, estuarine and island locations in Western Australia, over a distance of up to 1400 kilometres. The estuaries differed in the extent to which they were connected to the ocean. They were classified as permanently open, seasonally closed, normally closed or permanently closed to the ocean.

The level of genetic subdivision among populations was inversely related to the species capability for long distance dispersal. The standardised variance in allelic frequencies (F_{ST}) ranged from 0.010 in *M. cephalus*, which has a high capability for dispersal, to 0.385 in *A. rueppellii*, which has a low capability for dispersal.

The extent of genetic divergence was also examined between pairs of populations separated by distances less than 50 kilometres. At this scale there was limited genetic divergence between marine populations (F_{ST} ranged from 0.002 to 0.013). A greater range of F_{ST} values was obtained for the comparisons between marine and estuarine populations (0.001 to 0.174) and for the comparisons between marine and island populations (0.026 to 0.168). An interaction was observed between the two factors examined in this study: dispersal capability and degree of population isolation. For example, although all four species showed similar levels of genetic divergence for the comparisons between marine populations, the low dispersal species showed higher levels of divergence for the comparisons between marine and estuarine or island populations than did the high dispersal species. This study shows that the genetic structure of a species is influenced not only by its life history characteristics, but also by the types of habitats it utilizes.

A C-banding and electron microscopic study of the holocentric chromosomes of the earwig *Labidura truncata*

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The work presented was done at the Australian National University, Canberra, ACT, in the Departments of Population Biology and Human Genetics of the Research School of Biological Sciences and the John Curtin School of Medical Research respectively

The earwigs, Order Dermaptera, are unique among orthopteroid insects in having holocentric chromosomes, they share this characteristic with the bugs, Order Hemiptera.

The earwigs of the genus *Labidura* have diploid numbers ranging from 8 to 14 and an XY-male, XX-female sex determining mechanism in the few species investigated. The most widely distributed Australian species, *Labidura truncata* Kirby, 1903, has 5 pairs of chromosomes, including the sex pair (Giles and Webb, 1972).

In *Labidura truncata* mitotic centromeric activity is distributed across a broad central region of each chromosome and at anaphase the separated chromatids have the shape of a shallow "U" (rather than the "V" seen at this stage with monocentric chromosomes). The ends of the chromosomes, which trail at anaphase because they have no centromeric activity, are heterochromatic and positively C-banded after barium hydroxide treatment of air-dried chromosomal preparations. There is sufficient variation in the size of these C-banded regions to distinguish all of the pairs of chromosomes in *L. truncata* ; this has not been previously possible for any earwig. Transmission electron microscopy of sectioned testes verifies the holocentric nature of the mitotic chromosomes of spermatogonial divisions in *L. truncata* , showing the spindle fibres to be inserted across the whole width of the broad centromere.

During both spermatocytic divisions the chromosomes of earwigs and bugs show a localized centromeric activity which is termed "neocentric". The purpose of neocentric activity is clear during first meiosis because holocentric activity on each side of a chiasma would destroy the chiasma during anaphase. Neocentric activity is continued into second meiosis although it is not clear why this occurs. The insertion of the spindle fibres into male meiotic chromosomes showing neocentric activity in *Labidura truncata* was explored using transmission electron microscopy.

Giles ET and Webb GC (1972) The systematics and karyotype of *Labidura truncata* Kirby, 1903 (Dermaptera: Labiduridae). *J Austral ent Soc* **11**: 253 - 256.

In situ hybridization using probes derived from laser microdissected chromosomes

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Laser microdissection of specific chromosome regions followed by PCR amplification and subsequent cloning enables rapid generation of region specific genetic probes[1]. To date this approach has been trialed on human and marsupial target material. Marsupial chromosomes, because of their size and ease of identification, provide useful material for evaluating this technique. We have microdissected chromosome 4 of the marsupial *Sminthopsis crassicaudata* into 5 overlapping regions (chromosome 4 was chosen as it is thought to contain the ß-globin gene family and 3 other linked genetic markers - *TRF*, *SOD1* and *PGD*). DNA from each of 5 isolated regions from a single chromosome was PCR amplified using non-specific priming, EcoR1 digested, size selected and cloned into the vector λgt10. Recombinant clones contained inserts of average size 500bp. Fluorescent *in situ* hybridization has been used to map the clones. The results of our preliminary analyses of these clones will be presented. A brief summary of the potential use of laser microdissection for human genetic analysis will also be given.

Reference: 1. Hadano, S. et al., *Genomics* **11**: 364-373 (1991).

Key Words: *In situ* hybridization, laser microdissection, genetic probe, human, marsupial, PCR.

supernova: A Gene Involved in the Cleavage Divisions of the *Drosophila* Embryo

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The cleavage divisions of the *Drosophila* embryo appear to be under the control of homologues of the universal set of mitotic regulator genes first identified in the yeast *Schizosaccharomyces pombe*. Two other groups of loci are also involved in the orderly division of nuclei. On group of genes regulate the organization and structure of the mitotic apparatus whereas the second group maintains the orderly segregation of chromosomes during cleavage.

This work describes a recessive maternal lethal, *supernova*, which disrupts normal nuclear division during cleavage in the *Drosophila* embryo. Deletion mapping and *in situ* hybridization to polytene chromosomes localizes *supernova* to region 89A1/2 on the cytological map. Subsequent to this, we have generated mutant alleles within this region using P Element and EMS mutagenesis screens.

Following fertilization, nuclei attempting division produce chromosomal ring-like structures and DNA bridges. These divisions are characterized by mitotic asynchrony amongst dividing nuclei with abnormalities in spindle organization. The terminal phenotype is characterized by the presence of many variable sized nuclei distributed randomly throughout the egg cytoplasm. These embryos eventually cellularize, but cells are irregular in shape and size as compared with normal blastoderm cells.

Fluctuating Asymmetry and Genetic variation in relation to pollution impacts on *Uca Coarctata*.

M. Wiggins Griffith University

Pollution stress on invertebrates has been shown to cause differential genetic selection and affect developmental stability, observed as variations in bi-lateral symmetry (fluctuating asymmetry).

Sampling of fiddler crab(*Uca Coarctata*) sub-populations from four Moreton Bay estuaries, Brisbane, Logan, Caboolture rivers and Tingalpa creek, representing different pollution regimes, was conducted. Genetic variation was measured by electrophoresis on PGI, EST-B and GOT enzymes. Morphological measurements of meristic characteristics (eyestalks, ridging, hair grouping, leg segments) were assessed for, firstly variation between sub-populations, secondly, correlations with levels of genetic heterozygosity.

Preliminary data and results are presented.

THE MOUSE X-Y SHARED GENE UBE1 DETECTS HOMOLOGUES ON THE MARSUPIAL X AND Y CHROMOSOMES.

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The X and Y chromosomes of humans and other eutherian mammals differ greatly in size and gene content. However, they share a small pseudoautosomal region, and recent evidence also suggests that several genes on the X of man and/or mouse also have apparently active alleles on the Y; these homologous regions may be relics of the originally homologous sex chromosomes. One of these genes is UBE1, which codes for a ubiquitin acivating enzyme and may be involved in spermatogenesis[1]; in man this gene is located only on Xp, but in mouse and other non-primate eutherians there are active alleles on both X and Y.

We have used a mouse cDNA probe to screen DNA from male and female marsupials, identifying male-specific sequences as well as shared bands, some of which show female:male dosage differences consistent with X linkage. We have cloned one of these sequences, and mapped it to the X and Y chromosomes, using Southern analysis of rodent-cell hybrids, as well as *in situ* hybridization. This suggests that marsupials, like mice, retain copies of the UBE1 gene on both sex chromosomes, and suggests that this gene was shared by both sex chromosomes in the common ancestor of eutherian and marsupial mammals.

This localization of a UBE1 homologue also refines the breakpoint of an ancient X-autosome rearrangement which added a large autosomal region to the ancient X and Y[2]. Whereas other human Xp genes are autosomal in marsupials (as well as monotremes), and are thought to be part of this added region, the UBE1 gene (which maps to human Xp11.1-11.3), and presumably the whole centromeric region, must have been part of the original mammalian X.

[1] Mitchell, M. et al (1991) Nature 354:483.
[2] Graves, J.A.M. and Watson, J.M. (1991) Chromosoma 101:63.

Phospho-Glycerate Kinase Polymorphism in the scallop *Pecten fumatus* : selection in natural and experimental populations.

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Studies of marine molluscs have revealed substantial evidence for the action of selection on allozyme polymorphisms, or on loci closely linked to them. In mussels, oysters, and clams, correlations have been found between growth rates, survival, or reproductive output with one or more of particular alleles, single locus heterozygosity, and multi-locus heterozygosity. In scallops (family Pectinidae) only survival has been thus correlated. Many of these studies are difficult to interpret because of inadequate distinctions between age-classes, and the difficulty of following individual growth rates in the marine environment.

Here we describe the results of a study of *Pecten fumatus* in Bass Strait. Genotypic (twelve polymorphic allozyme loci) correlates of survival in a natural population have been explored by repeated sampling of a recognizable cohort of scallops. As well, a reciprocal transplant of caged scallops has allowed a parallel analysis of individual growth and survival.

The results of each experimental approach agree, and implicate strong selection influencing *Pgk* genotypes. In the natural cohort, large changes in *Pgk* allele frequency and heterozygosity are only explicable by strong viability selection against a particular homozygous genotype. In the cage experiment, similar viability selection was detected, as well as an effect of *Pgk* genotype on individual change in weight. No significant correlations involving multi-locus heterozygosity were found.

These results emphasize the need for careful interpretation of field studies on marine invertebrates and provide the first example of strong single-locus selection in a pectinid.

cog and _sor_; two elements involved in the control of genetic recombination in the _histidine-3_ region of _Neurospora crassa_.

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cog is a recombinator located between *his-3* and *ad-3*[1]. Two alleles are known; *cog* and *cog+*. In a cross homozygous *rec-2*, *cog+* gives higher levels of recombination both between alleles of *his-3* and between *his-3* and *ad-3*. *cog* has been shown to be 3′ of *his-3*, with respect to *his-3* transcription[2]. Transformation of *his-3 ad-3* spheroplasts with DNA including *his-3*$^+$ and 3′ sequences shows that *ad-3*$^+$ and therefore also *cog* is within the DNA we have mapped. Comparison of the sequences 3′ of *his-3* between Neurospora wild-types shows substantial restriction site polymorphism. These RFLPs are being used to place *cog* on the physical map by following the segregation of each RFLP and *cog* in the progeny of crosses heterozygous for *cog*, RFLPs, *his-3* and *ad-3* amongst those segregants that are recombinant for *his-3* and *ad-3*.

Crosses of *his-3* mutations derived in the St Lawrence (ST74A) wild-type to *ad-3* strains show low levels of genetic recombination between *his-3* and *ad-3* even when *rec-2* is homozygous. The gene responsible has been designated *sor*, suppressor of recombination. The relationship between *cog* and *sor* is under investigation.

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1 Catcheside, D.G. & Angel, T. (1974) A *histidine-3* mutant, in *Neurospora crassa*, due to an interchange. Aust. J. Biol. Sci., **27**: 219-229.

2 Bowring, F.J. & Catcheside, D.E.A. (1991) The initiation site for recombination *cog* is at the 3' end of the *Neurospora crassa his-3* gene. Mol. Gen. Genet. **229**: 273-277.

ASSOCIATION OF INSERTION/DELETION POLYMORPHISM OF *ACE* WITH HUMAN ESSENTIAL HYPERTENSION Robert Y.L. Zee, Yi-Kun Lou, Li-Hua Ying, Lyn R. Griffiths and Brian J. Morris. *Molecular Biology & Hypertension Laboratory, Department of Physiology, The University of Sydney, NSW 2006*

Essential hypertension (HT) is a polygenic disorder. A number of genes whose products control blood pressure have been cloned. These include hormone, enzyme, adrenoceptor and hormone receptor genes. In addition, one or more polymorphisms have been described for many of these genes. The present cross-sectional study examined whether alleles of such polymorphisms segregate with HT. The study used leukocyte DNA from 85 HT and 100 normotensive (NT) caucasian subjects of British descent. To increase the power of the analyses, we used only HTs who had two HT parents and NT offspring of NT parents who were older than 50.

No association with HT was seen for alleles of a *Dra*I RFLP of the α_2-adrenoceptor gene (frequency of minor allele = 0.17 in HT and NT groups; $P = 1.0$ by χ^2-analysis), a *Bgl*I RFLP of the β_1-adrenoceptor gene (0.10 in HT cf 0.12 in NT; $P = 0.44$), a *Xho*I RFLP of the atrial natriuretic factor gene (0.50 cf 0.46; $P = 0.67$), a *Bgl*I RFLP of the insulin gene (0.36 cf 0.43; $P = 0.20$), a *Hin*dIII RFLP of the renin gene (0.76 cf 0.74; $P = 0.85$) and a *Pst*I RFLP of the antithrombin III gene (0.49 cf 0.54; $P = 0.37$).

In contrast, using PCR, we found a highly significant difference in the frequency of alleles of a 287 bp insertion/deletion ('I'/'D') polymorphism of the angiotensin I-converting enzyme gene (*ACE*) (0.56 in HTs cf 0.40 in NTs, for the 'I' allele: $P = 0.0018$). The 'I' allele has previously been linked to lower and the 'D' allele with higher plasma ACE activity. Whether the insertion polymorphism, which is located in intron 16 close to exon 17, is associated with differential splicing, leading to a form of ACE with higher specific activity, or whether the effect in HT is mediated by formation or degradation of hormones or neurotransmitter peptides unrelated to angiotensin and kinins remains to be demonstrated. Interestingly, recent mini- and microsatellite mapping studies of genetically hypertensive rats has led to the identification of 3 of the loci responsible for the elevation of blood pressure in the SHR-SP strain [1,2]. One of these loci contains the rat ACE gene and is sensitive to elevation in NaCl intake. Studies by sib-pair analysis by another group have failed, however, to show linkage of ACE with essential hypertension [3], suggesting that their choice of subjects, many of whom did not have two hypertensive parents, and their use of GH, which is linked to ACE, rather than ACE itself may have reduced the chance of success in their study.

In conclusion, the gene for ACE is either a cause of elevated blood pressure in essential hypertension or is in linkage disequilibrium with causative DNA.

1. Hilbert P, *et al.* (1991) *Nature* 353: 521-529
2. Jacob HJ, *et al.* (1991) *Cell* 67: 213-224
3. Jeunemaitre X, *et al.* (1992) *Nature Genetics* 1: 72-75

THE FORMATION OF PSEUDOGENES IN MITOCHONDRIAL DNA FROM PARTHENOGENETIC LIZARDS (*Heteronotia binoei*; Gekkonidae)

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The *H. binoei* species complex consists of several bisexual chromosome races and numerous triploid parthenogenetic lineages which arose by repetitive hybridization between two of the bisexual forms (CA6 and SM6 races). The parthenogenetic forms have two distinct types of mtDNA- the 3N1 type derived from the CA6 sexual parent and the 3N2 type from the SM6 sexual parent. RFLP analysis has revealed the presence of large tandem duplications in the mtDNA genomes of most of the parthenogens but not in any of the sexual races, which suggests that the duplications arose in the parthenogenetic forms. Cleavage site mapping of the 3N2 mtDNA revealed a 5-kb direct tandem duplication spanning the 16S and 12S rRNA genes, the control region and most of ND1. Modifications to each copy of this duplicated sequence include four large deletions but each gene affected by a deletion is complemented by an intact version in the other copy of the sequence. Sequencing of a fragment from one copy of the duplication revealed mutations expected to disrupt gene function. Post-duplication evolution has therefore resulted in mitochondrial pseudogenes.The redundant copy of a duplicated gene is expected to be under relaxed selection and to show a different mode of sequence evolution. The mtDNA duplications in these parthenogenetic geckos provides a unique opportunity to investigate how mtDNA sequences evolve without strong functional constraints.

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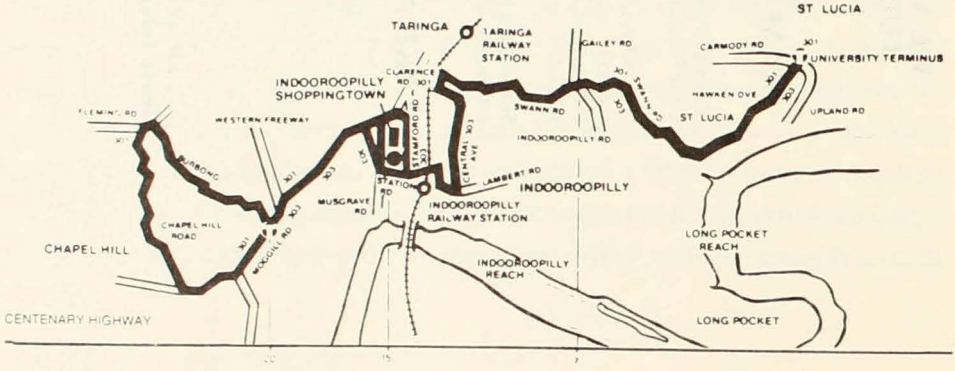

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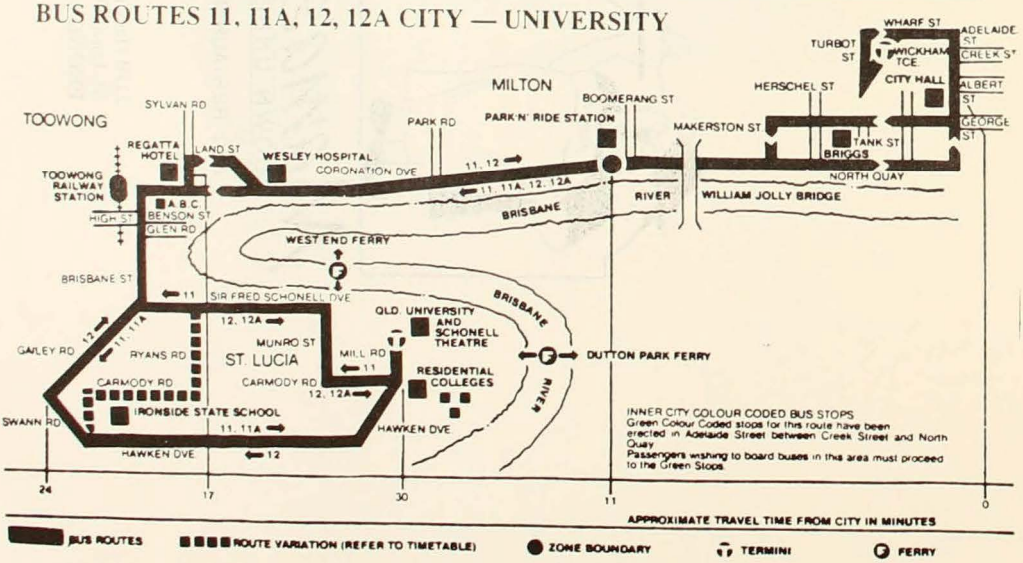

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