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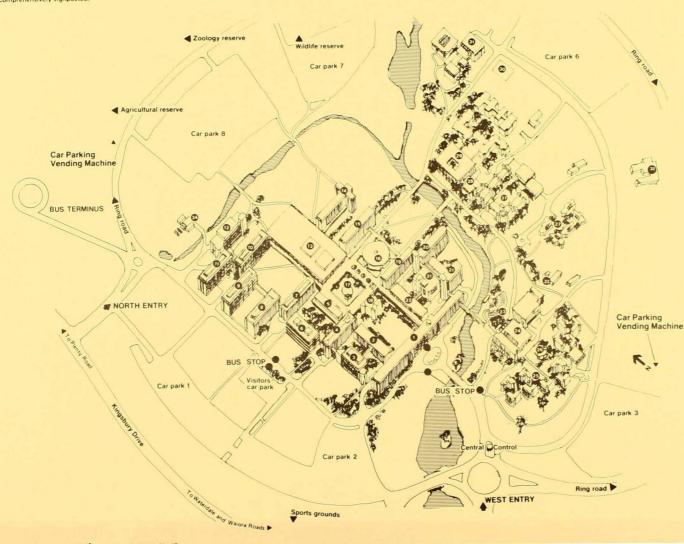
35TH ANNUAL CONFERENCE OF THE GENETICS SOCIETY OF AUSTRALIA

at La Trobe University 15-18 May 1988

PROGRAM & ABSTRACTS

La Trobe University : a campus guide

La Trobe University's campus was laid out in the mid-1960s on a master plan with the concept of a central Library, with adjoining space for social and communal use, closely surrounded by academic buildings with ready access to one another and the Library, separated by a green belt from the colleges. It was decided that the academic and living areas should constitute a walking campus, with vehicle access available only to service traffic. The campus covers some 200 heectares — this map concentrates on the area within the ring road and provides visitors with a ready reference to the buildings. You will find that the campus is comprehensively signosted.





1 David Myers Building

Administration, History Information, Undercroft Theatre, Vice-Chancellor Registrar, Business Manager Art Gallery

2 Peribolos West Computer Centre

3 Physical Sciences 2 Mathematics, Electronic and Communication Science

4 Physical Sciences 1 Physics, Computer Science 5 SRC Office

6 Thomas Cherry Building 1st Year Science Microbiology

7 Physical Sciences 3

8 Physical Sciences 4 Geology Biochemistry

9 Agriculture Biochemistry

10 Biological Sciences 1 Zoology, Genetics and Human Variation, Botany

11 Biological Sciences 2

12 Behavioural Sciences Psychology, Social Work, Human Resource Centre, Psychology Clinic 13 Borchardt Library

14 Social Sciences Legal Studies, Politics, Sociology 15 Donald Whitehead Building

Economics 16 East lecture theatres 1 to 6

17 Agora (University Centre) Banks, Bookshop, post office restaurants, shops

18 Agora Theatre Agora Cinema

19 Humanities 3 Language Centre: English: French Spanish: Italian

20 Humanities 2 Art History Music, Philosophy 21 Education

Hu/Ed theatre, Comparative Centre, Curriculum Centre, Innovation Centre, Media Centre, Teaching Centre, Urban Centre

22 Peribolos East Careers, Dental, Counselling, Health, Chaplains, Archaeology Housing

23 Moat Theatre

24 Union Union Hall, Activities, Bar and Bistro 25 Chisholm College

26 Chisholm College Arts Centre

27 Menzies College Menzies College Theatre 28 Staff Club

29 Gienn College Glenn College Theatre, Archaeology Anthropology Museum

30 Tennis courts 31 Sports and recreation centre

Indoor swimming pool, squash courts, field house, gymnasium, sauna

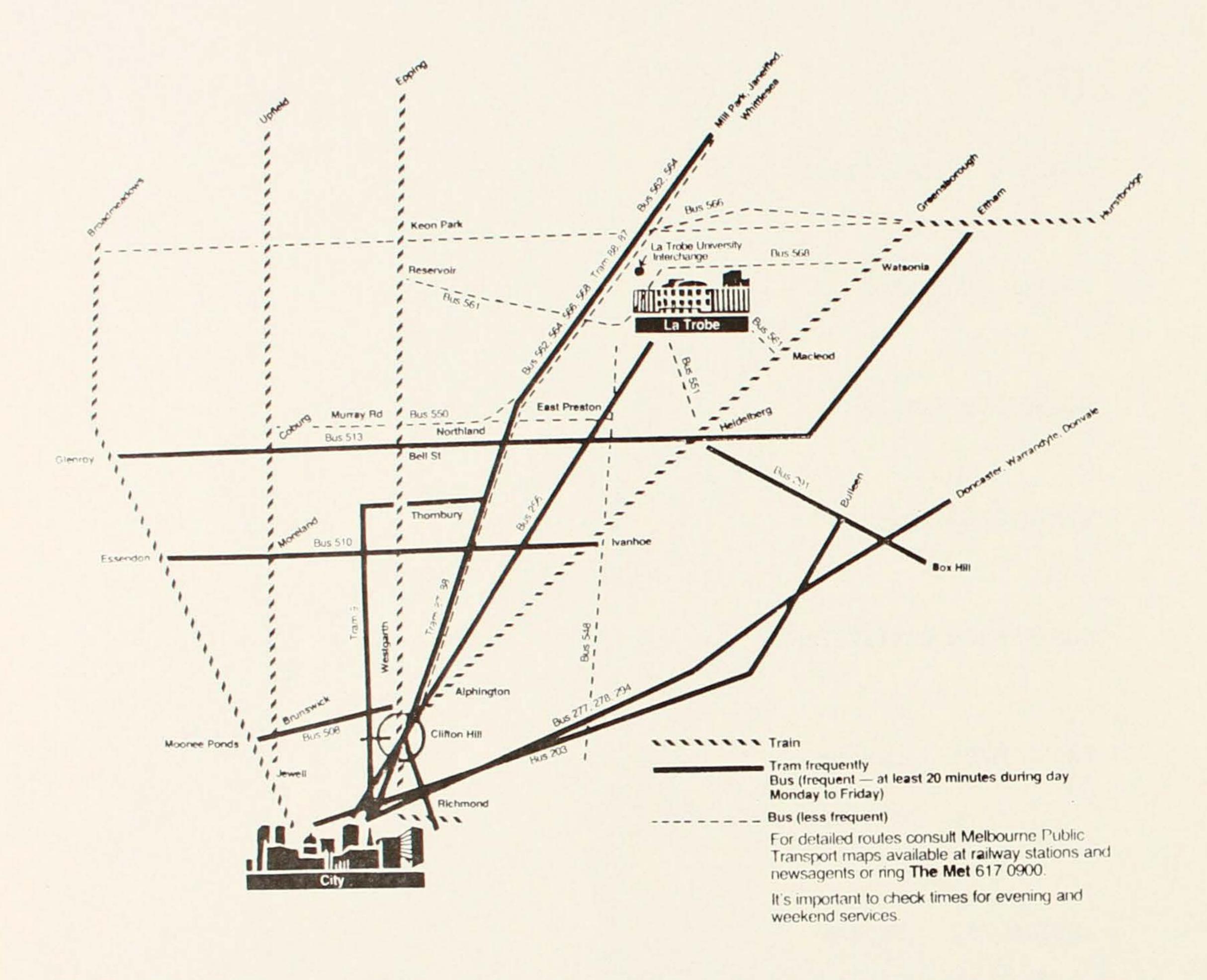
32 Children's Centre 33 Child Minding (Union)

34 Animal and Glass house complex

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How to get to La Trobe by public transport



TRAM ROUTES 87/88 BUS ROUTES 256 BUS ROUTES 259 BUS ROUTE 551 BUS ROUTE 550 BUS ROUTE 561 BUS ROUTE 568 BUS ROUTE 561 BUS ROUTE 562 BUS ROUTE 562 BUS ROUTE 566 City (Bourke Street) to La Trobe University City (Corner Russell/Bourke Streets) to La Trobe University City (Corner Russell/Bourke Streets) to La Trobe University via Freeway (peak hour) Heidelberg Railway Station to La Trobe University Northland Shopping Centre to La Trobe University Macleod Railway Station to La Trobe University Watsonia Railway Station to La Trobe University Reservoir Railway Station to La Trobe University Kew to La Trobe University (leaves from the corner of Cotham/Burke Roads) Humevale (infrequently) Whittlesea, Plenty Road/Wallan Road (via Plenty Road) to Regent Station Mill Park West, C/R Cuthbert Drive/Roycraft Avenue (via Plenty Road) to Greensborough Railway Station



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:

> Yvonne Fripp (Local Secretary) Clinton Hale (Local Treasurer) Jenny Graves Neil Murray

REGISTRATION

Registration is \$30 for non-students and \$20 for students, except for students presenting a paper or poster for whom the registration fee will be waived. Non-financial members of the Society are encouraged to pay their membership fees with their registration. (The standard membership fee is \$21 per annum, with a reduced fee of \$7 for students, overseas members and members not in full-time employment.)

The registration desk will be located in the Airport Lounge, Glenn College throughout the Conference. Registration times are 7.00pm to 10.00pm on Sunday 15th May and 8.15am to 9.00am on Monday 16th May. A member of the Department of Genetics and Human Variation will also be present at the desk during the morning and afternoon tea breaks for those wishing to register after these times.

ACCOMMODATION

Single rooms have been allocated in Glenn 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 only, \$20 per night; Bed and Breakfast, \$28.50). Rooms should be vacated by 10.00am on the day of departure but baggage may be left at the College until final departure.

PARKING

Parking at La Trobe University, except at night or weekends, costs \$1 per day. Parking permits for the duration of the Conference may be purchased at the registration desk, or daily tickets may be obtained from the vending machines located on the University Ring Road (see map on inside front cover of this book). Car Park 6 is the closest to Glenn College.

MEALS

BREAKFAST

For those staying in Glenn College, breakfast is served between 7.30am and 8.30am in the Main Dining Room of the College. Breakfast is \$8.50 and those who registered for bed and breakfast have been issued with breakfast tickets.

The Theatre Restaurant located in the Agora opens at 8.30am serving coffee and snacks.

LUNCH

Lunch is available at a number of venues on campus. <u>Union Bar</u> (licensed): located downstairs in the Union Building; hot meals <u>available</u> in the bar area; food obtained elsewhere may eaten there. <u>Staff Club (licensed)</u>: located next to Glenn College; hot meals and bar <u>snacks; reciprocal rights for members of other University Staff Clubs</u> including the right to purchase liquor to be consumed off the premises; delegates who are not members of a University Staff Club will need to be signed in by a member of the La Trobe University Staff Club. <u>Theatre Restaurant</u>: located in the Agora; hot meals, pies, sandwiches, cakes, hot and cold drinks. <u>Health Food Shop</u>: located in the Agora; soup, hot and cold foods and drinks. <u>Delicatessen</u>: located in the Agora; pizza, sandwiches, hot snacks.

EVENING MEALS

<u>On Campus</u> : Little interest was expressed in the meal offered at Glenn College on Monday evening and it is unlikely that it will be economical for the College to provide this service.

The Theatre Restaurant in the Agora will be open until 7.30pm each evening with a limited range of hot food available.

<u>Off Campus</u> : Trams and buses run from the University to nearby suburbs and also to the city. See the diagram "How to get to La Trobe by public transport' opposite page 1. Tram and bus timetables and maps of Melbourne will be on display near the registration desk.

<u>City</u> (About 1 hour on a tram or bus, 30 minutes by car) There are many good restaurants in the city and two useful publications 'Cheap Eats in Melbourne' and 'The Age Good Food Guide' can be viewed at the registration desk. Trams and buses to the city usually go to Bourke Street. From there it is only a short walk to Melbourne's China Town in Little Bourke Street.

Bundoora Shopping Centre (About 5 minutes on a tram) A hotel bistro and chinese restaurant are located in the shopping centre.

There is a McDonald's restaurant on the corner of Plenty Road and Albert Street, Reservoir, just past the 'Target' supermarket (about 5 minutes by tram).

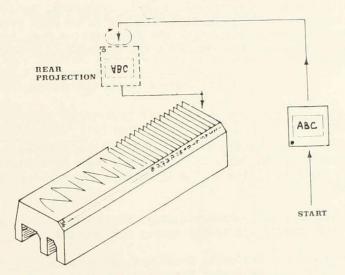
There are also restaurants in local suburbs such as Greensborough, Heidelberg and Ivanhoe.

SCIENTIFIC SESSIONS

The Conference Opening and all sessions of papers will be held in the Eastern Lecture Theatre (ELT) complex, about 2 minutes walk from Glenn College. Tea, coffee, posters and the Trade Display will be in the Airport Lounge, Glenn College.

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 35mm slides (see diagram below for details of loading slides for rear projection). Slides should be taken to the technician's room located between the ELT2 and ELT3 lecture theatres 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 will be on view on both Monday 16th and Tuesday 17th May in the Airport Lounge, Glenn College. Authors are asked to set up their displays by 1.30pm on the Monday at the latest and to be in attendance at the poster sessions on both afternoons. Drawing pins will be available at the registration desk.

STUDENT SUBSIDY

Student members of the Society who present a paper or poster and who are not from Melbourne are eligible for a travel subsidy based on the distance travelled to the meeting. Cheques will be available from Clinton Hale during afternoon tea on the afternoon of Tuesday 17th May. Cheques not collected then will be posted subsequently.

TELEPHONES AND MESSAGES

Public telephones are located in Glenn College and in the Agora. During normal office hours messages for delegates can be received on (03) 479 2265 (General Office, Department of Genetics and Human Variation).

La Trobe	University	Numbers	are:	Telephone	(03)	479	1111
	Let a Statistical			Fax	(03	478	5814

TAXIS

345 3455 (Silver Top Taxi Service) 480 2222 (North Suburban Taxis Ltd)

LA TROBE UNIVERSITY SPORTS AND RECREATION CENTRE

This centre, which has an indoor swimming pool, gymnasium, squash and tennis courts, is close to Glenn College. The Centre is open from 6am to 8pm each day. See the notice-board near the registration desk for full details of opening hours and facilities. A 15% discount is available to Conference delegates.

SHOPPING AND BANKS

Branches of the State Bank of Victoria and Westpac are located in the Agora. Both are open from 9.30am until 3.00pm and both have automatic teller machines. The Post Office in the Agora is an agency for the Commonwealth Bank.

A post-office, pharmacy, bookshop/newsagency, fruit shop, clothes shop, jewellery shop, hairdresser and travel agency are located in the Agora.

Local shopping centres are Bundoora, Northland, Greensborough and Heidelberg. See the Melbourne Street Directory and tram and bus timetables available at the registration desk.

EMERGENCY

Fire Brigade 11441 Ambulance 000 Police 11444

NB: Notification of any emergency can take place by calling extension 2222 on an internal phone. The operator will then inform the service you require. This number (2222) is linked directly to the University switchboard between 8.30am and 5.30pm, and to the Central Control gatehouse at all other times.

Emergency medical assistance can be obtained during normal office hours from the University Health Service (extn 2967 or 2970) located on the ground floor of the Peribolas East Building (Bldg 22 on map inside front cover of this book).

At other times contact:

Austin Hospital <u>450 5111</u> Corner of Burgundy St (Route 40) and Upper Heidelberg Rd (Route 46), Heidelberg. (J4, Map 31, Melway Melbourne Street Directory)

Preston and Northcote Hospital <u>487 2345</u> 205 Bell St., Preston. Near the corner of Bell Street (Route 40) and Plenty Road (Route 27), Preston) (H2, Map 30, Melway Melbourne Street Directory)

Heidelberg Repatriation Hospital 490 2111 Corner of Waterdale Road and Banksia Street, West Heidelberg. (G4, Map 31, Melway Melbourne Street Directory)

SOCIAL FUNCTIONS

MIXER

7.00-10.00pm on Sunday 15th May in the Airport Lounge, Glenn College. Drinks and light snacks will be provided free to registered delegates.

WINE TASTING

5.15pm to 6.45pm on Monday 16th May in The Carvery, Glenn College. Tickets are \$6 and may be purchased at the registration desk on Sunday night and Monday morning. A selection of high quality wines from a number of Victoria's wine growing areas will be available for tasting. The wines are from Gatehouse Cellars, Albert Park and Peter Mitchell from this company will provide information about the wines to be sampled.

A number of Australian cheeses will also be provided for tasting.

ANNUAL SOCIETY DINNER

7.10pm for 7.30 pm on Tuesday 16th May at the La Trobe University Union. Tickets are \$21 and can be obtained at the registration desk on Sunday and Monday. The ticket covers pre-dinner drinks, a three course buffet meal and a band. The Union has a 'Bring Your Own' liquor licence and those going to the dinner will need to purchase their liquid refreshments beforehand and take them to the dinner.

Delegates staying on campus who are members of a University Staff Club may purchase liquor for the dinner at the La Trobe University Staff Club. This club is located next to Glenn College and will be open at lunch-time each day. See the Staff Club wine list on the Conference notice-board near the registration desk. Purchases of wines on the Staff Club list can be arranged for those who are not members. Ask at the registration desk. There are also a number of bottle-shops in nearby suburbs.

BARBEQUE

A barbeque will be held on Wednesday afternoon at the Craiglee Winery, Sunbury, not far from Tullamarine Airport. Tickets are \$10 which includes transport, drinks and food.

Transport to the barbeque will depart from Glenn College at 12.30pm. Those driving there should obtain directions from the registration desk or from Neil Murray. The winery may be found at E10, Map 113 in the 'Melway Greater Melbourne Street Directory' 1988 Edition.

Delegates who require transport to Tullamarine Airport from the barbeque should see Yvonne Fripp and let her know the departure time of the flight.

ACKNOWLEDGEMENTS

On behalf of the Genetics Society of Australia, the organising committee wishes to thank La Trobe University for hosting the Conference. Secretarial and technical staff, in particular Leah Earl, of the Department of Genetics and Human Variation are thanked for their invaluable contributions. Mrs J.A. Hannah is gratefully acknowledged for coordinating the accommodation, morning and afternoon teas and social functions at Glenn College.

The committee thanks Australian Airlines who provided financial and other assistance.

The Victorian Institute of Marine Sciences is thanked for their financial contribution to the Symposium on Genetics of Marine Species.

The committee thanks Mr Peter Mitchell of Gatehouse Cellars for coordinating the Wine Tasting. We are also extremely grateful to Pat Carmody of Craiglee Winery for inviting us to hold our barbeque there.

We also thank the following Sustaining Members for supporting the Conference with their trade displays:

> Cambridge University Press Pharmacia (Aust.) Pty Ltd. Trace Scientific Pty. Ltd. Wild Leitz (Aust.) Pty. Ltd.

SUSTAINING MEMBERS FOR 1988

A.E. Stansen & Co. Pty. Ltd., P.O. Box 118, MT. WAVERLY, VIC. 3149

Annual Reviews Inc., 4139 El Camino Way, PALO ALTO, CALIFORNIA, U.S.A. 94306

Blackwell Scientific Publications (Australia) Pty.. Ltd., 107 Barry Street, CARLTON, VIC. 3053

Boehringer Mannheim Australia Pty. Ltd., P.O. Box 316, NORTH RYDE, N.S.W. 2113

Cambridge University Press, P.O. Box 85, OAKLEIGH, VIC. 3166

Carl Zeiss Pty. Ltd., P.O. Box 147, CAMPERDOWN, N.S.W. 2050

D.A. Book (Aust.) Pty. Ltd., P.O. Box 163, MITCHAM, VIC. 3132

F.S.E. Pty. Limited, Locked Bag 9, STRATHFIELD, N.S.W. 2135

Gelman Sciences Pty. Ltd., P.O. Box 456 LANE COVE, N.S.W. 2066 Genesearch, Technology Drive, Gold Coast Technology Park, ERNEST, QLD. 4210

John Morris Scientific Pty. Ltd., P.O. Box 447, WILLOUGHBY, N.S.W. 2068

Oxford University Press, G.P.O. Box 2784Y MELBOURNE, VIC. 3001

Oxoid Australia Pty. Ltd., 104 Northern Road, WEST HEIDELBERG, VIC. 3081

Pharmacia (Aust.) Pty. Ltd., P.O. Box 289 CARLTON SOUTH VIC. 3053

Promega Pty. Ltd., P.O. Box 10, ROZELLE, N.S.W. 2039

Trace Scientific Pty. Ltd., P.O. Box 494 BAULKHAM HILLS, N.S.W. 2153

<u>Wild Leitz (Australia) Pty. Ltd.</u>, Private Bag 6, CAMBERWELL, VIC. 3124

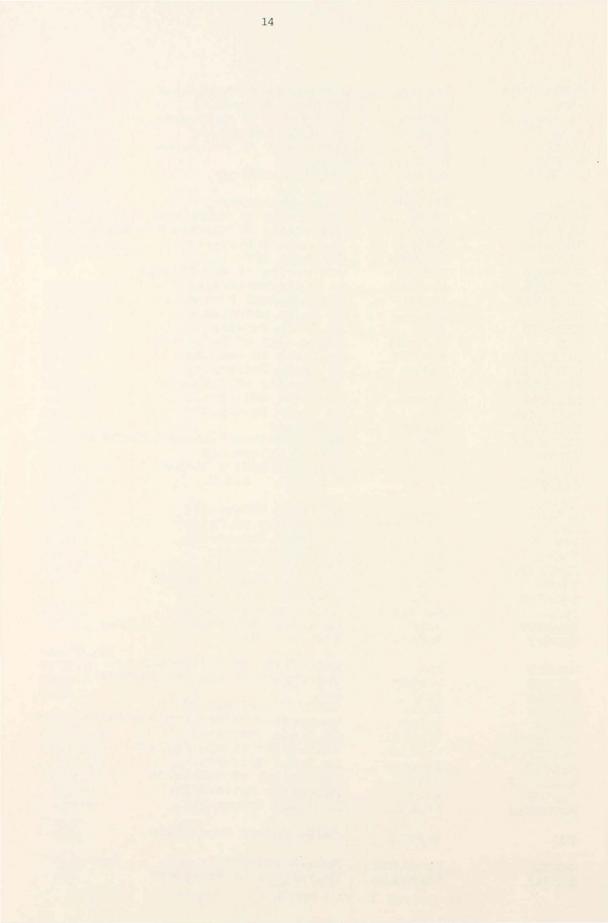
CONFERENCE PARTICIPANTS

ADCOCK	Greg	University of Melbourne
	Alex	Monash University
AGROTIS		
AITKEN	MaryAnne	University of Melbourne
ANDERSON	Marilyn	University of Melbourne
ANDERSON	Peter	CSIRO Plant Industry
	Justen	University of Melbourne
ANDREWS		
ANDRIANOPOULOS	Alex	University of Melbourne
ARMSTRONG	Tim	University of New England
ATLEY	Lynne	La Trobe University
	And the second se	University of Wollongong
AYRE	David	University of worrongoing
BAKER	Louise	University of Melbourne
BAKER	Wendy	R.S.B.S. A.N.U.
	Stuart	University of New England
BARKER		
BATEMAN	Carol	Calgene Pacific
BATTERHAM	Phil	University of Melbourne
BAVERSTOCK	Peter	SA Museum
BEDO	Dan	CSIRO Entomology
BEILHARZ	Rolf	University of Melbourne
BENNETT	Cathy	La Trobe University
	-	La Trobe University
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BLOWS	Mark	La Trobe University
BOCK	Ian	La Trobe University
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	Dick	CSIRO Plant Industry
BROCK		
BROWN	Lindsay	La Trobe University
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BUTLER	Margaret	University of Otago NZ
CARTHEW	Sue	University of Wollongong
CASSIDY	Susi	Flinders Medical Centre
CENTER	Rob	La Trobe University
CERTOMA	Andrea	Royal Children's Hospital
CHAPLIN	Jennie	University of Wollongong
CHEN	Zhenzhong	University of Melbourne
	-	
CHRISTIAN	Pete	CSIRO Entomology
CHRISTIDIS	Les	Museum of Victoria
COATES	David	W.A. Wildlife Research Centre
COGAN	Peter	Melbourne
COLLET	Chris	
		CSIRO Wildlife & Ecology
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COOPER	Steve	University of Adelaide
CORNER	Brian	University of Otago NZ
CORRICK	Cathie	Walter & Eliza Hall Institute
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CROSSLEY	Stella	Monash University
CROZIER	Ross	University of New South Wales
		inter bouch hares
DANTETT	A duri a m	T- T I II I III
DANIELL	Adrian	La Trobe University
DAVIDSON	Julia	La Trobe University
DAVIES	Andrew	University of Melbourne
DAVIS	Meryl	
DAVEY	and the second	University of Melbourne
	Anna	Monash Medical Centre
DAWSON	Snorky	University of New England
DEHAAN	Judy	Monash Medical Centre
DENNINGTON	Simone	University of Melbourne
DICKINSON	Matt	Carbo Bl vi Merbourne
		CSIRO Plant Industry
DIVER	Bill	Peter McCallum Cancer Institute
DIXON	Pat	University of New South Wales
DONNELLAN	Steve	SA Museum
DRIVER	Chris	
DRUITT		Victoria College Rusden
DRUITI	Jenny	University of New England

EAST Peter University of New England EDWARDS David La Trobe University ELIZUR Abigail CSIRO Entomology EWENS Warren Monash University FISCHER Miriam A.N.U. La Trobe university FOLEY Debra FOSTER La Trobe University Jamie FOSTER Melissa Brookfield Zoo FRIPP Yvonne La Trobe University GAME Annie CSIRO Entomology GATFORD Helen University of Melbourne GEORGIOU Jasmine University of Melbourne GILLIES Chris University of Sydney GOLLMANN Gunter University of Melbourne GORDON Caroline La Trobe University GRAHAM Anne University of New England GRAVES Jenny La Trobe University GRIFFITHS Bob Monash University GUTOWSKI Steve Forensic Science Lab., Vic. HALE Clinton La Trobe University HAMMOND Laurie Vict. Inst. Marine Sciences HANNA Peter Deakin University HANNAN Frances University of Melbourne HARGREAVES Jenny A.N.U. Marion CSIRO Entomology HEALY HOFFMANN La Trobe University Ary CSIRO Molecular Biology HOLLIDAY Robin HOLLOWAY Bruce Monash University University of Adelaide HOPE Rory W.A. Wildlife Research Centre HOPPER Steve HOWARD Jane La Trobe University HOWDEN Ross University of Melbourne HOWELLS Tony A.N.U. University of Melbourne HOXLEY Edyta Alison University of Wollongong HUNT University of Melbourne HYNES Michael La Trobe University INCERTI Paula CSIRO Biotechnology JAGADISH M.N. R.S.B.S. A.N.U. JIANG Chenshan University of Western Australia JOHNSON Mike CSIRO Entomology KAROTAM Jill University of Melbourne University of Melbourne Margaret KATZ KELLEHER Richard University of Melbourne KELLY Len La Trobe University KING Leanne Darwin Max KING CSIRO Plant Industry Wayne KNIBB Monash Medical Centre Ismail KOLA

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LEE	Barry	University of Melbourne
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LINSENMEYER	Martha	La Trobe University
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LITTLEJOHN	Tim	University of Melbourne
LOW	Kheng	University of Sydney
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MCKENZIE	John	University of Melbourne
MACLEAN	Helen	University of Melbourne
MACPHEE	Donald	La Trobe University
MADGWICK	Peta	Melbourne
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MARTIN	Paul	CSIRO Animal Health
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MAYO	Jean	University of Adelaide
MAYO	Oliver	Waite Institute
MERAKOVSKY	John	University of Melbourne
MILLER	Katie	A.N.U.
MITCHELL	John	La Trobe University
MORAN	Chris	University of Sydney
MORGAN	Tony	Monash University
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MURRAY	Jim	CSIRO Animal Production
MURRAY	Neil	La Trobe University
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RAMSBOTHAM	Rebecca	University of Melbourne
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	Stephen	
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SHAW	Tim	La Trobe University
SHERWIN	Bill	La Trobe University
SIN	Michael	University of Melbourne
SINCLAIR	Andrew	La Trobe University
SINCLAIR	Sally	La Trobe University
SMOLENSKI	Adam	University of Tasmania
SPENCER	James	La Trobe University
STAUNTON	Tim	La Trobe University
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SYMONDS	Jane	R.S.B.S. A.N.U.
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TAKIS TAN	Helen Mui-keng	La Trobe University University of Sydney
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TAKIS TAN TAYLOR TEARLE TEMPLETON THANOU THOMSON TODD TOMASOV TWOMEY WALKER WALLIS WARREN	Helen Mui-keng Andrea Rick Alan Annie John Maryanne John Andrea Bev Graham Bill	La Trobe University University of Sydney La Trobe University CSIRO Entomology Washington University, St. Louis Flinders Medical Centre University of Sydney La Trobe University La Trobe University Balwyn Vic. University of Wollongong University of Western Australia A.N.U.
TAKIS TAN TAYLOR TEARLE TEMPLETON THANOU THOMSON TODD TOMASOV TWOMEY WALKER WALLIS WARREN WATSON	Helen Mui-keng Andrea Rick Alan Annie John Maryanne John Andrea Bev Graham Bill Jacki	La Trobe University University of Sydney La Trobe University CSIRO Entomology Washington University, St. Louis Flinders Medical Centre University of Sydney La Trobe University La Trobe University Balwyn Vic. University of Wollongong University of Western Australia A.N.U. CSIRO Biotechnology
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TAKIS TAN TAYLOR TEARLE TEMPLETON THANOU THOMSON TODD TOMASOV TWOMEY WALKER WALLIS WARREN WATSON WATTS WESTERMAN	Helen Mui-keng Andrea Rick Alan Annie John Maryanne John Andrea Bev Graham Bill Jacki Robyn Michael	La Trobe University University of Sydney La Trobe University CSIRO Entomology Washington University, St. Louis Flinders Medical Centre University of Sydney La Trobe University La Trobe University Balwyn Vic. University of Wollongong University of Western Australia A.N.U. CSIRO Biotechnology University of Western Australia La Trobe University
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PROGRAMME

Names of presenters are underlined

SUNDAY, 15TH MAY

REGISTRATION AND MIXER 7.00-10.00 pm Airport Lounge, Glenn College

MONDAY, 16TH MAY

REGISTRATION 8.30 am Airport Lounge, Glenn College

OPENING ADDRESS 9.00 am ELT 3 Lecture Theatre

Chairman, Ian Bock

Professor J.F. Scott, Vice-Chancellor, La Trobe University.

PRINCIPAL GUEST SPEAKER 9.10 am ELT 3 Lecture Theatre

Chairman, Ian Bock

Alan Templeton (Washington University, St. Louis) Molecular analyses of genotypic variation and their importance in ecological, quantitative and human genetics

MORNING TEA 10.10-10.40 am Airport Lounge, Glenn College SESSION 1A ELT 2 Symposium on Species and Speciation

Chairman, Murray Littlejohn

- 10.40 <u>Hugh Paterson</u> (Invited Speaker, University of Queensland) The recognition concept: 10 years on
- 11.20 I.R. Bock
 - Interspecific hybridisation in Drosophila
- A. Templeton The meaning of species -- a genetic perspective

 12.00
 S.D. Hopper The recognition concept and plant speciation

SESSION 1B ELT 3 Symposium on Mutagensis in Prokaryotic and Eukaryotic Cells

Chairman, Donald MacPhee

- 10.40 <u>Alexander Morley</u> (Invited Speaker, Flinders Medical Centre) Somatic mutation in human cells
- 11.20 D.M. Podger Mutagenesis and DNA repair - an update 11.40 T. Shaw
- Nucleoside metabolism in eukaryotes 12.00 W.P. Diver
- Methylation-instructed mismatch repair in chinese hamster ovary cells

LUNCH 12.20-1.20 pm

SESSION 2A ELT 2 Symposium on Species and Speciation (continued)

Chairman, Hugh Paterson

1.20 M. Littlejohn

Search for the origins of homogamy

1.40 <u>S. Crossley</u> The genetic basis of behaviour in the Drosophila bipectinata complex

2.00 Discussion

Chairman, Bruce Holloway

- 1.20 A.D.M. Strom and A.F. Morgan
- Gene replacement in Pseudomonas putida PPN 1.40 C. Zhang and B. Holloway

An ordered cosmid library of the catA region in Pseudomonas aeruginosa

- 2.00 S. Rogers, G. Adcock, N.L. Brown, J. Camakaris, B. Lee, D. Rouch and J. Williams Copper homeostasis in E. coli
- 2.20 J. Saleeba, P. Atkinson, C. Cobbett, and M.J. Hynes aciA, a gene of known regulation but undefined function in Aspergillus nidulans
- 2.40 T. Littlejohn and M.J. Hynes Molecular mechanisms of amdS regulation in Aspergillus nidulans

SESSION 2C ELT 4

Chairman, Jenny Graves

- 1.20 A.H. Sinclair and J.M. Graves The sex-determining gene in mammals: radical new clues from marsupials
- 1.40 J. Watson and J.M. Graves Sex chromosome evolution in mammals
- 2.00 M. Schmidt, <u>A. Certoma</u>, D. Du Sart, K. Fowler, M. Leversha, H. Dahl, I. Jack and L. Sheffield An interstitial deletion Xq27 that interferes with the proper inactivation of the X chromosome in man
- 2.20 D.W. Cooper, A.M. Van Daal, A.A. Piper, E.A. Holland, L.M. McKenzie, P.L. Molloy, and J. Fleming Sex-specific methylation differences for two X-linked genes in the tammar wallaby (*Macropus eugenii*)
- 2.40 C. Collet, R. Joseph and K.R. Nicholas Isolation and characterisation of marsupial milk protein genes

AFTERNOON TEA, POSTERS, TRADE DISPLAY 3.00 -4.00 pm Airport Lounge, Glenn College

M.J.D. WHITE PRESIDENTIAL ADDRESS 4.00 - 5.00 pm ELT 3

John Langridge (CSIRO Division of Plant Industry, Canberra) Molecular Processes in Species Differentiation

Chairman, Michael Hynes

WINE TASTING 5.15 - 6.45 pm The Carvery, Glenn College

TUESDAY, 17TH MAY

SESSION 3A ELT 2 Symposium on Genetics of Marine Species

Jointly sponsored with the Victorian Institute of Marine Sciences

In addition to the papers in this session and session 4A, the following posters and paper are presented as contributions to this symposium:

Poster 20. Hybridisation and gene introgression in two species of abalone; Poster 21. Genetic variation within and among populations of reef corals; Poster 22. Mitochondrial DNA polymorphism in the deep sea fish, orange roughy, (Hoplostethus atlanticus);

'The evolutionary genetics of Galaxias truttaceus, G. auratus, and G. tanycephalus' (Session 6A).

Chairman, Neil Murray

- 9.00 <u>Michael S. Johnson</u> (Invited Speaker, University of Western Australia) Larval dispersal and genetic patchiness
- 9.50 P.I. Dixon, R.H. Crozier and M. Black Pattern of genetic variation within and between populations of whiting species
- 10.10 R. Watts The effect of estuaries on the genetic structure of coastal fishes

SESSION 3B ELT 3

Chairman, John Thomson

- 9.00 <u>Marilyn Anderson</u> (Invited Speaker, University of Melbourne) Molecular aspects of self-incompatibility
- 10.00 Roger Parish (Invited Speaker, La Trobe University) Structure and regulation of ribosomal genes in Dictyostelium

MORNING TEA 10.30 - 11.00 am Airport Lounge, Glenn College SESSION 4A ELT 2 Symposium on Genetics of Marine Species (cont.) Chairman, Michael Johnson

- 11.00 L. Woodburn Shellfish genes: flow between populations of Pecten in southern Australia
- 11.20 L. Hammond and J.F. Tomasov Electrophoretic variation between populations of the inarticulate brachiopod Lingula in the Indo-West Pacific region
- 11.40 A. Hunt and D.J. Ayre Geographic variation in the sea anemone, *Oulactis muscosa*
- 12.00 P. Hanna Production of species-specific monoclonal antibodies for use in marine sciences

SESSION 4B ELT 3

Chairman, Dick Brock

- 11.00 N. Brown and A. Morby Genes which move to heavy metal: the molecular genetics of transposable mercuric ion resistance
- 11.20 **P.R.J. Leeton** and D.R. Smythe Variation within a retroviroid element of *Lilium* species
- 11.40 A.L. Lavelle and S.M. Leahy Highly repeated DNA sequences in Lilium
- 12.00 C.B. Gillies Telomeric C band heterozygosity and pairing in rye

SESSION 4C ELT 4

Chairman, Steve McKechnie

- 11.00 J.A. McKenzie and G.M. Clarke
- Lopsided blowflies: an asymmetrical view of fitness 11.20 L. Baker, P. Batterham and J.A. McKenzie
- Developmental asymmetry and the Noich locus of Drosophila melanogaster
- 11.40 <u>H. Saad</u> and J.G. Oakeshott Esterase 6 and reproductive fitness in Drosophila melanogaster
- 12.00 J.Karotam and J.G. Oakeshott Nucleotide variation in 5' regulatory sequences of esterase-6 in sibling *Drosophila* species

LUNCH 12.20 - 1.20 (or 1.30) pm 19

SESSION 5A ELT 2 Symposium on Conservation Genetics

Chairman, Ross Crozier

- 1.20 Warren Ewens (Invited Speaker, Monash University) Minimum viable population sizes
- D. Coates
 Patterns of genetic diversity in populations of two rare and endangered plant species (Acacia anomala & Stylidium coroniforme)

 J.F. Sampson, S.D. Hopper and S.H. James
 Genetic diversity and the conservation of Eucalyptus crucis Maiden.
- 2.30 J.M. Graves, N.D. Murray, <u>W. Sherwin</u> and A. Taylor Conservation genetics of koalas
 2.50 R. Lacy, M.L. Foster and B.A. Brewer Theorem in pairland and insular perulations
 - Inbreeding depression in mainland and insular populations of *Peromyscus* mice
- SESSION 5B ELT 3

Chairman, Len Kelly

- 1.30 A. Elizur, T. Lockett, W. Knibb and R. Saint A homeobox gene is involved in pattern formation in the developing eye of *Drosophila melanogaster*
- 1.50 A.J. Howells, A. Elizur, Y. Haupt and R.G. Tearle A molecular comparison of the *scarlet* eye colour gene and its homologues in three dipteran species
- 2.10 C.N. Chen, M. Eberwine, M.J. Healy and R.L. Davis Molecular analysis of the memory gene, dunce, of Drosophila melanogaster
- 2.30 S.W. Mckechnie, B.W. Geer and J.G. Oakeshott Developmental profile of *Adh* mRNA under ethanol induction in larvae of *Drosophila melanogaster*

SESSION 5C ELT 4

Chairman, John Mitchell

P.J. Martin and J.A. McKenzie				
Inheritance	of anthelmintic resistance in			
 Trichostrongylus	colubriformis			

- 1.50 <u>B. Corner</u> and R. Poulter Genetic mapping and electrophoretic karyotyping in Candida albicans
- 2.10 R. Poulter

Genetic mapping and directed mutagenesis in Candida albicans 2.30 M. Fischer, M.J. Howell and J.J. Hargreaves

Isolation and characterization of Taenia ovis antigens 2.50 M.N. Jagadish, P. Vaughan, A. Azad and I. Macreadie Heterologous expression of viral proteins in yeast

20

AFTERNOON TEA, POSTERS AND TRADE DISPLAY 3.10-4.10 pm Airport Lounge, Glenn College

SESSION 6A ELT 2

Chairman, Michael Westerman

- 4.10 <u>Susan M. Carthew</u> Breeding systems in *Banksia spinulosa*4.30 <u>C. Moritz</u> and W.M. Brown Evolutionary genetics of parthenogenetic lizards
- 4.50 J.R. Ovenden and R.W.G. White The evolutionary genetics of *Galaxias truttaceus*, *G. auratus* and *G. tanycephalus* (Pisces : Galaxidae)

SESSION 6B ELT 3

Chairman, Oliver Mayo

4.10 M. Lafranchi

Isonymy, social class and mating structure in late nineteenth century Tasmania

- 4.30 R.J. Mitchell, M. Kosten and J. Williams
- Genetic structure in the white population of Tasmania 4.50 S. Gutowski and B. Atchison

DNA and forensic science in Victoria.

ANNUAL GENERAL MEETING ELT 3 5.10 pm

ANNUAL SOCIETY DINNER 7.10 FOR 7.30 pm La Trobe University Union

WEDNESDAY, 18TH MAY

SESSION 7A ELT 2

Chairman, John McKenzie

9.00 J.K. Davidson Quantitative genetics of cold tolerance in natural populations of Drosophila melanogaster and D. simulans 9.20 J.S.F. Barker Genetic variation in Drosophila buzzatii for oviposition on natural substrates 9.40 C. Jiang and John Gibson Comparison of allozyme frequencies in Chinese and Australian populations of Drosophila melanogaster 10.00 A. Agrotis and S.W. McKechnie Four base-cutter analysis of allelic variation from Australian and American populations of Drosophila melanogaster J.E. Symonds and J.B. Gibson 10.20 Biochemical characterisation of low activity variants of glycerol-3- phosphate dehydrogenase from natural

populations of Drosophila melanogaster

SESSION 7B ELT 3

Chairman, Ismail Kola

9.00 <u>A. Davey</u>, A. Sinclair, and I. Kola Chromosomal localization of the genes encoding heat shock protein 70 (HSP70) in human, mouse and marsupial

9.20 J. Mann

Genomic imprinting with special reference to the effect of two maternally derived X-chromosomes on parthenogenetic development in the mouse.

10.00 J. Dehaan

Cellular DNA methylation levels in normal and transformed cells after various drug treatments

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SESSION 7C ELT 4

Chairman, Yvonne Fripp

- 9.00 D.M. Rowell The origin of complex sex-linked translocation heterozygosity in Delena canderides (Sparassidae: Arachnida)

 9.20 J.A. Chaplin Environmental stability and the mode of reproduction of the freshwater ostracod, Candocypris novaezealandiae

 9.40 R.G. Beilharz, B.G. Luxford and J.L. Wilkinson The inheritance of reproduction.
- 10.00 S.N. Ethier and <u>R.C. Griffiths</u> The two-locus infinitely-many-alleles model with recombination

MORNING TEA 10.40 - 11.10 am

GUEST SPEAKER ELT 3

11.10 -12.10 pm

Robin Holliday (CSIRO Laboratory for Molecular Biology) DNA methylation and epigenetic mechanisms

Chairman, Des Cooper

BARBEQUE Craiglee Winery, near Tullamarine Airport

- 12.30 Transport departs from Glenn College for Winery
- 2.45 Transport to Tullamarine Airport if required for Canberra delegates to catch 3.40 flight
- 3.45 Transport departs for Tullamarine Airport and La Trobe University

POSTERS

- 1. M.A. Davis and M.J. Hynes Nitrogen control of the amdS gene of Aspergillus nidulans
- M. Katz and M.J. Hynes Two divergently transcribed Aspergillus nidulans genes under coordinate control
- A. Andrianopoulos and M.J. Hynes Physical characterization of the positively acting regulatory gene, amdR, of Aspergillus nidulans
- 4. I.B. Richardson, M.J. Hynes and M.Katz Characterisation of the gatA gene of Aspergillus nidulans
- 5. J. Williams, G. Adcock, N.L. Brown, J. Camakaris, B. Lee, S. Rogers and D. Rouch Plasmid-mediated copper resistance in *E. coli*
- A. Wilton and R. Hope Progress towards cloning an X-linked gene for a human nerve cell antigen
- T. Armstrong and J.S.F Barker Habitat selection: olfactory responses of Drosophila buzzatii to naturally occurring yeast resources
- 8. C.J.I. Driver, S.W. McKechnie, M. Nagy and K. Turney Transposable elements in ageing *Drosophila melanogaster*
- 9. A. Urban and S.W. McKechnie, A. Agrotis and K. Turney Mitochondrial DNA variation in a winery population of *D. melanogaster*
- 10. P. East and G. Whitington Isolation and preliminary characterisation of a duplicated esterase locus in Drosophila buzzatii
- 11. W.D. Warren and A.J. Howells Characterisation of the Cinnabar locus of Drosophila melanogaster
- 12. W.R. Knibb, A. Elizur, N. Brink, R. Tearle and R. Saint. Genetics of the rough eye mutation in Drosophila melanogaster
- 13. K. Miller, M.J. Howell and M. Fischer Characterisation of the genome of the liver fluke
- 14. <u>B.T.O. Lee and J. Martin</u> Cloning and molecular characterization of a dominant-male sex determining gene

- 15. D.L. Hayman, J. Richter and C.R. Leach Somatic crossing over in a family of somaclonally derived plants
- 16. Mui-keng Tan and J. Thomson Evolutionary relationships of bracken fern (<u>Pteridium</u>) taxa based on analysis of the chloroplast genome
- 17. V.H.K. Low and J. Thomson
 - Genetics of cyanogenesis in Australian bracken (Pteridium esculentum) : an update
- 18. A.J. Daniell

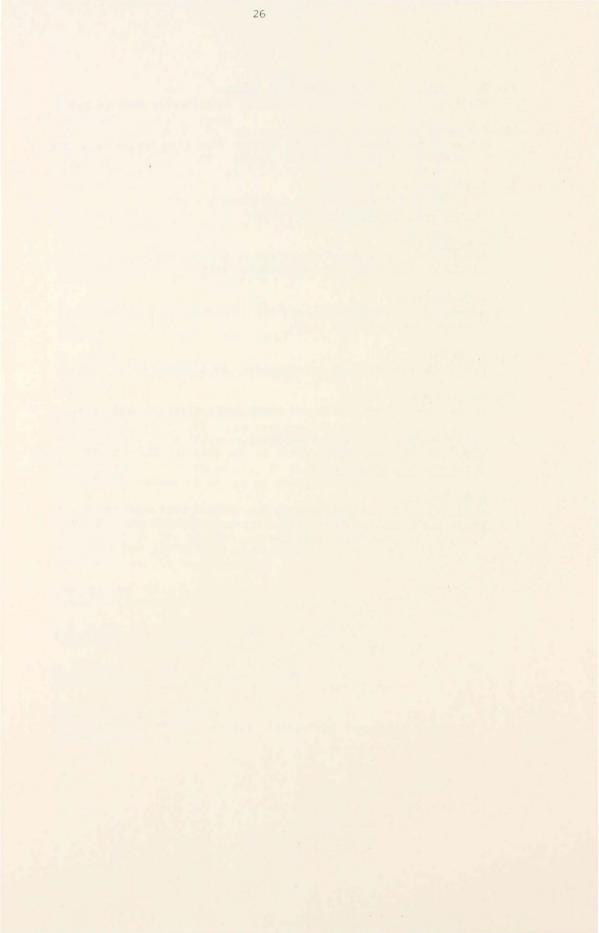
Genetics and population structure in the endemic Australian slug family Cystopeltidae (Mollusca:Pulmonata)

- 19. A.D. Marchant Zonemodel: a computer package for investigating genetic interactions in hybrid zones
- 20. L. Brown

Hybridisation and gene introgression in two species of abalone

- 21. D. Ayre and S. Dufty Genetic variation within and among populations of reef corals
- 22. J.R. Ovenden, A.J. Smolenski and R.W.G. White Mitochondrial DNA polymorphism in the deep sea fish, orange roughy (Hoplostethus atlanticus).
- 23. G.P. Wallis

Mitochondrial DNA variation in the crested newt superspecies (Caudata: Salamandridae): insertion polymorphism and limited cytoplasmic gene flow among species



PRINCIPAL GUEST SPEAKER

MOLECULAR ANALYSES OF GENOTYPIC VARIATION AND THEIR IMPORTANCE IN ECOLOGICAL, GUANTITATIVE AND HUMAN GENETICS

Alan R. Templeton

Department of Biology Washington University St. Louis, Missouri 63130

Traditional quantitative genetic analyses treat the genotype as an ill-defined black box. An alternative approach is possible when the phenotype of interest has a plausible biochemical or physiological basis controlled by known genetic loci. Genetic variation at these loci can be studied using molecular techniques and related directly to the phenotypic variation. This measured genotype approach has several advantages over the traditional biometrical approach.

First, because the genotypes are measured directly, there is no need to perform genealogical analyses. This means that rigorous quantitative genetic studies can be performed on surveys of individuals of unknown relationship. This is particularly important in ecological genetic studies that attempt to understand the genetic basis and ecological significance of phenotypic variation found in natural As an example, molecular genetic variation in ribosomal populations. DNA and its somatic amplification is shown to influence several life history phenotypes in a natural population of Drosophila mercatorum. Ecological studies predict that this life history variation should cause fitness variation as a function of changes in the age structure of the population that are induced by changes in humidity. Because the genotypes and environments are measureable, a predictive ecological genetic study is possible. Hence, one can directly test the role and impact of natural selection in a rigorous manner with the measured genotype approach.

A second advantage of this approach is that it allows a direct study of interaction effects. For example, the evolutionary significance of epistasis is illustrated by powerful interactions between the molecular components of the rDNA system found in *D. mercatorum*. Genotype-by environment interactions are also easily examined. For example, we have shown that various lipid phenotypes in humans (which are predictive of coronary heart disease risk) are influenced by genetic variation at loci coding for apoproteins. Moreover, different measured genotypes are shown to respond differently to cholesterol lowering drugs.

Third, certain classes of phenotypes are now amenable for study that were impossible to even define with the traditional biometrical approach. For example, the cholesterol studies show that different measured genotypes have different levels of phenotypic variance and patterns of correlation between lipid phenotypes. Hence, the genetic basis of homeostasis and pleiotropy can be studied directly.

THE RECOGNITION CONCEPT AFTER 10 YEARS

Hugh Paterson

Department of Entomology, University of Queensland St Lucia, Queensland 4067

Ten years have passed since the publication of the first detailed paper pointing out new, and re-emphasizing some older shortcomings to the Biological Species Concept, and offering an alternative path. The opportunity will be taken to deal with a number of criticisms of the newer concept and defences of the BSC. The present status of the Recognition Concept will be reviewed.

INTERSPECIFIC HYBRIDIZATION IN DROSOPHILA

Ian R. Bock

Department of Genetics & Human Variation La Trobe University Bundoora 3083, Melbourne.

Interspecific hybridization in animal species has been known for many decades. The phenomenon was first summarised in 1922 by J.B.S. Haldane, who noted the prevalence of sex ratio aberrations and unisexual sterility amongst hybrid offspring, and provided the empirical generalization ("Haldane's Law") that the absent, rare or sterile sex is the heterogamic one.

Interspecific hybridizations in Drosophila have been attempted for several decades, and over 260 successful crosses involving almost 200 species have now been reported amongst the few hundred species which have been cultured. The ability to cross in the laboratory with one or more other species appears to be more the rule than the exception; in the virilis and cardini species groups, every species is crossable with at least one other species of its group. Interspecific hybridization has, however, been achieved only between close relatives, that is, between members of the same species group. Haldane's Law is observed in the progeny of the great majority of Drosophila interspecific crosses.

The results obtained for the genus Drosophila suggest that natural interspecific isolating mechanisms may be broken down relatively easily in the laboratory. Substantial evidence from the Lepidoptera also indicates that hybridization between closely related species is easily accomplished, and the same conclusion is suggested by less comprehensive studies on other groups. The question of to what extent the phenomenon is a general one for the animal kingdom is otherwise rendered unanswerable by the impossibility of breeding most species (even of Drosophila) artificially.

THE MEANING OF SPECIES -- A GENETIC PERSPECTIVE

Alan R. Templeton

Department of Biology Washington University St. Louis, Missouri 63130

The "biological species concept" defines species as reproductive communities that are separated from other such communities by intrinsic isolating barriers. However, there are other "biological" concepts of species, so the classical biological species concept is more accurately described as the "isolation" species concept. The purpose of this paper is to give a biological definition of species that follows directly from the evolutionary mechanisms responsible for speciation and their genetic consequences.

The strengths and weaknesses of the evolutionary, isolation and recognition concepts are reviewed and all three are judged to be inadequate for this purpose. As an alternative, I propose the cohesion concept which defines a species as the most inclusive group of organisms having the potential for genetic and/or demographic exchangeability through genetically based cohesion mechanisms. This concept borrows from all three biological species concepts. Unlike the isolation and recognition concepts, it is applicable to the entire continuum of reproductive systems observed in the organic world. Unlike the evolutionary concept, it identifies specific mechanisms that drive the evolutionary process of speciation. The cohesion concept both facilitates the study of speciation as an evolutionary process and is compatible with the genetic consequences of that process.

THE RECOGNITION CONCEPT AND PLANT SPECIATION

Stephen D Hopper

Western Australian Wildlife Research Centre, Department of Conservation and Land Management, PO Box 51, Wanneroo, 6065

Paterson (1985) defines a species as "that most inclusive population of individual biparental organisms which share a common fertilization system". This Recognition Concept of species has been developed to rectify logical and heuristic difficulties of the Biological Species or Isolation Concept, largely in the context of the zoological literature. Paterson argues persuasively that a focus on who mates with who is needed to advance understanding of speciation, rather than the past preoccupation with so-called isolating "mechanisms". Further, he proposes that divergence in the premating or fertilization system delineates speciation in a "comprehensively adequate way". Divergence in postmating systems is irrelevant. Paterson considers the Recognition Concept to be applicable to most eukaryotes, and thus challenges the current consensus of many authors that several species concepts and modes of speciation need to be recognized to encompass all entities and processes in plants and animals.

Botanists in the main advocate that a pragmatic Taxonomic Species Concept is the only universally applicable one, and that the Taxonomic Concept needs to be divorced explicitly from concepts that embody explanations of evolutionary processes. The Recognition Concept may well coincide with and therefore replace the Taxonomic Concept in many plant groups, although quantitative questions concerning biparentality and the level of divergence of fertilization systems required for speciation leave room for debate. For example, if strict biparental sexuality is a requirement of the Recognition Concept, species could not be recognized in most eucalypts, since they appear to have mixed mating systems that involve predominant outcrossing with some selfing. Similarly, if natural hybridization is taken to be indicative of conspecifcity under the Recognition Concept, extensive lumping of well-accepted species in outbreeding plant genera would be required (e.g. almost all species of the kangaroo paw genus Anigozanthos).

Modes of hybrid speciation, especially allopolyploidy, may well be accomodated by the Recognition Concept, but they challenge Paterson's thesis that postmating events have no relevance to speciation. The need for binomials for asexual organisms is an issue that the Recognition Concept explicitly does not embrace, but nevertheless exists. These concerns aside, the Recognition Concept provides a fresh perspective on the species problem, and warrants critical evaluation across the broad spectrum of plant groups studied by evolutionary systematists.

SESSION 1B

SOMATIC MUTATION IN HUMAN CELLS

Alexander A. Morley

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Methods have been developed for studying mutation in human somatic cells using the lymphocyte as the cell model and detecting and expanding mutant cells by the use of limiting dilution cloning. Two gene loci have been used for selection - the HPRT locus on the X chromosme and the HLA-A locus on chromosome 6. In young adults the mutation frequency at the HPRT locus is 1×10^{-6} to 2×10^{-5} and at the HLA locus it is $1 \text{ to } 5 \times 10^{-5}$; at both loci mutation frequency increases with age. Spontaneous and induced mutagenesis can be studied at both loci both in vivo and in vitro. Molecular analysis of in vivo mutations indicates that approximately one-third of HPRT mutations are associated with gene deletion. Although gene deletion also frequently occurs with HLA-A mutations, it is nearly always associated with homozygosity of the non-selected HLA-A allele, which appears to be due to mitotic recombination, including gene conversion, or whole chromosome loss with reduplication.

SESSION 1B

MUTAGENESIS AND DNA REPAIR - AN UPDATE

Denis M. Podger

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DNA repair processes can be divided into three broad categories:

a. Removal Repair,

b. Recombinational Repair, and

c. Replicative Repair.

Recent interest in research on damage removal has focussed on the biochemistry and physiology of the proteins which are responsible for methyladenine-directed mismatch repair, and a role in repair has been identified for cytosine methylation. The functions of the various genes and their products that form the adaptive response repair network have been further characterized. In the area of recombinational repair, genetic studies have identified a large number of genes whose products are induced in response to DNA damage, and contribute to what is commonly called the RecF pathway. Experiments on replicative repair have concentrated on the regulation of the SOS response and on the role of repair proteins in the misincorporation and bypass steps of UV-mutagenesis. Examples of recent work in each category will be discussed, and important areas of DNA repair and mutagenesis which have received little attention will be addressed.

Session 1B

NUCLEOSIDE METABOLISM in EUKARYOTES

Tim Shaw

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Adequate, balanced pools of (deoxy)ribonucleotides are required for accurate nucleic acid synthesis in all types of cells. Starvation or imbalance can cause all known types of genetic damage, but, more significantly, can also initiate normal processes such as differentiation.

Nucleic acid precursors may be produced by *de novo* synthesis or acquired by salvage of preformed nucleosides or nucleobases. The metabolic pathways for nucleoside metabolism are complex and stringently regulated. The relative contribution which each pathway ultimately makes to intracellular nucleic acid precursor pools varies with organism, tissue and cell type as well as with cell cycle status and degree of differentiation.

A multitude of factors controls the relative contribution of each pathway. Some of these will be reviewed in the eukaryote context. METHYLATION-INSTRUCTED MISMATCH REPAIR IN CHINESE HAMSTER OVARY CELLS

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We isolated derivatives of a CHO cell line with lower levels of DNA 5-methylcytosine. We reasoned that, if hemimethylated sites acted as signals for mismatch repair (as is the case in *Escherichia coli*), then permanent removal of these signals would lead to an elevated spontaneous mutation rate and increased sensitivity to DNA base analogs which provoke base mismatches. This proved to be the case.

SESSION 2A

SEARCH FOR THE ORIGINS OF HOMOGAMY

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The reinforcement model of speciation, wherein homogamic mechanisms (resulting in mate choice) arise, or homogamy is perfected, as a direct consequence of natural selection operating on interactions between individuals of two previously separated daughter-populations currently is very much out of fashion. This unpopularity stems from several serious theoretical predictions and a paucity of robust examples. Accordingly, the origins of homogamy must be sought in an earlier (i.e. non-interactive) stage of divergence. Thus the precise study of geographic variation in potential homogamic systems is now most appropriate.

The communication system used in mate-choice (i.e. the distinctive signal produced by an breeding individual of one sex, and the response of a reproductively mature conspecific individual of the opposite sex) is a very suitable system for such a study. The four obvious ways in which geographic variation in a reproductive communication system could arise are as follows.

- A. INCIDENTAL
 - 1. Random drift
 - 2. Pleiotropy
- B. PRODUCTS OF DIRECT SELECTION
 - 1. Sexual selection
 - Differential effects of natural selection on communication systems in diverse reproductive environments involving:

 (a) physical, and
 (b) biological
 (i.e. other signallers). factors.

Acoustic communication is the principal means of attraction and choice of mates by frogs, and is mediated through the distinctive advertisement call of a male and the associated positive phonotactic response of a female. Examples from studies of geographical variation in the acoustic signalling systems of south-eastern Australian frogs (e.g. the <u>Geocrinia laevis</u> complex and the <u>Litoria ewingi</u> complex) will be discussed in the light of the indirect origins of homogamy in continuous and disjunct distributions.

SESSION 2A

THE GENETIC BASIS OF BEHAVIOUR IN THE Drosophila bipectinata COMPLEX

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The genetic basis of differences in behaviour between the four species of the *D*. *bipectinata* complex was studied by analysing the behaviour of reciprocal F_1 hybrids and backcross hybrids. The inheritance of species specific male tarsal sex-comb patterns was also investigated.

The four species (D. bipectinata, D. malerkotliana, D. parabipectinata, and D. pseudoananassae) differed mainly in their male courtship songs. Each song was characterized by the number of pulses in a burst (p/b), the time between pulses (inter-pulse interval ipi), and inter-burst interval (ibi). Pairs of species differed in one, two, or all three of these song parameters.

The pattern of inheritance of song types and of tarsal sex-comb differences suggested a polygenic model with genes located on the autosomes. Some dominance of *D*. malerkotliana genes was indicated in both genetic analyses : song and sex-comb pattern.

Finally, the function of song types was investigated by observing the behaviour of courting pairs in the presence of simulated songs. The results supported the view that male species specific songs function as ethological isolating mechanisms within the *D*. bipectinata complex.

GENE REPLACEMENT IN PSEUDOMONAS PUTIDA PPN.

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A general technique for site-directed mutagenesis of <u>Pseudomonas</u> <u>putida</u> chromosomal genes has been developed. This involves (i) the identification of cloned PPN markers by complementation, (ii) Tn5 mutagenesis of these markers in <u>Escherichia coli</u> and (iii) the conjugal transfer to PPN resulting in gene replacement. Therefore, wild-type sequences can be replaced by the Tn5 mutated homologous sequence. In particular, this paper will present the generation of a <u>recA</u>-like mutant of PPN using this method. The genetic consequences of this work and future research will be discussed.

The availability of a Tn5 mutagenesis system of selected PPN chromosomal genes has an additional advantage. It has been previously demonstrated that PPN Hfr donor strains can be generated which mobilize chromosomal DNA in either direction from the site of a Tn5 insertion. This has allowed rapid and accurate mapping of Tn5-induced chromosomal mutations. The PPN <u>recA</u>-like mutant generated by gene replacement, will also be used to illustrate that the proposed Tn5-homology mediated Hfr formation system is dependent on a Rec+ background.

AN ORDERED COSMID LIBRARY OF THE CatA REGION IN PSEUDOMONAS AEURIGINOSA

Chunfang Zhang and Bruce Holloway

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pMO1811, the R prime plasmid employed to develop a region selective transposon mutagenesis system to study the catA region of P. aeruginosa PAO, was used as the DNA source for a cosmid bank. Fourteen complementation patterns for the available mutants were identified from 500 cosmid clones. The genes identified in the region of the chromosome covered by pMO1811 were organized in two clusters. Each cluster occupied a fragment less than 25 kb long. Between the two clusters, there was a region of at least 25 kb in which no markers were detected. Cosmid clones covering this region were identified by three chromosome walking steps, enabling an ordered array of cosmids to be identified. A restriction map using EcoRI, HindIII and KpnI was constructed for the whole chromosomal region covered by pMO1811 using 22 overlapping cosmid clones identified by both complementation and chromosome walking and the region shown to be 125 kb long. By Southern hybridization, it was confirmed that a copy of IS21 flanked each end of the chromosomal insert in pMO1811, the copies being on the same orientation. Attempts were made to identify ancestral insertion sequences flanking the ben ant cat gene cluster. While no homology was detected between the two flanking areas of this gene cluster under the Southern hybridization conditions used, homology was demonstrated between the ben area of P. aeruginosa and P. putida, implying the evolutionary relationship for this region of these two pseudomonads.

COPPER HOMEOSTASIS IN E. COLI.

S. Rogers, G. Adcock, N.L. Brown, J. Camakaris, B. Lee, D. Rouch and J. Williams

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Copper is an essential metal, yet is toxic at high concentrations. A number of copper-sensitive mutants of <u>E. coli</u> have been isolated and analysed by biochemical and genetic methods (Rouch <u>et al</u>, MS in preparation). These studies have allowed a model for the mechanisms of copper homeostasis in <u>E. coli</u> to be postulated. The results of experiments to clone the chromosomal genes involved in copper metabolism and to determine their functions will be described.

ACIA, A GENE OF KNOWN REGULATION BUT UNDEFINED FUNCTION IN ASPERGILLUS NIDULANS

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<u>aciA</u> is an acetate inducible gene in <u>Aspergillus nidulans</u>. Acetate induction is mediated by the regulatory gene, <u>amdA</u>, which may also regulate <u>amdS</u>. <u>amdS</u> allows <u>A</u>. <u>nidulans</u> to grow on acetamide</u>. 5' sequences of <u>amdS</u> and <u>aciA</u> have been compared allowing an homologous site to be identified. This may be the binding site of the <u>amdA</u> regulatory product.

This model has been tested by use of titration studies. A series of subclones were made to represent 1.5kb around the transcription start point of <u>aci</u>A. Each clone has been transformed into <u>A</u> <u>nidulans</u> in multicopy form. In isolates of sufficiently high copy number, available <u>amd</u>A regulatory product was present in limiting quantities if the subclone included the approapriate binding site. In these strains growth of <u>A</u>. <u>nidulans</u> on acetamide was reduced because little <u>amd</u>A product was available to induce <u>amd</u>S expression. This method allowed the <u>amd</u>A binding site to be located to a region of DNA.

The function of <u>aci</u>A has been investigated by innactivating <u>aci</u>A with inserted <u>lac</u>Z sequences. The phenotype of this loss of function mutant has been studied. This innactivated strain has also allowed the <u>aci</u>A locus to be mapped.

MOLECULAR MECHANISMS OF amdS REGULATION IN Aspergillus nidulans.

Tim G. Littlejohn and Michael J. Hynes University of Melbourne

The picture of how eukaryotic genes are regulated is slowly coming into focus. As this is happening, however, our understanding of how multiple regulatory circuits control the expression of a single gene is still unclear. Whether these different activators of transcription bind to DNA simultaneously or separately, cooperatively or independently, at overlapping or adjacent sites, interacting with each other once bound or not, and how they then activate transcription are still unclear. amdS is one such multiply regulated gene (5 known control circuits) and has been used to answer some of these begging questions.

Through use of cotransformation and two step gene replacements of in vitro mutagenised controlling regions of amdS-lacZ fusion genes, how and where two of the genes regulating amdS act has been studied. This analysis has shown that:

(i) The facB gene product binds to, and exerts its major regulatory effect at, a 38 bp fragment found centered around -201 bp from the startpoint of transcription. The mutant facB88 gene product appears to have a different site of regulatory influence.

(ii) The amdR gene product binds to, and exerts its major regulatory effect at, an 18 bp fragment found centered around -182 bp from the startpoint of transcription. The mutant amdR^c gene product acts at the same site.

(iii) A general promoter element that affects the level of amdS expression is found at a site centered around -177 bp from the startpoint of transcription. The similarity between this element and other eukaryotic promoter elements will be discussed.

THE SEX DETERMINING GENE IN MAMMALS: RADICAL NEW CLUES FROM MARSUPIALS

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We have used in situ hybridization to study genes associated with sex chromosomes of marsupials. Initially we began a study of genes found on the short arm of the human X chromosome including DMD, OTC and SYNI, spanning the region Xp21-11. In the Tammar wallaby, <u>M. eugenii</u> OTC and SYNI are syntenic on chromosome 1 while DMD is asyntenic on chromosome 5 (Sinclair <u>et al.</u>, (1987) <u>Genet. Res. 50</u>: 131-136 and unpublished data).

Recently, the putative testis-determining factor gene (TDF) has been cloned (Page et al., (1987) <u>Cell 51</u>: 1091-1104; Goodfellow pers. comm) and has been shown to be Y chromosome specific in a wide variety of mammals. Furthermore the gene detects homologous sequences on the short arm of the human X chromosome at Xp21.

As this region of the X chromosome is apparently missing in marsupials, we decided to investigate the location of the putative TDF gene. The TDF clone mapped to two sites in the Tammar, on the short arm of chromosomes 1 and 5. This finding, though unexpected, locates the TDF gene exactly to the sites of OTC, SYNI and DMD in this marsupial. This suggests that in the proposed mammalian ancestor a large portion of the X chromosome, including these four genes, was translocated to marsupial autosomes while remaining X-linked in eutherians.

The classical explanation for all male sexual dimorphisms is that they result from the presence of a single TDF gene on the Y chromosome, which transforms the undifferentiated gonad into a testis. With the formation of the testis the whole hormonally directed cascade of events unfurls leading to maleness. In the absence of testis the female form develops. However, it has recently been reported that sexual differentiation of the external genitalia precede gonadal differentiation in the Tammar and as such cannot be controlled by hormones. This suggests that genes other than TDF are responsible for these early sexual dimorphisms in marsupials and eutherians (0 et al., (1988) <u>Nature</u> 331: 716-717).

In the light of these observations our localization of the putative TDF gene to two autosomal sites suggests we may have found the gene(s) responsible for the sexual dimorphisms which precede gonadal development in males and females.

It also poses the questions as to what remains on the punctiform marsupial Y chromosome. Presumably the "real" TDF gene still resides on the Y chromosome and the minute size of this chromosome in marsupials should make it easier to locate.

These results from marsupials offer new clues to sex determination in mammals and suggest a re-evaluation of the single TDF trigger hypothesis.

SEX CHROMOSOME EVOLUTION IN MAMMALS

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The finding that the sex chromosomes of the monotremes are largely undifferentiated and that X-chromosome inactivation involves only the differential region of the monotreme X has led us to suggest a model for sex chromosome differentiation and X-chromosome inactivation in mammals. The model involves a gradual reduction of the Y accompanied by a spread of X-inactivation to newly unpaired loci on the X. The monotremes represent an intermediate stage in this process with the X and Y chromosomes being largely homologous. In the Metatheria (marsupials), the Y is much reduced and the pairing region has been lost from the sex chromosomes, while in the Eutheria the pairing region is retained and the activity of loci such as Sts suggest that genes are still being recruited into the Xchromosome inactivation system.

CHARACTERISATION OF THE Xq27 REGION OF THE HUMAN GENOME

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A de novo deletion Xq27, spanning approximately 600-1000kb has been found in a mentally retarded female. The deleted chromosome is preferentially early replicating in 3 different cell types analysed: T cells (75%), B cells (98%), and skin fibroblasts (100%). The other, structurally normal, late replicating X chromosome contains an abnormally early replicating region within the area corresponding to the deletion, in B cells and in fibroblasts. Southern analysis of the lymphoblasts of the patient revealed that the loci: HPRT, DXS 144, F9, DXS 105, F8, DXS 52, DXS 15, DXS 152, DXS 157, G6PD were all present in a double copy, while only DXS 98 was deleted. These results indicate that apart from sequences involved in X chromosome inactivation, the deletion most likely covers the fragile site-mental retardation syndrome, mapping between the F9 and F8 genes. This region of the human genome is of considerable interest and it has been studied extensively for the content of transcribed sequences, with no effects so far. Screening of the available genomic probes for the location within the deletion may greatly facilitate these efforts. Alternatively a specific library from the region may now be constructed.

SEX SPECIFIC METHYLATION DIFFERENCES FOR TWO X-LINKED GENES IN THE TAMMAR WALLABY (MACROPUS EUGENII)

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In marsupials, females are XX and males XY in sex chromosome constitution . Their X chromosome is partially homologous with the X of eutherian mammals. In both groups, only one of the two X chromosomes in the sama of females is active, and so both males and females have the same level of activity for X linked cenes. This method of dosage compensation is called X-inactivation, which may be of two kinds paternal or random. Inactivation of the paternally derived X (XP) occurs in marsupials and in the extraembryonic membranes of eutherians. Random X-inactivation occurs in the eutherian organism proper, in which either the X^{P} or the maternally derived X (X^{M}) is inactivated on a random basis such that half of the somatic cells of a female have an active X^P , the other an active X^M . Much effort has been directed towards discovering the molecular basis for the establishment and maintenance of this form of genetic inactivity. Here we report that HpaII, a methylation sensitive restriction enzyme, generates female specific fragments from the HPRT and PGK gene sequences in a number of tissues of the tammar wallaby (Macropus eugenii). These results, obtained using kangaroo genomic probes, suggest that there are methylation differences between the X^M and X^P in macropodid marsupials (kangaroos and wallabies), and hence that differential cytosine methylation might characterise active and inactive genes in their paternal X-inactivation system. Sperm do not possess the female specific fragment recognised by the PGK probe, which suggests that the methylation differences between chromosomes are established after fertilisation. The relevance of these findings to models of X-inactivation will be briefly discussed.

ISOLATION AND CHARACTERISATION OF MARSUPIAL MILK PROTEIN GENES

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In contrast to eutherians, the marsupial neonate is very immature and development continues within the pcuch. Milk composition changes to meet the changing growth and energy requirements of the pouch young during the different phases of development and correlates with changing nutritional requirements. The two kinds of milk differ in all their main components of lipid, carbohydrate, and protein as well as electrolyte concentrations. This contrasts with eutherians, where milk composition remains constant throughout lactation.

Lactogenesis and the re-direction of milk protein synthesis mid-lactation are significant as they involve the redifferentiation of the gland and are accompanied by dramatic changes in gene expression. Both the intiation of milk protein synthesis at parturition and the redirection of milk protein synthesis mid-lactation are under the control of a single hormone, prolactin. Lactogenesis in eutherians, in contradistinction to marsupials, is a complex two component process involving an intricate combination of steroid and peptide hormones.

As an essential step towards an understanding of the mechanisms of prolactin control in the regulation of cellular differentiation and the modulation of gene expression in the marsupial mammary gland we are cloning various milk protein genes which are expressed at different phases of lactation.

The caseins represent 50% of the total milk proteins during early and late stages of lactation of the tammar wallaby and can be used as markers to examine the control of lactogenesis. A single protein, late lactation protein (LLP), accounts for most the increased protein content during the latter phase of lactation. LLP appears to be a novel protein and serves as a marker to monitor the transition from early to late lactation.

The cloning of marsupial milk protein genes and the progress towards the characterisation of putative LLP and casein cDNA clones will be presented.

M.J.D. WHITE PRESIDENTIAL ADDRESS

MOLECULAR PROCESSES IN SPECIES DIFFERENTIATION

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Most species differ from each other in morphology brought about by changes in development. But an alteration in a developmental process itself may have multiple effects which are so drastic in total that the organism is severely handicapped or fails to survive. However, changes in the timing, placement or intensity of developmental gene expression are more frequent, better tolerated and meet most adaptive purposes at the species level. These regulatory genetic changes should not affect manifoldly acting elements of the gene switching system as a hormone, receptor or other transacting factor, but rather the transcriptional signals flanking individual genes. These small DNA sequences are arranged on a modular basis so that the regulatory and protein-coding regions of genes may evolve independently. It is not yet known with certainty how transcriptional specificity is altered.

SESSION 3A

LARVAL DISPERSAL AND GENETIC PATCHINESS

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A unique aspect of the marine environment is its habitable continuity over vast distances. Through planktonic dispersal, the potential for genetic connectedness among marine populations is far greater than that in terrestrial or lacustrine systems. Since many marine species combine a sessile or sedentary adult phase with a planktonic dispersal phase, the genetic composition of local populations depends on both local, post-settlement events and those leading up to settlement, which may be affected by what happens far from the local population of adults. While the sedentary habit favours local genetic differentiation, planktonic dispersal favours genetic homogeneity over large distances.

These contrasting genetic effects require that genetic differentiation be studied at local and geographic scales. Intensive local studies of limpets and urchins at Rottnest Island, Western Australia, have revealed a consistent pattern of:

 a) Significant genetic heterogeneity among samples of adults from sites separated by short distances;

b) Significant genetic heterogeneity among different cohorts.
 The heterogeneity among sites is the result of different histories of recruitment, so that an understanding of the differences among cohorts is essential.

The temptation is to explain variation among cohorts as a result of variation in the geographic source of the recruits. Extension of sampling over more than 1500 km, however, shows that there is very little geographic variation, and that the differences among sites a few km apart are as great as those over large distances, as expected with large-scale gene flow. Geographic variation is insufficient to explain the differences among cohorts, and consequently the fine-scale genetic patchiness among adults. Similar results have been found in other studies of marine invertebrates, and it is unlikely that planktonic dispersal can cause both large-scale homogeneity and fine-scale heterogeneity.

The implication is that selective mortality, either in the plankton or soon after settlement, is the major determinant of genetic differences among cohorts. Because of the homogenizing effects of planktonic dispersal, the fine-scale heterogeneity must be renewed each generation, implying considerable genetic death.

PATTERNS OF GENETIC VARIATION WITHIN AND BETWEEN WHITING SPECIES

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Whiting are small to medium sized fish which inhabit the shallow coastal waters of the Indian and western Pacific Oceans. This paper arises from studies which originally aimed to investigate the population structure of <u>Sillago bassensis</u> and <u>Sillago robusta</u>.

We studied liver and muscle enzymes from the two sub-species of <u>Sillago</u> <u>bassensis</u> (school whiting) described by McKay (1985), using starch gel electrophoresis. The difference in genetic makeup between them was highly significant (p<0.0001, Gtest), as reflected by the fact that twelve out of the forty- three loci examined were fixed for different alleles in the two sub-species. There was no evidence for introgression between them at the two localities where they were sympatric.

Similar studies were carried out on <u>Sillago robusta</u> in which morphological differences between eastern and western forms were reported by McKay (1985). Again, large differences were found between the two forms; thirteen out of the twenty-seven loci examined were fixed for different alleles in the two forms. These two forms are almost certainly distinct species.

Two sub-species of <u>Sillago maculata</u> (trumpeter or winter whiting) were examined in the same way. Twenty-three enzyme loci, as expressed in liver and muscle samples, were compared. No fixed differences were observed. McKay's (1985) sub-specific status for the two forms of <u>S</u>. maculata is appropriate.

The population structure of the eastern form of <u>S. bassensis</u>, <u>S. bassensis</u> flindersi, was examined in detail. Seven polymorphic loci were used in the comparisons between samples from sites between Yamba (N.S.W.) and Anxious Bay (S.A.). These studies revealed a large amount of population sub-structuring, particularly in northern New South Wales waters. However the genetic relationships between the samples were not as expected on the basis of their geographic location: the samples were related in a haphazard manner. In northern New South Wales, repeat sampling from the same locality did not always yield the same result and the variability between samples at some sites was as great as the variability observed over the whole range of the species.

Models to explain the observed genetic relationships between the samples are discussed.

Reference: McKay, R.J. (1985). A revision of the fishes of the Family Sillaginidae. Mem. Qld Mus. <u>22</u>(1): 1-73.

SESSION 3A

THE EFFECT OF ESTUARIES ON THE GENETIC STRUCTURE OF COASTAL FISHES

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Marine organisms which have pelagic larvae and/or a migratory adult stage have a large potential for dispersal. Dispersal provides the opportunity for gene flow which can have a homogenising effect on a species, reducing genetic differentiation over a large range. Conversely, species which have a limited dispersal capacity will experience restricted gene flow. When gene flow is limited there is a greater potential for selection to act independently in each part of a species range and increase local differentiation.

Species which utilize estuaries may be subjected to reduced gene flow and increased local differentiation as a result of the spatial discontinuity of estuaries and the uniqueness of the estuarine habitat.

Using a multispecies approach and comparing species with differing reproductive styles and dispersal capacities I intend to evaluate the effect of estuaries on gene flow in a variety of coastal fishes. One of these species is <u>Apogon rueppellii</u>, a mouthbrooding cardinalfish which ranges from Cape Leeuwin in the south-west corner of Western Australia into the Northern Territory. This species utilizes estuaries and coastal embayments within its range, and has a small dispersal potential due to a combination of its mouthbrooding habit and lack of adult migration. Samples of this species were collected from rivers, estuaries, coastal embayments and from two offshore islands over a distance of 1500km along the coast of Western Australia.

The effect of distance and estuaries on the genetic variation of allozymes at 7 polymorphic loci was investigated using starch gel electrophoresis. Nei's D was calculated for each pair of populations and gene flow was estimated using F_{st}.

A north-south cline in allelic frequencies was detected at several loci and heterozygosity increased from north to south along this cline. On a large scale there was considerable divergence between populations whereas over short distances there was relatively little divergence. These data suggest that this species fits the isolation by distance model of population structure which allows gradual changes to occur in allele frequencies when there is only local exchange between adjacent subpopulations. When comparing estuarine and marine populations separated by small distances there were significant differences in gene frequencies suggesting that there is less gene flow between estuarine and marine sites than over similar distances within a habitat.

SESSION 3B

MOLECULAR ASPECTS OF SELF-INCOMPATIBILITY

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The interacting partners during fertilization in higher plants are pollen grains and the female pistil. If mating is compatible, pollen produces a tube which grows through the pistil to the embryo sac. In many plant families, inbreeding is prevented by rejection of pollen tubes after they grow some distance down the style. Rejection is controlled by the product of the *S*-gene, which has multiple alleles, S_1 , S_2 , S_3 and S_4 .

We have isolated cDNA clones encoding the putative S_2 -, S_3 - and S_6 - allele products. Overall, the sequences are approximately 57% homologous at the nucleic acid level and 54% homologous at the amino acid level. The sequences are punctuated with four highly variable regions which encode hydrophylic amino acids which are predicted to be on the surface of the protein. Southern analysis of *N*. alata genomic DNA using the S_2 -, S_3 - and S_6 - cDNA probes indicates:

- * the gene is restricted to a single locus;
- * the gene is present in low copy number (probably single copy);
- * characteristic restriction fragment length polymorphisms for the different S-alleles.

An S_2 -specific antibody has been raised using a synthetic peptide that corresponds to one of the variable hydrophilic regions in the *S*-associated molecules. Using electron-immunocytochemical techniques the antibody has been shown to bind specifically to the intercellular fluid of the transmitting tissue of the S_2 -styles, that is, the site of the incompatibility reaction.

SESSION 3B

STRUCTURE AND REGULATION OF RIBOSOMAL GENES IN DICTYOSTELIUM

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In the chromatin of Dictyostelium ribosomal RNA genes the gene and a region extending 2.3 kb upstream from the transcription initiation site are DNase I sensitive and appear to be free of nucleosomes when the genes are being strongly transcribed (1-4). We characterized a structural boundary at -2.3 kb. On the gene distal side of this boundary nucleosomes are positioned whereas on the gene proximal side they are either absent or randomly distributed. Abutting the gene proximal side of the boundary is a chromatin region which appears to be free of nucleosomes regardless of the structure of adjacent chromatin. This region contains a duplication of the putative promoter located 29 bp distant from the nearest of four clustered topoisomerase I recognition sequences. The latter occur on both strands and are occupied by topoisomerase I - like activity in vivo. An additional recognition sequence beyond the structural boundary is not cleaved. The structural boundary may delimit transcriptionally poised chromatin, the putative upstream promoter acting as an RNA polymerase I preloading site with stress release mediated by local topoisomerase I. Five clustered topoisomerase I recognition sequences are clustered near the start site of transcription and are also cleaved in vivo by an enzyme with the properties of topoisomerase I.

- 1. Ness et al. (1983) J. Mol. Biol. 166, 361-381.
- 2. Sogo et al. (1984) J. Mol. Biol. 178, 897-928.
- 3. Parish et al. (1986) Nucleic Acids Res. 14, 2089-2107.
- 4. Ness et al. (1986) J. Mol. Biol. 188, 287-300.

SESSION 4A

SHELLFISH GENES: FLOW BETWEEN POPULATIONS OF PECTEN IN SOUTHERN AUSTRALIA

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Members of the genus Pecten comprise the majority of commercially - exploited scallops in south - eastern Australia. The fishery is characterised by highly variable catches, both temporally and spatially, reflecting fluctuations in the successful recruitment of young scallops into the fishery.

Following confirmation of the existence of a single species of Pecten in south-eastern Australia (where three were previously recognized), the aim of this study has been to determine the relationship between beds. Are beds "self-sustaining", or does extensive larval transport contribute to the establishment and maintenance of stocks over large areas? What is the population structure of the species?

The distributions of rare allozymes and allozyme frequency variances are used to describe patterns of gene flow.

The implications of these patterns for the fishery's management are discussed.

Electrophoretic variation between populations of the inarticulate brachiopod Lingula from the Indo-West Pacific region.

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ABSTRACT

The inarticulate brachiopod Lingula, the classic "living fossil", is widely distributed throughout the Indo-West Pacific. Previous studies demonstrate no morphometric basis for recognizing most of the 14 or so extant species (e.g. Hammond & Kenchington 1978, J. Zool. 184: 53). Allozyme data for widely separated Queensland populations indicate a close genetic relatedness (Hammond & Poiner 1984, Lethaia 17: 139) probably as a result of dispersal mechanisms which effect high gene flow (Hammond 1982, J. Zool. 198: 183). The present study describes allozyme variation within and among populations from Australia, Philippines, New Caledonia and Hawaii, to resolve the taxonomy of extant Lingula and test hypotheses concerning specific or generic longevity through geological time (bradytely). The proportion of polymorphic loci is high (up to 0.56) and consistent with that observed for other brachiopod genera. Average heterozygosity is also high in most of the populations examined. The Hawaiian sample exhibited fixed differences at a number of loci, confirming that it represents a separate species, L. reevil King. The other three populations are likely to belong to L. anatina Lamark. The data do not support either of the conflicting generalizations in the literature that bradytelic species harbour exceptionally high levels or conversely, very low levels of genetic variation.

SESSION 4A

GEOGRAPHIC VARIATION IN THE SEA ANEMONE, OULACTIS MUSCOSA

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Oulactis muscosa is an intertidal sea anemone which is dioecious and sexually mature throughout the year. Biochemical genetic evidence was used to infer the mode of reproduction and to provide data on the genetic structure of populations and the contribution of reproduction to recruitment. This was carried out by sampling six loci on a total geographic scale of 770km to compare the levels and patterns of genetic variation within and amongst populations.

The genetic structure of the populations studied was consistent with recruitment by sexually produced individuals. The observed genotypic frequencies for most loci closely matched those expected under Hardy-Weinberg equilibria, however, consistent deficits of heterozygotes were detected for all loci. Low levels of genetic differentiation were found between populations and F_{s_T} values were similar to those for other species with planktonic dispersal of larvae.

SESSION 4A

PRODUCTION OF SPECIES-SPECIFIC MONOCLONAL ANTIBODIES FOR USE IN MARINE SCIENCES

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Over the last decade there has been a considerable expansion in monoclonal antibody technology. One of the main themes of this expansion has been the development of rapid and highly specific identification tests for viral or cell types in biomedical and veterinary sciences. Underlying the principles of monoclonal antibody production is the potentiality of each mammalian Blymphocyte to make an antibody against a particular site (epitope) on a particular antigen. Typically, the site is only several amino acids or carbohydrate residues in length and provides the basis for isolating species-specific monoclonal antibodies. Alternatively, the total production of antibodies by all B-lymphocytes against antigens results in polyclonal serum, which in many cases, exhibits a high degree of cross-reactivity with different antigens.

A fundamental problem in marine sciences has been the inability to identify the early planktonic stages of a particular species amongst others of closely related species. In particular, the problem exists because of very similar morphology. Lack of material of a given species does not allow for the electrophoretic isoenzyme comparisons of species often carried out in genetic studies of speciation.

At Deakin University, we have a strong research group which have made species-specific monoclonal antibodies against a variety of marine organisms including Crown of Thorns starfish larvae, commercial bivalve larvae, commercial prawn larvae from the Gulf of Carpentaria and <u>Vibrio</u> bacteria which cause serious problems in aquaculture. New research to identify fish eggs using the same procedures is planned. The report will give an outline of the procedures, problems and outcomes of the research to produce species-specific monoclonal antibodies.

Genes which move to heavy metal: the molecular genetics of transposable mercuric ion resistance.

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Resistance to mercuric ions is one of the most widespread determinants of resistance to antimicrobials. Many of the mercury resistance determinants occur on transposable elements. The transposon Tn<u>501</u> provides a model system for the study of the mechanisms of mercuric ion resistance in bacteria and of the mechanism of transposition of Class II transposons.

A molecular genetic approach has allowed a model for mercuric ion resistance in Gram-negative bacteria to be formulated, and the results of recent experiments to test this model will be discussed. The model can be extended to describe the mechanisms of mercury resistance in Gram-positive bacteria and of resistance to organomercurials.

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VARIATION WITHIN A RETROVIROID ELEMENT OF LILIUM SPECIES

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A 9 kbp dispersed sequence has been isolated and characterised from the very large genome of <u>Lilium henryi</u>. This sequence (called "<u>del</u>" from dispersed element in lilies") resembles a retrovirus in having very long (2 kb) terminal repeats and in possessing sequence motifs characteristic of reverse transcriptase. <u>del</u> is present in all of the <u>Lilium</u> species examined, although it varies considerably between species in its abundance: EMBL3 library data from <u>L</u>. <u>henryi</u> indicate a <u>del</u> copy number of 50000; while <u>L</u>. <u>longiflorum</u> possesses some 500000 copies per haploid genome.

<u>del</u> elements from <u>L</u>. <u>henryi</u> and <u>L</u>. <u>longiflorum</u> (with close similarities in restriction sites) have been compared at the DNA sequence level. The degree of sequence conservation between the elements is high, especially in the region of the RNA binding protein motif and the protease motif, as would be expected of functional domains.

Contrasting this, <u>del</u> sequence heterogeneity, based on restriction sites as well as gross arrangement, is higher in <u>L</u>. <u>longiflorum</u> than in <u>L</u>. <u>henryi</u>. This intra-species variation could be related to the copy number, in which case the time and frequency of infection and amplification, the rate of mutational decay and the effects of selection must be considered.

HIGHLY REPEATED DNA SEQUENCES IN LILIUM

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Plants belonging to <u>Lilium</u> species have very large genomes, a substantial proportion of which consists of repeated sequences. The repeated sequences of <u>Lilium henryi</u> have been isolated using <u>Cot</u> analysis, where the reassociation rate of DNA is proportionate to the concentration of each complementary sequence type and therefore provides some measure of sequence frequency. The rapidly reannealing <u>Cot</u>IMs probe was used to identify fragments released from genomic DNA following digestion with the restriction enzyme <u>KpnI</u>. The repeated <u>KpnI</u> sequence does not hybridize to any other known repeated sequence in <u>Lilium</u> such as 5S, 18S, 23S or <u>del</u> sequences.

<u>KpnI</u> sequences have been isolated from an EMBL3 library of genomic L. <u>henryi</u> DNA. Restriction mapping of several phage clones has shown that the <u>KpnI</u> element is at least 8 kb in length. We have estimated the copy number in <u>L. henryi</u> to be in the order of 50 x 10^4 copies per haploid genome.

The repeated sequence has been found in a number of other species including distantly related species such as <u>L</u>. <u>longiflorum</u>. Evidence suggests that the <u>KpnI</u> sequence is dispersed. Coupled with the variation in copy number between species, the data obtained so far is not inconsistent with KpnI sequences being mobile elements.

TELOMERIC C BAND HETEROZYGOSITY AND PAIRING IN RYE

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Surface spreading electron microscopic techniques have been used to study the effects on meiotic chromosome pairing (synaptonemal complex formation) of telomeric C band differences in rye (Secale cereale, 2n=14). The material studied included a hybrid between the cv. E line, which has very little telomeric heterochromatin, and the cv. Dankowskie Zlote, which has prominent C bands on 12 of the 14 telomeres. This represents an approximately 2-fold difference in telomeric C band content, about 2pg of DNA. At pachytene in the hybrid most synaptonemal complexes were found to have unequal lateral elements, with unpaired lateral element protrusions at telomeres. Synaptonemal complex formation was, however, often complete to the end of the shorter lateral element.

This result was confirmed in crosses between the E line and lines with single telomeric C bands on 1RS or 1RL. Internal loops were sometimes seen in the synaptonemal complex of bivalent 1 in these crosses. The results support a pairing model in which synaptonemal complex formation commences distally, but not at telomeric nuclear envelope attachments. The possibility of synaptic adjustment effects will be discussed, and the relationship of these findings to crossing over will be examined.

LOPSIDED BLOWFLIES : AN ASYMMETRICAL VIEW OF FITNESS

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Genetic evidence suggests that the evolution of resistance to the insecticide diazinon by L. cuprina initially produced an increase in fluctuating asymmetry. At that time resistant flies were presumed to be at a selective disadvantage in the absence of diazinon. Subsequent evolution in natural populations selected modifiers to ameliorate these effects. The fitness and fluctuating asymmetry levels of resistant flies are currently similar to those of susceptibles. Previous genetic analyses have shown the fitness modifier to co-segregate with the region of chromosome III marked by the white eyes, w, locus, unlinked to the diazinon resistance locus, Rop-1, on chromosome IV. This study maps the asymmetry modifier to the same region, shows, as in the case of the fitness modifier, its effect to be dominant and presents data consistent with the fitness/asymmetry modifier being the same gene (gene complex). These results suggest changes in fluctuating asymmetry reflect changes in fitness.

DEVELOPMENTAL ASYMMETRY AND THE Notch LOCUS OF D. melanogaster

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It is possible that the fitness/asymmetry modifier (see McKenzie and Clarke abstract) may be an allele at the *Scalloped wings* (*Scl*) locus. *Scl* is considered to be homologous to the *Notch* locus in *Drosophila melanogaster* on the basis of recombinational and phenotypic data. Since D. *melanogaster* has a well defined genetic system, the study of *Notch* (*N*) may more readily yield information regarding *Scl*.

The likelihood of Scl and N being homologous was investigated using Fluctuating Asymmetry (F.A.), since Scl alleles had previously been shown to increase F.A. We report here that many *Notch* alleles also cause a similar effect. This strengthens the possibility of N and Scl being homologous.

Molecular studies reveal that clones spanning the N locus hybridize to *L. cuprina* genomic DNA in Southern analysis. *Scl* mutants have been analyzed using these probes.

ESTERASE 6 AND REPRODUCTIVE FITNESS IN DROSOPHILA MELANOGASTER

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In <u>Drosophila</u> <u>melanogaster</u>, Esterase 6 (EST 6) is produced primarily in the anterior ejaculatory duct of the adult male, from where it is transferred to the female during mating. This suggests a role for the enzyme in reproductive fitness. Here we report two experiments investigating this role, one investigating the effects of variation in the EST 6 activity of males on the latency to mating, the second investigating the effects of this variation on the productivity of these matings. The experiments are based on a set of forty two EST 6 isollelic lines extracted from a single, natural population of <u>D. melanogaster</u>. These lines show inherited three-fold variation in ejaculatory duct EST 6 activity.

In the first experiment, virgin males with greater EST 6 activity were found to take longer to initiate a mating with virgin females from a standard line. This effect varied with the time the males had 'conditioned' the mating chambers before the females were introduced. This suggests that the effect is pheromonally mediated. It is not clear how EST 6 might interact with mating pheromones but we will discuss some possible mechanisms.

The second experiment monitored the time course of fertile and total egg production of females of a standard line mated with males from the different EST 6 lines. No effect of the male donated EST 6 was found on the total number of eggs laid at any time in the females reproductive life. Nor was there any effect of EST 6 on the proportion of eggs that were fertile in the first two to five days after mating, when the proportion was high in all females. However the proportion of fertile eggs declined after this time and the decline was greater for females that had been mated with high EST 6 males. We propose that this reflects a primary effect of EST 6 on the release of the sperm stored after mating in the female's spermathecae. The rate of this release is known to depend on the lipid content of the stored semen and we propose that EST 6 catabolises these lipids to facilitate sperm release. We further propose that in females mated to high EST 6 males the rate of release in the first few days after mating is more rapid than necessary to fertilise all the mature eggs then available, so that fewer sperm are retained to fertilise eggs maturing later.

The results of both experiments thus indicate disadvantages to high EST 6 males in their first mating. These disadvantages will be discussed in relation to the mating and remating behaviours and the high levels of EST 6 activity variation found in natural populations of <u>D. melanogaster</u>.

NUCLEOTIDE VARIATION IN 5' REGULATORY SEQUENCES OF ESTERASE-6 IN SIBLING DROSOPHILA SPECIES

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In Drosophila melanogaster and its sibling species <u>D.simulans</u> and <u>D.mauritiana</u>, Esterase-6 is produced primarily in the ejaculatory duct of the adult male and is transferred to the female during mating. Once in the female, EST6 has been shown to stimulate oviposition and delay her time to remating. In the present study, the nucleotide sequences of the <u>Est-6</u> genes and 5' flanking sequences of <u>D.simulans</u> and <u>D.mauritiana</u> were obtained for comparison with D.melanogaster.

Comparison of the coding regions of the three <u>Est-6</u> genes produced three lines of evidence for selective constraint against amino acid changes in the EST6 protein among these three species in which its physiological role has been conserved.

Firstly, the level of replacement site variation is only one quarter that of silent site variation.

Secondly, most (76%) of the amino acid replacements which have occurred are physicochemically conservative.

Thirdly, physicochemically non-conservative amino acid replacements do not occur in regions of putative structural or functional importance.

1.1kb of Est-6 5' flanking sequences were also compared among the species. The region -1 to -500bp from the start of translation shows a high overall level of conservation. The percentage nucleotide difference for the region is 2.5% as compared to exon replacement sites (2.2%) and exon silent sites (9.4%). Moreover, the region includes two subsequences, each over 100bp long (-1 to -106 and -219 to -334), which are absolutely conserved across the three species. The former includes the non-consensus TATA box (AATAAAA) at -69 to -63bp and seven contiguous nucleotides (-41 to -35bp) shown by S1 nuclease mapping to be transcription initiation sites in D.melanogaster.

For sequences upstream of -500bp the percentage nucleotide difference increases sharply to around 13%, insertion/deletions are larger and more common and there are no long regions (over 50bp) of perfect homology.

Thus it seems likely that sequences required for regulation of $\underline{\text{Est-6}}$ gene expression are concentrated in the region -1 to -500bp 5' of the coding region. It is possible that the two perfectly conserved regions are necessary for the tissue, sex and stage specific expression of EST6 in these three species. Germline transformation experiments using 5' deletions, currently being carried out within the group, may confirm these hypotheses.

SESSION 5A

MINIMUM VIABLE POPULATION SIZES

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Many people who are involved with populations which are subdivided into small sub-populations (for example in national parks or in zoos) are concerned at the possible loss of genetic variation in these subpopulations through the effects of inbreeding. The concept of the "Minimum Viable Population Size", taken to be a population size for which this effect is at an acceptably low level, has been put forward. However, the theory involved in this concept is not well-defined. A discussion of the appropriate theory, together with general comments concerning genetic processes in subdivided populations, will be given.

SESSION 5A

PATTERNS OF GENETIC DIVERSITY IN POPULATIONS OF TWO RARE AND ENDANGERED PLANT SPECIES (ACACIA ANOMALA AND STYLIDIUM CORONIFORME). David J. Coates W.A. Wildlife Research Centre, Department of Conservation and Land Management, PO Box 51 Wanneroo, W.A. 6065

The effect of factors such as population size and population isolation on the level and distribution of genetic diversity within populations is of particular interest because of implications relating to the conservation of genetic resources and the survival of rare and endangered species. Many of these species have suffered rapid reductions in population size and increased population isolation in recent times. The significance of these effects, on both the short term and long term survival of populations has been addressed to some extent by population genetic theory. However, there is a clear lack of empirical data concerning the genetic consequences of rapid decline in population size and increased population isolation in natural populations of most plant and animal species.

The two species investigated in this study (Acacia anomala and Stylidium coroniforme) are extremely rare and threatened due to habitat destruction and disturbance. Acacia anomala occurs in two small disjunct groups of populations (Chittering and Kalamunda) some 30 km apart. The Chittering populations reproduce sexually whereas the Kalamunda populations appear to reproduce solely by vegetative means. The level and distribution of genetic variation was studied at 15 allozyme loci. In the Chittering populations the mean number of alleles per locus was 2.0 and the expected panmictic heterozygosity, H (genetic diversity index) 0.209. This H is high for plants in general even though these populations have recently become reduced in size and more isolated. In the Kalamunda populations the mean number of alleles per locus was 1.2 and H 0.079 although the observed heterozygosity of 0.150 was only marginalfy less than the Chittering populations (0.177). These data, in addition to Nei's diversity measures and Wright's F statistics, indicate that the Chittering populations are primarily outcrossing whereas the Kalamunda populations are clonal with each population consisting of one or more groups of individuals with identical multilocus genotypes.

Stylidium coroniforme is known from only two wild populations which have quite different histories. Population 1 has been through a severe bottleneck and in 1980 consisted of two plants. This number had increased to 83 in 1986. Population 2 has remained virtually undisturbed over the past 10 years and consists of some 400 plants. The level of genetic variation was studied at 15 polymorphic allozyme loci. The mean number of alleles per locus was 3.2 in population 1 and 2.9 in population 2 and H was 0.369 in population 1 and 0.327 in population 2. The observed heterozygosity was 0.289 and 0.286 in populations 1 and 2 respectively. Theoretical expectations that the bottleneck suffered by population 1 would result in reduced H and H are clearly not met.

In both <u>Stylidium coroniforme</u> and the Chittering populations of <u>Acacia</u> <u>anomala</u> there appears good evidence to indicate that their genetic systems are adapted to small population conditions and that genetic diversity levels remain virtually unchanged even under conditions of rapidly reduced population size and increased population isolation.

SESSION 5A

GENETIC DIVERSITY AND THE CONSERVATION OF EUCALYPTUS CRUCIS MAIDEN

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E. crucis Maiden occurs as small, isolated populations confined to granite outcrops in southwestern Australia. The level and distribution of genetic diversity at 11 allozyme loci in 10 populations were estimated. Ten loci were polymorphic. However, many alleles were fixed in populations and occurred at low frequencies in others. The mean expected panmictic heterozygosity for populations was low when compared with tree species in general but similar to other tree species occurring in small, isolated populations. The level of population differentiation was high as expected for small, isolated populations undergoing genetic fixation through genetic drift. The majority of the differentiation was attributable to between-population rather than between-subspecies differentiation. Analyses of allozyme data suggest that the mating system of *E. crucis* may be adapted to maintain diversity within populations by selection favouring heterozygous, presumably outcrossed progeny. The optimal strategy for the conservation of the genetic resources of *E. crucis* and other eucalypts with similar distribution patterns are considered in the light of this and previous studies.

SESSION 5A

CONSERVATION GENETICS OF KOALAS

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Koala populations are subject to large fluctuations on the scales of centuries and of a few years. Because of the perceived general rarity of koalas and periodic availability of excess animals from particular populations, especially French Island, a widespread program of relocations and re-stocking attempts has taken place throughout South-Eastern Australia since the 1920's.

The pattern of re-locations, which has been described in some detail, is expected to result in reduced genetic variation in some populations, especially those which have passed through a number of severe bottlenecks. We aim to determine whether this loss of variation has actually occurred. Some variation has been detected in DNA and proteins.

The number of koalas showing symptoms of Chlamydial disease (which reduces female fertility) is known to vary considerably between populations. This study will be able to detect any association between levels of genetic variation and the severity of the effects of this disease on the population.

Possible genetic implications for future management of koalas will be outlined.

SESSION 5A

INBREEDING DEPRESSION IN MAINLAND AND INSULAR POPULATIONS OF PEROMYSCUS MICE

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A reduction in fecundity and survival of inbred organisms (inbreeding depression) is a very widespread phenomenon in both domesticated and wild populations. If deleterious recessives are the primary cause of inbreeding depression, as is generally believed, then prolonged inbreeding should purge a population of its genetic load. Small, isolated populations with past histories of bottlenecks and consequent inbreeding should be relatively unaffected by future inbreeding. Alternatively, if general heterosis accounts for inbreeding depression, fitness should decline with a loss of heterozygosity, regardless of the past history of a population.

We examined the effects of inbreeding on reproduction of deermice (Peromyscus). Eight populations of two species of Peromyscus, ranging from continental populations high in genetic variability to island populations depauperate in variability, were sampled to establish laboratory breeding stocks. The expected deleterious effects of inbreeding were observed in most populations, but effects were not universal across populations nor across reproductive parameters. For some populations and some aspects of reproduction even extensive inbreeding produced no discernible effects. Island populations, low in variability and with probable past histories of inbreeding, did not tolerate inbreeding better than did more heterogeneous populations. The complex responses to inbreeding do not support any simple theory of inbreeding depression, nor do they allow prediction of the severity of inbreeding depression within a population based on knowledge of the size, isolation, and genetic diversity of the wild population from which it was obtained.

A homeobox gene is involved in pattern formation in the developing eye of Drosophila melanogaster.

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We have previously described the isolation and preliminary characterization of a homeobox gene involved in eye development in Drosophila melanogaster. We have demonstrated that the gene corresponds to the rough gene, by EMS mutagenesis of an isochromosomal line. Specifically, we have generated a rough allele in which a single base change in the homeobox domain of the open reading frame would result in premature termination during translation. The viability of this mutation suggests that the action of this gene is restricted to eye development. This is the first example of a homeobox gene which is not involved in major pattern forming events that occur up to the cellular blastoderm stage of embryogenesis, raising significant questions about the nature and evolution of this class of genes.

In situ hybridization to tissue sections has shown that expression of this gene correlates with the region affected by the mutation. Expression is restricted to the developing eye-antenna disc of the third instar larva, specifically within and posterior to the morphogenetic furrow that traverses the eye-antennal imaginal disc during development. It should be stressed, however, that rough is involved in developmental events, since rough mutants alter the pattern of differented cells in this tissue. Expression of the gene occurs prior to final events of cell proliferation and differentiation in this terminally differentiating tissue, events that are better defined than related events of post-blastoderm embryogenesis. Molecular analysis of this gene may provide clues to the regulation of these events.

A MOLECULAR COMPARISON OF THE <u>SCARLET</u> EYE COLOUR GENE AND ITS HOMOLOGUES IN THREE DIPERAN SPECIES

A.J. Howells, A. Elizur, Y. Haupt and R.G. Tearle. Department of Biochemistry, Faculty of Science, Australian National University and CSIRO Division of Entomology.

We have used sequences from the cloned scarlet gene of Drosophila melanogaster to isolate the homologous scarlet gene from D.buzzatii and topaz gene from Lucilia cuprina. Sequencing of the topaz and scarlet (D.melanogaster) genes is now almost complete. As reported previously, these genes have virtually identical exon-intron structures. The exons are identical in size, are interrupted by introns at the same places and are 80-90% homologous at the predicted amino acid level. At the nucleotide level the homology of the exons is less (about 70%), which reflects the marked differences in codon usages in the two genes. In topaz more codons have A or T in the third position than G or C, whereas in scarlet the converse is found. The introns show virtually no sequence homology (except at the splice junctions) and are generally longer in topaz. In addition, three of the topaz introns contain moderately repeated DNA sequences, whereas no repeated DNA is found in scarlet. As a consequence of the longer introns, the topaz transcription unit appears to be about 13 kb, at least four-times longer than that of scarlet. We are currently sequencing the restriction fragment from topaz which we anticipate contains exon 1; we hope to be able to compare the upstream regulatory regions of the two genes in the near future.

Work on the <u>scarlet</u> gene of *D.buzzatii* is less advanced. Data obtained from cross-species hydridizations indicates that the transcription unit is probably about 3kb in length, similar to that in *D.melanogaster*. Interestingly, we have evidence indicating the presence of repeated sequence DNA within the *D.buzzatii* clones but they appear not to be located within the <u>st</u> transcription unit. The repetitive frequency of these sequences in the genome seems to be of the order of one hundred. Sequencing of restriction fragments from within the <u>scarlet</u> transcription unit commenced recently.

MOLECULAR ANALYSIS OF THE MEMORY GENE, DUNCE OF DROSOPHILA MELANOGASTER

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The dunce gene of Drosophila melanogaster encodes a protein that influences learning and memory processes and plays a role in cyclic nucleotide metabolism. Flies carrying the dunce mutation exhibit defective learning and memory in both associative and non-associative learning paradigms. In addition, the mutant flies show reduced levels of the enzyme cAMP phosphodiesterase and elevated levels of cAMP. Analysis of cDNA clones that define the dunce open reading frame reveal that the deduced amino acid sequence is strikingly homologous to the amino acid sequence of a bovine brain Ca^{2+} calmodulin dependent cyclic nucleotide phosphodiesterase and more weakly related to the predicted amino acid sequence of a yeast cAMP phosphodiesterase. Characterisation of the 5' untranslated region of the dunce transcription unit has identified five structurally distinct classes of transcripts. At least two overlapping transcription units have been defined, one of which extends for 54kb and the other for more than 107kb. The latter transcription unit defines 16 dunce exons, with exons 1 and 2 being separated by an intron of 79kb. At least two other genes reside within this large intron, including the glue protein gene Sgs-4.

DEVELOPMENTAL PROFILE OF Adh mRNA UNDER ETHANOL INDUCTION IN LARVAE OF D. melanogaster

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On a defined food medium, low in sucrose and fats, ADH activity and ADH protein levels in larvae are elevated markedly by the presence of dietary ethanol. During normal larval development two <u>Adh</u> mRNA's are produced. One, from a proximal promotor, increases in concentration to a peak at mid-3rd-instar then declines to very low levels through the pupal stage. The second, from a distal promotor, increases from being absent in mid-3rd-instar to moderate levels, before declining to low levels during the pupal stage. Our data demonstrate that both <u>Adh</u> mRNA's are specifically elevated in larvae by dietary ethanol. The pattern and extent of increase in <u>Adh</u> mRNA's excludes the possibility that the elevated mRNA levels are a simple consequence of the slowing of development which occurs when dietary ethanol is added to the medium.

FINE RESOLUTION MAP OF THE SATELLITE DNAS ON THE SECOND AND Y CHROMOSOMES OF DROSOPHILA MELANOGASTER

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The heterchromatic regions of the second and Y chromosomes of <u>D. melanogaster</u> encompass about 15,000 kb and 40,000 kb of DNA, respectively. Although much of this DNA is comprised of the tandemly repeated, satellite DNAs, numerous genetic functions map within the heterochromatin of these chromosomes. These include 15 vital loci (associated with lethality, in late embryogenesis or in larvae and pupae) in the chromosome 2 heterochromatin, and six fertility factors on the Y chromosome. We are investigating the relationship between these genetic functions and the satellite DNAs present in heterochromatin using a combined genetic and molecular approach.

As a first step, we have constructed a map of nine simple sequence satellites using cloned probes and in situ hybridizations to mitotic chromosomes. The linear arrangement of different satellites was determined by a combination of two strategies. The first was to map satellite sequences (using biotin probes and colorimetric detection) to small heterochromatic regions delimited by the breakpoints of translocation chromosomes. These reciprocal translocations move blocks of heterochromatin away from neighbouring blocks and onto other, easily identifiable chromosomes. Since the heterochromatic breakpoints of the translocations can be ordered using simple banding procedures, a crude satellite map can be constructed. The second strategy was to correlate different satellite sequences with specific fluorescent or banded regions in the heterochromatin: N-bands, Hoechst 33258 bands ("AT-rich" regions) or chromomycin A-bands ("GC-rich"). The banding map was determined using sequential fluorescent and N-banding regimens on the same prometaphase chromosomes, thereby allowing ordering of the bands along the heterochromatin (Pimpinelli, Bonaccorsi and Gatti, personal communication). Each block of heterochromatin has a specific "signature" as defined by these three banding techniques.

We find that N-bands always contain repeats of AAGAG (the major component of the 1.705 satellite), and AAGAC repeats (only one nucleotide in five different) do not N-band but instead fluoresce brightly with chromomycin A. Other satellite repeats can be easily identified by their characteristic patterns. A fine scale map of the heterochromatin of chromosomes 2 and the Y will be presented, and the relationship of the satellite repeats with each other and with the genetic functions in heterochromatin will be discussed.

INHERITANCE OF ANTHELMINTIC RESISTANCE IN TRICHOSTRONGYLUS COLUBRIFORMIS

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Trichostrongylus colubriformis is a nematode parasite that lives in the small intestine of sheep. Resistance to the two commonly used groups of anthelmintics for the control of this parasite, is widespread in Australia. Reciprocal matings between thiabendazole (TBZ) resistant and susceptible *T. colubriformis* were done to produce an F_1 generation which was intercrossed to produce an F_2 generation or backcrossed to both parental strains. Eggs from the parental, F_1 , F_2 and backcross populations were tested for resistance to TBZ by *in vitro* egg hatch assays and the parasitic stages by an anthelmintic efficiency assays using guinea pigs as a model system.

TBZ resistance in *T. colubriformis* was found to be controlled by more than one gene acting additively with strong maternal effects. With respect to its fitness in the presence of the anthelmintic, TBZ resistance was found to be incompletely recessive. Where the population was expected to contain 25% or less of the resistance genome, the anthelmintic removed 73% or more of the worms. However, where the population contained greater than 75% of the resistance genome, the anthelmintic was useless for control purposes.

The development of polygenic resistance to TBZ, resulted from using a non-persistent anthelmintic at an efficiency level below the LD₁₀₀. This polygenicity is consistent with a model of selection from within the normal distribution of drug tolerances, which would channel variation at many loci.

In contast, similar selection for resistance using the chemically unrelated anthelmintic, levamisole, has resulted in resistance in *T. colubriformis* which is controlled by a single, sex-linked, recessive gene, with some minor contribution from a number of other genes.

Despite contrasting genetic systems for resistance, these results support the strategy of high dose rates as a method of delaying the onset of anthelmintic resistance in nematode populations with low frequencies of resistance alleles, provided the control strategy is integrated with knowledge of the population biology of the parasite.

Genetic Mapping and Electrophoretic Karyotyping in *Candida albicans*

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The yeast *Candida albicans* is a frequent human pathogen, causing superficial infections (often known as thrush) as well as severe systemic infections. Genetic analysis of *C. albicans* is complicated by its lack of a sexual cycle, and by its habitual diploidy. Previous work has established a parasexual cycle based on protoplast fusion, UV-induced mitotic recombination, and heatshock-induced chromosome loss. Recently, electrophoretic karyotypes obtained by field inversion gel electrophoresis revealed extensive chromosomal length polymorphisms between different strains of *C. albicans*.

The strain ATCC 10261 is of particular interest since it and its mutant derivatives have been used extensively in parasexual genetic analyses. Furthermore, it is naturally heterozygous at a *lys* biosynthetic locus. FIGE karyotyping reveals that this strain has a diverged pair of homologues. In some cases, selection for *lys* homozygotes from ATCC 10261 by UV-induced mitotic recombination alters the sizes of one or both members of the diverged pair of bands. This suggests that the diverged pair in the karyotype may correspond to the *lys* linkage group.

Parasexual genetic studies suggest that the *his2* and *pro1* loci are on the same linkage group as *lys*. However, two separate attempts to determine relative map positions using different ATCC 10261 derivatives gave conflicting results. In one instance, *pro1* and *his2* were closely linked and trans-centromeric to *lys*. In the other, *his2* was distal to *lys*, and both were trans-centromeric to *pro1*.

It is possible that the conflicting mapping results are a consequence of some form of rearrangement having generated divergent homologues of this chromosome in ATCC 10261. Future genetic studies in *C. albicans* should take account of strain-strain and homologue divergence.

GENETIC MAPPING AND DIRECTED MUTAGENESIS IN CANDIDA ALBICANS

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The pathogenic yeast <u>Candida albicans</u> is imperfect. All fully characterised clinical isolates are diploid. The system is of considerable genetic interest since it is the first diploid imperfect system to be analysed. A number of unusual features have been detected, for example, many clinical isolates are heterozygous for recessive auxotrophies.

A start has been made on analysing the genetic map of the yeast using protoplast fusion and induced mitotic crossing-over and chromosome loss. At least 6 linkage groups have been found and it is thought unlikely the final number will be much greater than 6. The most carefully analysed linkage group, the right arm of linkage group I, shows an unusual feature that may also be apparent in the other linkage groups. The 6 loci mapped to this linkage group all fall in the distal 25% of the mapped distance. That is to say the distance (frequency of mitotic crossing-over) between the centromere and 0.75 contains none of the auxotrophies while the distance 0.75 - 1.00 carries <u>adel arg met ura leu</u> and <u>tsl</u>. The term tooth-brush chromosome describes accurately the resulting genetic map.

Experiments are underway using FIGE karyotyping, transformation and gene disruption to attempt to answer the question of whether the abnormal map is a characteristic of just one arm of the map or is a more general feature.

ISOLATION AND CHARACTERIZATION OF TAENIA OVIS ANTIGENS

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Taeniid infections of sheep cause great losses to primary producers in Australia and world wide. One of these species *T. ovis* has been used for the present study.

The prime objective of this project is to obtain efficient expression of parasite antigens *in vitro*, and to isolate and characterize those antigens which are potential candidates for vaccines.

Double stranded DNA fragments complementary to polyA⁺ RNA isolated from *T. ovis* were cloned in the pEX1, pEX2 and pEX3 expression vector. Several recombinants expressing antigenic determinants were detected using *T. ovis* infected sheep serum. Of these, five clones were selected and studied further.

The hybrid proteins were analysed by polyacrylamide gel electrophoresis (PAGE) and Western blotting(WB) and were partially purified.

A "cocktail" of this protein was used to inoculate sheep to evaluate their antigenic potential. Dot blot tests showed that the animals had a positive response to the vaccination producing antibodies to adult and oncospheral *T. ovis* antigens.

The DNA of these five clones was isolated and characterized. Their sequences will be discussed, as well as more recent progress.

Heterologous Expression of Viral Proteins in Yeast

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Infectious Bursal Disease Virus (IBDV) causes severe immune deficiency in young chickens. IBDV is a bisegmented dsRNA virus in which the smaller segment (2.9kb) encodes the viral RNA dependent RNA polymerase (VP1/90-kDa) and the larger segment (3.4kb) encodes a precursor polyprotein N-VP2-VP4-VP3-C (115 kDa). The polyprotein undergoes proteolytic processing to generate the major structural components VP2 (52 kDa) and VP3 (32 kDa) and a minor non-structural component VP4 (28 kDa). VP4 appears to be a viral protease that is involved in processing of the polyprotein.

Saccharomyces cerevisiae (budding yeast) has been extensively used as heterologous host for production of economically significant proteins that include viral gene products. Schizosaccharomyces pombe (fission yeast) has recently become an alternate host because of its closer resemblance to the higher eukaryotes. To produce genetically engineered subunit vaccines against IBDV, we have transformed both S. cerevisiae and S. pombe with yeast expression vectors containing IBD viral RNA derived cDNA fragments in various forms. Our results suggest that either co- or post-translational processing of the large polyprotein occurs in both yeasts resulting in the generation of a stable C-terminal product VP3. However, the N-terminal product VP2 either in fully processed form or as part of the unprocessed polyprotein was not detected. Moreover, in S. cerevisiae when the processing of the polyprotein was prevented, because of an engineered mutation within VP4, even the C-terminal product VP3 was undetected. VP2 was detected only when fused to yeast pre-sequences at its N-terminal end suggesting that in S. cerevisiae VP2 or the unprocessed polyprotein without proper protection of its N-terminal amino acid residues is susceptible to proteolytic degradation. S. pombe is currently being tested for its ability to produce stable VP2 as a fusion protein.

SESSION 6A

BREEDING SYSTEMS IN BANKSIA SPINULOSA

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While some aspects of the reproductive biology of Proteaceous species have been investigated in recent years, knowledge of their breeding systems and the way pollen quality and availability affects them remains unclear. I am currently using both ecological and genetical methods to study this in Banksia spinulosa, a perennial shrub.

An estimate of outcrossing has been obtained using 4 polymorphic loci, and these plants appear to be almost totally outcrossed. Results for one locus are confounded by the possible presence of a null allele.

Work is currently being undertaken to determine whether this high outcrossing rate is a reflection of the type of pollen being received, or the result of physiological resource allocation or selfincompatibility within the plant.

EVOLUTIONARY GENETICS OF PARTHENOGENETIC LIZARDS

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We have been assessing mitochondrial DNA variation in parthenogenetic lizards and their bisexual relatives to investigate the approximate age of the parthenogens and the relatedness of the maternal founders of lineages. The most striking observation is the extreme homogeneity of mtDNAs from parthenogens relative to those from their bisexual relatives. This even holds for parthenogentic lineages with moderate-high genotypic diversity for nuclear-encoded loci. We conclude that there are strong geographic (and possibly temporal) constraints on the hybrid-origin of parthenogenetic lineages and that all extant lineages originated recently. Also, the bisexual parents of parthenogenetic lineages do not cluster phylogenetically, rather, they tend to be distantly related.

SESSION 6A

THE EVOLUTIONARY GENETICS OF GALAXIAS TRUTTACEUS, G. AURATUS AND G. TANYCEPHALUS (PISCES: GALAXIDAE)

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Galaxias truttaceus, *G. auratus* and *G. tanycephalus* are three monhpologically similar freshwater fish species. *G. truttaceus* is widely distributed in coastal drainages in Tasmania and Victoria and has a sea-going larval stage. In Tasmania it is also found in a few landlocked lakes. Lake and stream populations of *G. truttaceus* have different breeding strategies. *G. auratus* and *G. tanycephalus* are found only in one or two land-locked Tasmanian lakes and have similar breeding strategies to the lake form of *G. truttaceus*. We are using restriction enzyme analysis of mitochondrial DNA and allozyme gene frequency data to test the hypothesis that *G. auratus* and *G. tanycephalus* are recent allopatric derivatives of *G. truttaceus*. Genetic analyses of land-locked lake populations of *G. truttaceus* suggest that population bottlenecking and/or founder effects may have played an important role in the formation *G. auratus* and *G. tanycephalus*.

ISONYMY, SOCIAL CLASS AND MATING STRUCTURE IN LATE NINETEENTH CENTURY TASMANIA

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A major aim in population structure studies is to explain genetic variation. How genes and genotypes are distributed within and between populations are obviously influenced by many factors. Migration and mating behaviour are two aspects of human behaviour that directly influence genetic structure, the latter being the focus of this study.

All marriages (N= 4,258) registered in Tasmania between 1896-99 inclusive were analysed for mating structure. Surname analysis was used in the investigation. Theoretical and practical applications of surnames as genetic markers will be discussed. The application of surname analysis in population studies in Tasmania (1-4) have been very fruitful in revealing determinants of mating structure. Specifically, marital isonymy has shown that F coefficients were generally low and typical of other mainstream populations. The nonrandom component (Fn) was unusually high, particularly, in one region, the Midlands. Social class correlates revealed one class to be the predominate contributors to the high Fn values. The explanation of these results in the Midlands lies in the effects of a rigid socio-economic structure established during colonization.

1. Mitchell, R.J. etal. 1987. Epidemiology in Tasmania. ed. H. King, Brolga Press. ACT. pp 1-21.

2. Harding, R. 1985. Hum biol 57: 727-44.

3. Lafranchi, M. etal. 1988. Ann. Hum. Biol. (in press)

4. Kosten, M. etal. 1983. J. Biosoc. Sci. 15: 367-76.

GENETIC STRUCTURE IN THE WHITE POPULATION OF TASMANIA

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This paper attempts to answer two interrelated questions; firstly, what is the extent of genetic variation in the present-day population of Tasmania, and secondly, what mechanisms can explain the existence of sub-populations observed within the island. The first question is answered by an examination of the blood groups in a large sample of school children drawn from all over the island. Regional analysis via R matrix analysis indicated that the population of the Midlands region (one of the earliest settled) as the most divergent. To answer the second question the historical population structure of the Midlands region was examined. Evidence of significant deviation from random mating was found from an examination of vital records.

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DNA AND FORENSIC SCIENCE IN VICTORIA

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The individualisation of stains of biological fluid has long been of interest in forensic science, to include or exclude victims and suspects as possible sources of the stain. Recently restriction fragment length polymorphisms have begun to be used in forensic case work. In Victoria we have concentrated on single locus probes. We intend to combine several single locus probes, which all display RFLP with the same restriction enzyme, to give a complex pattern of high discriminating power but in which all allelic relationships are known.

One such probe, pDP34 (Page, D.C., Harper, M.E., Love, J. and Botstein, D. (1984) Nature, 311, 119-123) has been studied in detail. This probe displays an X chromosome polymorphism. Our results show that the female Melbourne population is in Hardy-Weinberg equilibrium for this RFLP (11 + 12 kb) (n = 70, X^2 = 1.26). In addition this probe hybridises to a 15 kb fragment from the Y chromosome and thus the presence of this fragment is diagnostic for male origin.

pDP34 has been successfully combined with a second probe, pEM36. Results of this and other probe combinations will be discussed.

QUANTITATIVE GENETICS OF COLD TOLERANCE IN NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER AND D. SIMULANS

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Genetic analysis of cold tolerance was applied to random samples of recently-collected isofemale strains of <u>Drosophila melanogaster</u> and <u>D.</u> <u>simulans</u> from diverse climates. The temperate-zone locality of Melbourne was sampled twice, once in 1986 and again in 1987. In 1987, collections were also made in the humid tropics at Townsville and the wet/dry tropical locality of Darwin. <u>D. simulans</u> was not found in Darwin. Seven symmetrical diallels were performed, each with from 9 to 12 randomly-chosen isofemale strains.

The pattern for the significant genetic components of the variance was generally consistent over time, across populations and across species. Cold tolerance was controlled by mainly additive genetic effects: there Was no evidence of directional dominance. Overall, it is proposed that natural populations of both members of the sibling species have the genetic architecture necessary for adaptive phenotypic response to the fluctuating selection pressure of extreme low temperature.

GENETIC VARIATION IN <u>DROSOPHILA BUZZATII</u> FOR OVIPOSITION ON NATURAL SUBSTRATES

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Theory shows that environmental heterogeneity in space or in time can maintain genetic polymorphisms, although the conditions may be stringent for some models of natural selection. Stable polymorphisms are more readily maintained if there is genotype specific habitat selection, but there is no evidence demonstrating such selection acting to maintain polymorphisms in natural populations.

For <u>D. buzzatii</u>, available evidence suggests a potential for habitat selection, in that adults discriminate among the yeast species found in natural cactus rots, and females prefer for oviposition those yeasts that in general are best for larval development (Vacek et al. 1985), cactus rots are spatially and temporally heterogeneous in abundance and frequency of cactophilic yeasts (Barker et al. 1983, 1987), and there is indirect evidence for genetic variation in oviposition preferences on different yeasts (Barker et al. 1986).

Direct evidence for such genetic variation was obtained from an experiment using eight isofemale lines from each of four widely separated natural populations. Females of each line were given a multiple choice of five yeast species, as oviposition sites, and the numbers of eggs laid on each yeast in a 30 hour test period were counted. The five yeast species were chosen from those known to be most abundant in cactus rots in Australia to provide a wide range of expected preferences.

The interaction term in ANOVA for yeast x isofemale lines was highly significant, that is, there are differences among isofemale lines within populations in preferences for these yeast species as oviposition substrates, which must be genetic, as all lines were grown and tested together under the same environmental conditions. An upper limit of the heritability of oviposition preference was estimated at 0.191.

Thus these results provide a basis for genotype specific habitat selection in natural populations. Whether this oviposition preference is a kind of habitat selection which maintains polymorphism will be discussed.

Barker et al. 1983.	Can. J. Microbiol. 29, 6-14.
Barker et al. 1986.	Aust. J. Biol. Sci. 39, 47-58.
Barker et al. 1987.	Microb. Ecol. 14, 267-276.
Vacek et al. 1985.	Biol. J. Linn. Soc. 24, 175-187.

COMPARISON OF ALLOZYME FREQUENCIES IN CHINESE AND AUSTRALIAN POPULATIONS OF Drosophila melanogaster

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To test inter-continental homogeneity of allozyme frequencies in Drosophila melanogaster populations variants at eight loci (Adh, Gpdh, Est-6, Pgm, Odh, Acp, G6pd and Pgd) were scored in eight Chinese populations covering similar latitudes to Australia.

The frequency of the $Est-6^{1.00}$ allele tended to show a decrease with increasing distance from the equator, but this pattern is opposite to that found on other continents.

Of the other loci scored $G \delta p d^{F}$ has a similar distribution to that found in Europe and, below 30°N, $G p d h^{F}$ shows a cline similar to that in Australia and North America.

The most consistent inter-continental pattern was found for alleles at the Adh locus. The frequency of Adh^5 decreased with increasing latitude, as previously observed in samples from North American, Europe and Australia. The southern Chinese populations, however, were remarkable in having a relatively high frequency (up to 36%) of a heat resistant Adh allele (with properties similar to those of $Adh^{F.Ch.D}$) which has previously only been found at low frequency in natural populations in Australia and North America.

FOUR BASE-CUTTER ANALYSIS OF ALLELIC VARIATION FROM AUSTRALIAN AND AMERICAN POPULATIONS OF DROSOPHILA MELANOGASTER

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A filter hybridization technique for identifying restriction-site and insertion/deletion variation within short regions of genomic DNA has been described by Kreitman and Aguade (1986). The technique uses restriction enzymes that recognize four-nucleotide sequences, denaturing polyacrylamide (sequencing) gels to separate fragments, and electroblotting to detect small insertion/deletion variation. In their study, 50 distinct haplotypes were detected among 87 isogenic lines of <u>Drosophila melanogaster</u> (representing two natural American populations) surveyed over a 2.7 kb region encompassing the alcohol dehydrogenase locus.

This study, using the same technique and restriction enzymes, describes the extent of variation within the same 2.7 kb region in a natural Australian population of <u>D</u>. <u>melanogaster</u> collected from the cellar of the Tahbilk winery. Haplotype frequency variation between the populations has been examined and compared so as to reveal the degree (if any) of genetic differentiation between the continents of North America and Australia.

Kreitman, M. and Aguade, M. (1986). Genetic uniformity in two populations of <u>Drosophila melanogaster</u> as revealed by filter hybridization of four-nucleotide-recognizing restriction enzyme digests. Proc. Natl. Acad. Sci. USA 83, 3562-3566.

BIOCHEMICAL CHARACTERISATION OF LOW ACTIVITY VARIANTS OF GLYCEROL-3-PHOSPHATE DEHYDROGENASE FROM NATURAL POPULATIONS OF Drosophila melanogaster

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GPDH is an important enzyme in insect flight metabolism and lipid biosynthesis. In natural populations of *D. melanogaster* the locus is relatively invariant with the two electrophoretic alleles. Within these two classes we have isolated a number of variants which are associated with low GPDH activity. Analyses of some of these variants suggest that the low activity is due, not to structural gene differences, but to linked modifiers. However one variant, *Cb62*, isolated from a population in Cardwell, Queensland, is interesting as it has altered electrophoretic mobility. This raises the possibility that a lesion in the structural gene of *Cb62* is responsible for the low activity.

GPDH activity has been assayed in adults from three homozygous low activity lines, *Cb62*, *T198* and *C22*; compared to a control homozygous *Gpdh*² line, their activities are approximately 32%, 24% and 5% respectively. In contrast to the control allele, and the other low activity variants, the level of activity in the abdomen in *Cb62* is very much reduced, whilst higher than expected levels are present in the thorax. *Cb62* is also remarkable in that its GPDH activity is unstable, both at high and low temperatures. At 0°C about 50% of activity is lost after 5 hours, but this is partially reversible after incubation of extracts at 20°C for 30 minutes. In heterozygotes between *Cb62* and the control line the level of enzyme activity is intermediate, and activity is stable at 0°C.

The properties of the *Cb62* enzyme are being investigated in relation to possible effects on fitness, particularly cold tolerance.

CHROMOSOME LOCALIZATION OF THE GENES ENCODING HEAT SHOCK PROTEIN 70 (HSP 70) IN HUMAN, MOUSE AND MARSUPIAL

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HSP 70 has been strongly conserved from bacteria to man, both in terms of nucleotide and amino-acid sequence homology. Although the exact function of the protein is unknown, it has been suggested that it plays some protective role in the cells response to stress. In this study we have mapped by <u>in-situ</u> hybridization the chromosomal localization of the HSP 70 genes in three different species. In the human at least one gene has been mapped to the region 6p12-6p21, and it is also possible that another gene is located in the region 5q35. In the mouse at least six HSP 70 genes have been mapped to the regions 4C7, 5B1, 5F1.1-G3.3, 14D1-D3 and 19B11-C1. In the the marsupial (<u>Macropus eugenii</u>) at least three genes have been localized in the regions 1q, 2q and 4p.

Thus, our data demonstrate that HSP 70 is a dispersed multigene family in all three species studied.

SESSION 7B

GENOMIC IMPRINTING WITH SPECIAL REFERENCE TO THE EFFECT OF TWO MATERNALLY DERIVED X-CHROMOSOMES ON PARTHENOGENETIC DEVELOPMENT IN THE MOUSE

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In certain extraembryonic tissues of normal female mouse conceptuses, X chromosome dosage compensation is achieved by preferential inactivation of the paternally derived X. Diploid parthenotes have two maternally derived X chromosomes, hence this mechanism cannot operate. To examine whether this contributes to the inviability of parthenotes, XO and XX parthenogenetic eggs were constructed by pronuclear transplantation and their development assessed after transfer to foster mothers. Consistent with the possibility that two maternally derived X chromosomes do contribute to parthenogenetic inviability was the result that the frequency of postimplantation development of XO parthenotes was much higher than that of their XX counterparts. However, both types of parthenotes showed similar developmental abnormalities at the postimplantation stage, demonstrating that parthenogenetic inviability is ultimately determined by the possession of two sets of maternally derived autosomes.

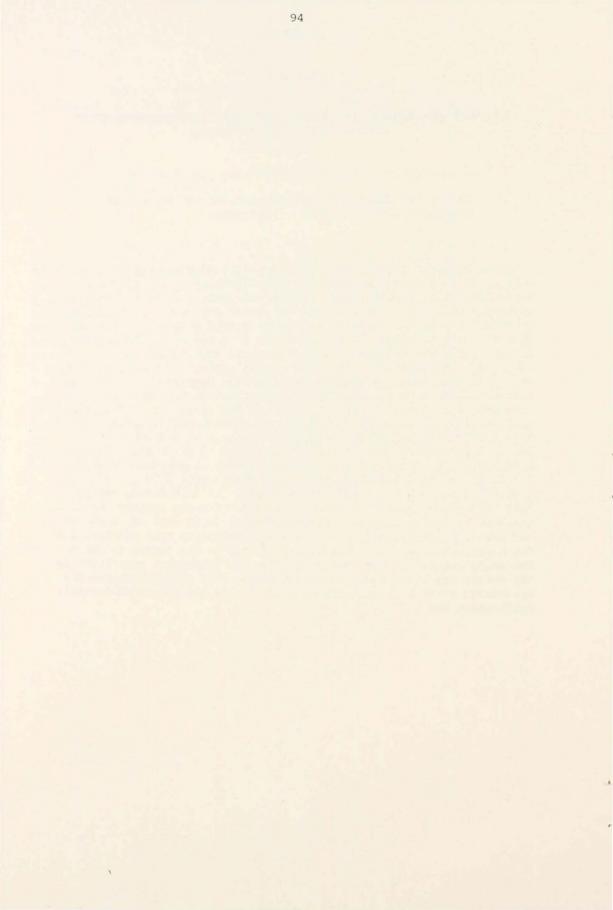
SESSION 7B

CELLULAR DNA METHYLATION LEVELS IN NORMAL AND TRANSFORMED CELLS AFTER VARIOUS DRUG TREATMENTS

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Sodium butyrate is very often used to alter gene expression in cultured cells. In this study, we examined the effects of this compound on various cellular events in WI-38 human embryonic lung fibroblasts in culture. During a 16-20 treatment at sodium butyrate concentrations of between 5 and 20 MM, no adverse effects on cell morphology were observed. However, cell division and DNA synthesis were reversibly inhibited, the latter by 85, 80, and 70% at sodium butyrate concentrations of 5, 10, and 20 MM, respectively. Although overall protein synthetic activity was not significantly affected, RNA synthesis decreased to 76% of the control values at a sodium butyrate concentration of 5 mM. Butyrate treatment also caused hypermethylation of DNA cytosines as determined by differential digestion by Mspl/HpaII restriction endonucleases and by high performance liquid chromatography analysis of the DNA. The 5-methylcytosine content of the DNA in untreated WI-38 fibroblasts was 2.94 + 0.46% of total cytosine residues, while in cultures treated with 5, 10, and 20 mM sodium butyrate, these values were 5.76 ± 0.28, 5.91 + 0.37, and 6.8 + 0.44%, respectively. An interesting feature is that this hypermethylation occurred in DNA which was synthesized in the presence of sodium butyrate (newly synthesized) as well as in DNA which had been synthesized before butyrate administration (pre-existing DNA). The hypermethylated state was conserved only in the former situation, since the methylcytosines were rapidly lost in the subsequent generation in the latter case. It would therefore appear that methylcytosines are maintained after cell replication only if they are generated on newly synthesized DNA.



THE ORIGIN OF COMPLEX SEX-LINKED TRANSLOCATION HETEROZYGOSITY IN DELENA CANCERIDES (SPARASSIDAE : ARACHNIDA)

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The Australian huntsman spider Delena cancerides possesses a number of distinct chromosomal races. The ancestral form has a telocentric karyotype consisting of 40 autosomes and 3 Xchromosomes, and forms normal bivalents at meiosis. In four other races, wholesale centric fusion has resulted in karyotypes of 21 metacentric chromosomes and a single telocentric. In one of these, ten metacentric bivalents are formed at meiosis, and the two remaining chromosomes are a metacentric X-X fusion product and a telocentric X. In the other three races, an X-chromosome has fused with an autosome and there is heterozygosity for autosomal fusions in the male. At meiosis, these males are characterised by the formation of sex-linked chains of 3, 5 or 9 chromosomes.

Half of the fusion products in these chains consistently migrate with the X-A fusion product and are homozygous in females, while the remaining chromosomes behave as neo-Y chromosomes and appear in males only. Owing to a high frequency of interstitial and proximal chiasmata in these chains, most of the genetic material is not sex-linked or sex-limited, however.

An analysis of electrophoretic data from these races suggests that the higher order chain races (5 and 9) arose from hybridisation between two fusion races homozygous for all, or almost all of their fusions. Furthermore, from an analysis of the hypothesised hybrid derivatives, it would appear that no selective advantage arising from chain formation is required for the *de novo* formation of these races. This model contrasts with the "speciation by monobrachial fusion" model of Baker and Bickham (1986), because here it is suggested that hybridisation between races possessing different fusions may result in selection for alternate segregation and subsequent complex sex-linked fusion heterozygosity rather than premating isolation.

ENVIRONMENTAL STABILITY AND THE MODE OF REPRODUCTION OF THE FRESHWATER OSTRACOD, *Candocypris novaezealandiae*.

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Most theoretical models predict that sexually reproducing individuals should have a selective advantage in unstable environments. In contrast with these predictions, anecdotal evidence suggests that *Candocypris novaezealandiae*, in common with many other species of freshwater ostracod, is reproducing asexually in temporary and permanent ponds (relativley unstable environments) and sexually in large lakes and reservoirs (relativley stable environments). Both genetic (electrophoretic) and sex ratio data were used to determine the reproductive mode of a small temporary pond, a large temporary swamp, eight permanent pond and a reservoir population of *C. novaezealandiae*. These data show that both the reservoir, and the temporary and permanent pond populations are mostly dependant upon clonal reproduction. In contrast, sexual reproduction is the dominant reproductive mode for the temporary swamp population. These results do not suggest an association between sexual reproduction and environmental stability.

THE INHERITANCE OF REPRODUCTION

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Animal breeders recognise reproduction as an important component of animal production and, hence, seek to improve it in their breeding programs. Yet, lifetime reproduction is the one trait through which all selection operates. Hence, lifetime reproduction has already been selected upwards (natural selection) since evolution of life began. It is, therefore, to be expected that, in animal species adapted to their environment, no further improvement of lifetime reproduction is possible, as the animals are already utilising all available resources of the environment optimally. Geneticists agree that fitness and its major components have heritability = 0 or very low.

This situation can be described by two equations:-F = A x B x C x ... r = a + b + c + ... where F is fitness or lifetime reproduction (defined widely), A,B,C,etc. are component traits of fitness, r is total of environmental resources. and a,b,c,etc. are the resources consumed by A,B,C,etc., respectively.

These equations imply, for an adapted population where r cannot be raised, that an increase in any component reproductive trait can only occur if values for other component traits fall. With F already maximised by previous selection, it can at best maintain its value and is likely to fall.

We present data from mouse selection experiments confirming these equations and we discuss the implications of these equations for the genetic improvement of animals by breeding. Environmental quality turns out to be critically important for what can be achieved by breeding programs.

THE TWO-LOCUS INFINITELY-MANY-ALLELES MODEL WITH RECOMBINATION

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A neutral 1-locus population genetics model is the infinitelymany- alleles model where each mutation produces an allele entirely new to the population. Random drift causes alleles to be lost, and with mutation produces stability in the population. A classical result is the probability distribution of the configuration of allele types in a sample of n genes, named the Ewens' sampling formula. In an extension to a 2-locus model one is interested in how the recombination rate affects the joint distribution of allele types at the two loci. Is the effect of recombination like mutation?

Of interest in the 1-locus model is the distribution of the lines of descent of a sample back to a common ancestor. What is an 'ancestor' when there is recombination, and what is the distribution of the lines of descent?

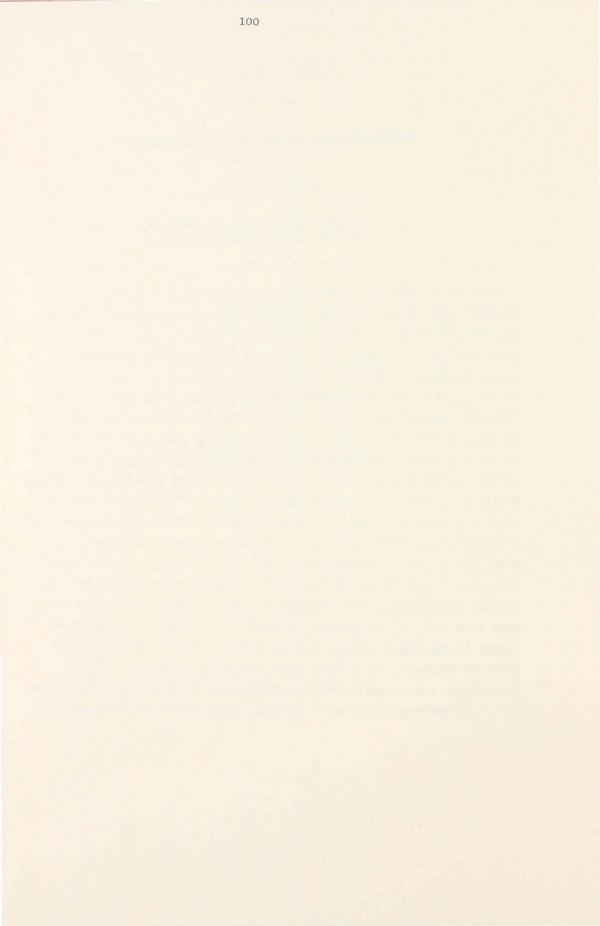
GUEST SPEAKER

DNA METHYLATION AND EPIGENETIC MECHANISMS

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Genes are essential for the transmission of genetic information from generation to generation, and this mechanism of inheritance is fully understood. Genes are also essential for unfolding the genetic programme for development, but the rules governing this process are quite obscure. Epigenetics comprises the study of the switching on and off of genes during development, the segregation of gene activities following somatic cell division and the stable inheritance of a given spectrum of gene activities in specific cells. Some of these processes may be explained by DNA modification, particularly changes in the pattern of DNA methylation and the heritability of that pattern. There is strong evidence that DNA methylation plays an important role in the control of gene activity in cultured mammalian cells, and the properties of a CHO mutant strain affected in DNA methylation will be described. Human diploid cells progressively lose cytosine methlyation during serial subculture, and this may be related to their in vitro senescence. There is also evidence that DNA modifications can be inherited through the germ line. Classical genetics is based on the study of all types of change in DNA base sequence, but the rules governing the activity of genes by epigenetic mechanisms are necessarily different. Their elucidation will depend both on a theoretical framework for development and on experimental studies at the molecular, chromosomal and cellular levels.



POSTER 1

Nitrogen control of <u>amd</u>S expression in <u>Aspergillus</u> nidulans

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Genetic studies have shown that the areA gene of <u>A</u>, <u>nidulans</u> codes for a positively acting regulatory protein which activates gene expression under nitrogen limiting conditions. The <u>amd</u>S gene of <u>A</u>, <u>nidulans</u> is one of many nitrogen catabolic enzymes regulated by the <u>areA</u> gene and nitrogen metabolite repression. No <u>cis</u>-acting mutants of <u>amd</u>S affecting nitrogen control have been identified. However, transformation studies have indicated that sequences downstream of a SmaI site at -114 are necessary for <u>areA</u> control. In <u>vitro</u> mutagenesis of this region of the <u>amd</u>S gene and cross-species comparisons between the <u>areA</u> gene and the functionally analagous <u>nit-2</u> gene of <u>Neurospora</u> <u>crassa</u> are being used to study both the <u>areA</u> gene product and its recognition sequences 5' to the <u>amd</u>S gene.

POSTER 2

Two divergently transcribed Aspergillus nidulans

genes under coordinate control

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The <u>Aspergillus nidulans lam</u>A gene is required for utilization of 2-pyrrolidinone as a nitrogen source. Previous work has suggested that lamA encodes a permease and/or lactamase which converts exogenous 2-pyrrolidinone to ö-amino-butyric acid (GABA). Expression of lamA is under the control of the amdR regulatory gene as are gabA, gatA and amdS. The co-regulation of these genes is under investigation in our laboratory.

The lamA gene was isolated from a cosmid gene bank by complementation of a lamA mutation. A 6 kb subclone which contains the lamA gene hybridizes to two divergently transcribed mRNAs of 0.9 and 4.0 kb. The larger transcript corresponds to the lamA gene. The smaller transcript has been designated the lamB message. Both transcripts appear to be similarly regulated. A closely related fungus <u>Aspergillus terreus</u> cannot metabolize 2-pyrrolidinone. We have shown that both lamA and lamB are required to allow <u>A. terreus</u> to utilize 2-pyrrolidinone as a nitrogen source.

Investigations are in progress to determine if one lam gene encodes the lactamase and the second a permease. We also hope to determine if the two genes share or have separate regulatory sites.

PHYSICAL CHARACTERIZATION OF THE POSITIVELY ACTING REGULATORY GENE, amdR, OF Aspergillus nidulans.

<u>Alex Andrianopoulos</u> and Michael J. Hynes. Department of Genetics, University of Melbourne.

The process of gene regulation is a complex interaction in which regulatory molecules, effector molecules, transcriptional machinery and 5' regulatory DNA sequences interact in a highly specific manner. As the number of cloned regulatory genes is increases, so is our understanding of the numerous ways in which regulation of gene expression is mediated. One interesting aspect of many regulatory genes is that they seem to possess distinct and separable functional domains for nuclear localization, DNA binding, inducer binding and activation function.

In Aspergillus nidulans, the utilization of certain amides, omega amino acids and lactams is controlled by the action of the positively acting regulatory gene amdR. The amdR gene positively activates the expression of the amdS, gatA, gabA and lamA structural genes in the presence of omega amino acid coinducers. In order to study the mechanism by which activation of structural gene expression is mediated by amdR, this regulatory gene was cloned.

Through the use of DNA-mediated transformation, the *amdR* regulatory gene was cloned using a cosmid based genomic library. The *amdR* gene has been shown to reside on a 4.2kb ClaI-EcoRI genomic fragment. Transcriptional analysis has revealed the presence of two *amdR* mRNA species of 2.7kb and 1.8kb in length, both of which are synthesized constitutively and which do not appear to require functional *amdR* product for their synthesis.

In order to define regions within *amdR* that account for its transcriptional activator properties, a series of deletions were made and analysed *in vivo* for both *amdR* mediated activation of structural gene expression and competitive inhibition of *amdR*⁺ activation. These results and their consequences will be addressed.

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POSTER 3

Characterisation of the <u>gatA</u> gene of <u>Aspergillus nidulans.</u> <u>I.B. Richardson</u>, M.J. Hynes and M.Katz Department of Genetics, University of Melbourne.

The <u>amdR</u> gene of the ascomycete fungus <u>Aspergillus nidulans</u> is known to regulate transcription of four genes: <u>amdS</u>, <u>gatA</u>, <u>lamA</u> and <u>gabA</u>. In addition it is likely that some or all of these genes are subject to regulation via the <u>areA</u> and <u>creA</u> genes. All of these genes except <u>gabA</u> have now been cloned and are under current study. The <u>gatA</u> gene was cloned by complementation in 1985.

Functionally, <u>gatA</u> has been localised to a 4kb genomic fragment. Within this the approximate extent of a 1.8kb transcript has been determined as well as the direction of transcription. 1.5kb has been sequenced. Preliminary S1 mapping has been done and a putative open reading frame, containing at least two introns has been found.

Multiple copies of the 5' region of <u>gatA</u> have been found to be capable of titrating amdR protein <u>in vivo</u>. This titrating capacity has been localised to an 87bp 5' fragment. Comparison of sequence between this 87bp region and a 100bp 5' fragment of <u>amdS</u>, also known to titrate, has revealed a possible <u>amdR</u> binding site.

PLASMID-MEDIATED COPPER RESISTANCE IN E. COLI

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The plasmid pRJ1004 (Tetaz, T.J. & Luke, R.J.K. 1983, J. Bacteriol 154: 1263-1268) confers increased copper tolerance in <u>E. coli</u>. This is due to two determinants; pco, which confers inducible resistance to copper, and <u>cdr</u>, which reduces the effect of copper on the cell by repair of copper-damaged DNA (Rouch <u>et al.</u>, MS in preparation; Rouch and Lee, MS in preparation). The two determinants have been cloned, and their properties are being characterized.

The pco determinant contains four genes; pcoR, which encodes a regulatory protein; pcoC, which encodes a cytoplasmic protein; and pcoA and pcoB of unknown function. The products of the pco determinant interact with the chromosomally-encoded products responsible for normal copper metabolism in E. coli. This interaction is being studied, with particular regard to the mechanisms of regulation of gene expression.

The <u>cdr</u> determinant confers resistance to several DNA-damaging agents, and its known properties will be presented.

PROGRESS TOWARDS CLONING AN X-LINKED GENE FOR A HUMAN NERVE CELL ANTIGEN.

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In an attempt to further study the genetics and molecular biology of the human nerve cell antigen R1 and its encoding X-linked gene MIC-5, we made a cDNA library (prepared from poly (A+) RNA isolated from the R1-positive melanoma cell line 1aR3) in the expression vector lambda gt11, and screened plaque lifts from this library for R1 expression using appropriate monoclonal and polyclonal antibody preparations. We also screened a commercially available lambda gt11 cDNA library made from human brain RNA. No R1expressing clones were detected in either library. We therefore decided to use a different approach to cloning MIC-5, involving the purification and partial amino acid sequencing of R1, followed by synthesis of complementary oligonucleotide probes suitable for screening human genomic and cDNA libraries. Detergent solubilized membrane preparations from human brain and cell lines were affinity purified on R1 columns and analysed using PAGE, Western blotting and immunological detection. These studies revealed the existence of various molecular components possessing R1 specificity. The major components had apparent molecular weights of approximately 200kd, 180kd and 150kd. Further studies will be aimed at isolating and sequencing peptides from the 150kd component.

HABITAT SELECTION : OLFACTORY RESPONSES OF DROSOPHILA BUZZATII TO NATURALLY OCCURRING YEAST RESOURCES

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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 previous studies indicate that the cactophilic <u>Drosophila buzzatii</u> shows considerable potential for habitat selection (see Barker, this meeting).

Long and short distance olfactory responses of female <u>D. buzzatii</u> to naturally occurring yeast resources were examined for six isofemale lines from each of two geographically isolated populations. Each line was tested for responses to pairwise yeast combinations in a vertical wind tunnel olfactometer, and in a Y-tube olfactometer.

Analyses of variance revealed that <u>D. buzzatii</u> did not choose yeasts randomly. Further, isofemale lines within populations showed differential olfactory attraction to the yeasts.

The results also suggest that long and short distance olfactory responses may be genetically independent.

The existence of genetic variation in olfactory preference for natural substrates in the laboratory indicates that genotype specific habitat selection may be important in natural populations of <u>D. buzzatii</u>.

TRANSPOSABLE ELEMENTS IN AGEING DROSOPHILA MELANOGASTER

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The most plausible explanation of the origin of many transposable elements is that they have arisen from infectious viruses analogous to Mouse Mammary Tumor Viruses that have become attenuated. When transmission occurs primarily through the germ line, selection would be expected to allow only minor damage to the host during their lifespan in their normal ecological niche, as a major reduction in the fitness of the host would reduce the spread of the virus genome. For most animals in the wild significant ageing related deterioration does not occur because they die while still "young". There should not be selection against damaging activity of these viruses in the period after the host would normally be dead, and viral action for example as mutagenic agents would be possible at this time in the lifespan. This activity may be responsible for ageing related somatic mutation and may explain the variation between species in the ageing rate.

We have investigated the elements <u>copia</u>, <u>412</u>, and <u>gypsy</u>. of <u>Drosophila</u> <u>melanogaster</u>. As most of the adult tissue of the fly is postmitotic, we reasoned that elements which are apparently dependent on host DNA polymerases would be less likely to replicate whereas elements such as the ones chosen, that are dependent on reverse transcription, could well be active in adult tissue.

For 412 we have used Southern blots of DNA from young and old flies cut with Eco RI. This enzyme cuts out a large internal fragment that will be the same for all elements independent of position. In addition there are a number of other bands arising from the ends of the elements and a section of the surrounding genome. We have used densitometry of the large internal band as one method of determination of copy number. To correct for amount of DNA on the membrane we have reprobed with plasmids containing either the ribosomal protein rp49 or the Adh gene. In addition we have run uncut DNA to check for the appearance of extrachromosomal circular DNA (ECC DNA) containing the element. This was also quantified. If 412 was active then an increase in copy number, not associated with ECC DNA might be expected to be found. If it moved into the same position frequently then new bands arising from the ends may appear on the Southern blot.

Our data indicates a greater than 100% increase in copy number of 412 with no apparent change in the band pattern, and no evidence of significant amounts of ECC elements. This suggests that 412 is active and may transpose into many different sites in different cells.

In similar experiments with <u>copia</u> we have found a smaller net increase in copy number again with no evidence of new bands or ECC elements.

With gypsy there were changes consistent with the possibility that gypsy excises or is excised.

Transposition in adult somatic tissues may be a common event among copialike elements in post-mitotic tissues. We speculate that this may be a major contributor to the phenotype of ageing.

MITOCHONDRIAL DNA VARIATION IN A WINERY POPULATION OF D. melanogaster

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Mitochondrial DNA haplotypes, involving two polymorphic restriction sites and length variation in the A-T rich region, were sampled in 52 isofemale lines established from the cellar and from the immediately adjacent orchard of the Tahbilk winery in 1987. Heritable differences in at least two quantitative traits have been reported from these two collection sites. Six haplotypes were identified and their distributions were homogeneous over the two sites.

Polymorphic length variation in the A-T rich region of mtDNA from D. melanogaster has recently been reported for 92 isofemale lines collected as small numbers of lines from many geographic locations around the world (Hale, L. R. and Singh, R. S. Proc. Natl. Acad. Sci. USA 83, pp 8813-8817; 1986). In this worldwide study 17 of the lines were heteroplasmic while at Tahbilk all 52 lines were homoplasmic. Possible explanations for this difference in frequency of heteroplasmy are discussed, along with possible reasons for the difference between the two studies in reported size of the most common mtDNA length variant.

Isolation and preliminary characterisation of a duplicated esterase locus in <u>Drosophila buzzatii</u>

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Carboxylesterases as a group appear to evolve rapidly, frequently exhibiting high levels of structural gene variation and very diverse patterns of developmental regulation. In insect species, esterases have been implicated in a variety of physiological functions including: neurotransmission, pheromone signalling, digestion, insecticide resistance and regulation of juvenile hormone titre. This great diversity of physiological and developmental specificity prompts the question - what is the relationship between the loci encoding these enzymes and how have the differences in catalytic activity and developmental expression evolved? Duplicated loci provide a powerful system for analysing mechanisms of molecular evolution, especially where it is possible to compare not only the paralogous homologues within a species but also the orthologous homologues in closely related species.

We are studying a β -esterase duplication in <u>Drosophila buzzatii</u> and related members of the mulleri sub-group of the repleta species complex. In these species one esterase locus is expressed throughout development, from the time of zygotic gene activation until death of the adult. In third instar larvae, the enzyme appears to be located almost exclusively in the haemolymph. In marked contrast, the other esterase of the duplicated pair first appears during mid third larval instar, activity peaks around the time of pupation and has disappeared by the time of eclosion of the mature imago. Thus, the enzymes differ completely in their temporal and spatial patterns of expression. Molecular analysis of this duplication provides a rare opportunity to study the acquisition of new regulatory information by one member of a duplicated gene pair. Amino terminal peptide sequence data for these two esterases from the related species D. mojavensis (Pen et al. 1986) indicated a high degree of homology (28/34 residues identical). More surprisingly however, these two enzymes showed a remarkable homology with the predicted N-terminal peptide of Esterase-6 in the very distantly related species D. melanogaster (25/34 residues identical between Est-6 and one or both of the D. mojavensis enzymes).

A <u>D. melanogaster Esterase-6</u> cDNA clone (Oakeshott <u>et al.</u> 1987) was used to screen a <u>D. buzzatii</u> genomic library at reduced stringency hybridisation and washing. Positive signals were obtained and two independent recombinant phages were isolated. Preliminary characterisation of these genes is the subject of this poster.

Oakeshott, J.G., Collet, C., Phillis, R.W., Nielsen, K.M., Russell, R.J., Chambers, G.K., Ross, V. and Richmond, R.C. (1987) Molecular cloning and characterisation of esterase-6, a serine hydrolase of <u>Drosophila</u>. Proc. Natl. Sci. USA <u>84</u>, 3359-3363.

Pen, J., van Beeuman, J. and Beintema, J. (1986) Structural comparison of two esterases from <u>Drosophila mojavensis</u> isolated by immunoaffinity chromatography. Biochem. J. <u>278</u>, 691-699.

CHARACTERIZATION OF THE CINNABAR LOCUS OF DROSOPHILA MELANOGASTER

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The *cinnabar* locus of *Drosophila melanogaster* encodes the enzyme kynurenine hydroxylase. The synthesis of this enzyme is rigidly controlled during development and is directly involved in the production of the brown eye pigment xanthommatin. We have undertaken the cloning and characterization of this locus to further our long term study of the coordinated regulation of the genes involved in xanthommatin production.

The *cinnabar* locus has been isolated following a short chromosome walk within polytene region 43E. Southern blot analysis of genomic DNA has shown that of the eleven *cinnabar* alleles investigated to date, seven have restriction pattern alterations. Four alleles have been shown to be associated with small deletions and three with insertions. All of these changes fall within a region of approximately four kilobases. This region is also absent in deletions that uncover *cinnabar* and neighbouring complementation groups. Northern blot analysis of this region is currently in progress.

GENETICS OF THE ROUGH EYE MUTATION IN DROSOPHILA MELANOGASTER

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A previously uncharacterized homeobox containing gene has been mapped to cytological location 97D2-6.

In order to elucidate the role of this new homeobox sequence we tested initially for correspondence with existing visible, lethal and deletion mutations in the region using Southern analysis. A correspondence with the eye mutation rough was implicated, and then confirmed by sequence analyis of our EMS induced rough allele from a defined isochromosomal line (see Elizur et al. this volume).

As part of the determination of the structure and organization of the <u>rough</u> gene our genetic strategy has been to induce a number of P-element <u>rough</u> alleles, and then to correlate the phenotype with the site of P insertion. That some of the alleles already generated differ in the degree to which they roughen the eye suggests this strategy will be informative.

Our evidence to date is that rough is different from all other homeobox genes in that it is not a vital locus - so far, all alleles generated are homozygous viable. To test this important point we are saturating the region of 97D with EMS and P-element induced lethal alleles. Such analyses also will better define the genetic neighbourhood of rough. Already from this screen we have identified one closely linked semi-lethal locus whose phenotype includes roughening of the eye in adults and abnormalities of anterior structures in embryos.

CHARACTERISATION OF THE GENOME OF THE LIVER FLUKE FASCIOLA HEPATICA

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Fasciola hepatica is an economically important parasite to the sheep and cattle industries in Australia causing millions of dollars loss in productivity per year. Cattle become resistant to reinfection but sheep develop no effective immunity and remain susceptible throughout life. Antibodies are produced by sheep but these have no deleterious effects on fluke survival which militates against production of a vaccine. Administration of anthelmintics has had little effect in controlling the parasite. This study was undertaken in order to gain a better understanding of the basic genomic structure and organisation of *F. hepatica* which may aid in the development of novel strategies for the control of this parasite.

Repeated sequences were isolated from a *F. hepatica* genomic library and one was partially characterised. The ribosomal RNA gene was isolated using *Drosophila melanogaster* ribosomal RNA genes and a preliminary restriction map was constructed and compared with a map of the ribosomal RNA gene of *Schistosoma mansoni* A cDNA clone of a sequence coding for a fluke antigen was used to isolate the genomic equivalent of the antigen coding sequence. A preliminary restriction map was constructed and compared with the restriction map of the cDNA clone to detect the presence of introns.

The basic structure of the genome of *F. hepatica* seems to be similar to that of other eukaryotes. Thus it would appear that techniques applicable to studies of gene regulation in a number of organisms could be applied to the liver fluke, with a view to gaining an understanding of how some control over gene expression in the organism might be achieved.

CLONING AND MOLECULAR CHARACTERIZATION OF A DOMINANT-MALE SEX DETERMINING GENE

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By microdissection and cloning of the arm G male determiner (MD) of *Chironomus oppositus* f. *tyleri*, a 4.1 kb clone was identified which showed differential binding to male and female *Chironomus* DNA on Southern analysis. Probing a second gene bank of *Chironomus* male DNA generated another clone which contained the original sequence and a 1.7 kb (EcoRI-BamHI) adjacent sequence which was subcloned into pUC19. In *in-situ* hybridization to polytene chromosomes from *Chironomus* species with the MD gene in different chromosomal positions, the 1.7 kb fragment hybridizes to one homologue only on the chromosome arm known to contain the MD (e.g. band 15D1 on the A2 homologue of *Ch. oppositus* f. *connori*), with further faint hybridization at about 18D4 on arm G. The fragment therefore presumably contains mostly the MD but also contains a short sequence not related to the MD gene.

When hybridized to male and female DNA of other species of Diptera and mammals on Southern transfers, there appears to be a band common to male DNA, which does not occur in female DNA. There are bands of hybridization in female DNA from *Chironomus* and other Diptera, but little or no homology in female DNA of mammals.

The DNA contained in this 1.7 kb fragment, as well as in the 4.1 kb adjacent EcoRI fragment, believed to contain a small part of the MD gene, is being further characterized by restriction mapping and sequencing. This MD, occurs in different chromosomal locations within and between species and forms.

The differential occurrence of somatic crossing over in somaclonal populations of different origins.

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Two populations of somaclonal plants of *Phalaris coerulescens* (a diploid perennial grass) have been derived. One of these of 37 plants was obtained from a callus culture initiated from an immature embryo. The other population of 99 plants was derived from a callus initiated from a germinated seedling.

Isozymic variation for four enzyme systems was examined in these populations.

The data are shown in Table 1.

Table 1.

Isozyme genotypes present in the two differently derived somaclonal

Source of somaclonal plants	Diaphorase		Leucine amino peptidase		Aconitase			Peroxidase		
	1-3	3-3	1-1	1-2	1-1	1-2	2-2	1-1	1-2	2-2
Immature embryo	13	24	-	24	1	10	26	26	11	-
Germinated seedling	99	-	99		-	99	-	-	-	99

populations of Phalaris coerulescens.

There is extensive variation present at three of the four loci in the population derived from the immature embryo callus. The most likely source of this variation is somatic crossing over during the initiation of the callus culture.

The much larger population derived from callus initiated from a germinated seedling shows no variation at two of the loci for which a similar potential exists.

Chromosome studies of the two populations are incomplete.

EVOLUTIONARY RELATIONSHIPS OF BRACKEN FERN (PTERIDIUM) TAXA BASED ON ANALYSIS OF THE CHLOROPLAST GENOME

M.K. TAN & J. THOMSON School of Biological Sciences University of Sydney NSW 2006

The cosmopolitan bracken ferns of the genus <u>Pteridium</u> comprise a conservative group of taxa variously regarded as monospecific or as an assemblage of perhaps 10-14 entities of undecided status. Major divergence in the genus appears to have occurred subsequent to the separation of the continental land masses derived from Laurasia in the Northern Hemisphere, where <u>P. aquilinum</u> <u>sensu stricto</u> predominates, and Gondwana in the Southern Hemisphere, where <u>P. revolutum</u> and <u>P.</u> esculentum are major taxa.

A clone collection of living bracken representative of the geographic distribution of the genus world-wide has been established in Sydney, chiefly from spore samples. The initial studies of the evolutionary relatonships amongst 8-10 putative taxa represented in this collection have centred on comparative restriction mapping of the chloroplast genome. P. aquilinum (Yamunotri Hills, India) and P. esculentum (Sydney, NSW), for example, appear similar for the regions of the chloroplast genome including the photosystem II genes Psb A, Psb B and Psb C/D, for which probes were derived from cloned spinach sequences kindly provided by Dr P. Whitfeld (CSIRO, Canberra). Some sequence divergence is apparent in the Psb B and Psb C/D regions of the chloroplast genomes from P. aquilinum (S. Africa) and P. esculentum (Sydney, NSW). In contrast the taxa examined so far show significant sequence divergence in other segments of the chloroplast genome. To facilitate study of the less conserved regions, 11-12 <u>Hind III fragments of P. esculentum</u> chloroplast DNA have been cloned into pUC 13 and are being used as probes on single and multiple restriction digests to map the regions distinguishing these Such taxa. comparisons will then be extended to other geographically separated bracken forms and the results will be correlated with sequence analyses of nuclear and chloroplast encoded rRNAs.

GENETICS OF CYANOGENESIS IN AUSTRALIAN BRACKEN (Pteridium esculentum): AN UPDATE

<u>V.H.K. Low</u> and J.A. Thomson, School of Biological Sciences, University of Sydney.

Preliminary studies of the cyanogenic potential of field-collected Pteridium from 30 localities throughout Tasmania and 79 throughout N.S.W. reported in 1987 showed that 41% of the Tasmanian fronds and 23% of the N.S.W. fronds were cyanogenic. Attention was drawn in our report last year to the much lower frequency of cyanogenic fronds from tub-grown clones compared to field stands. It was also shown that β -glucosidase was inducible by physical (shading, crushing, wilting) and chemical (p-phenylfluoroalanine and sodium arsenite) treatment in some genotypes, but constitutive or absent in others.

Here we present the result of more detailed characterisation of the representative clone collection with respect to the presence of enzyme and/or substrate. Attempts to identify factors responsible for controlling prunasin synthesis and accumulation are reported. These results contribute to a general model of cyanogenesis which may explain the widely disparate findings on the stability of cyanogenic/acyanogenic phenotypes reported in other species of Pteridium.

GENETICS AND POPULATION STRUCTURE IN THE ENDEMIC AUSTRALIAN SLUG FAMILY CYSTOPELTIDAE(MOLLUSCA:PULMONATA)

A. J. DANIELL

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The **Cystopeltidae** is one of the only two endemic Australian land snail families and its members are the only native slugs found in south eastern Australia. They are common throughout Tasmania, much of Victoria and along the Great Dividing Range and coastal areas of N.S.W. The slugs are found in a wide range of habitats from coastal swamps to mountain forests. Despite being the most abundant largesized mollusc in these habitats little is known of their biology or species composition. Current research shows them to be unusual in many aspects of their biology and behaviour, in particular their reproductive strategies. Allozymes have been used, along with other characters,

Allozymes have been used, along with other characters, to clarify species status as well as to investigate population structure. Species are readily distiguished by fixed allelic differences, and have been found to be generally parapatrically distributed. Populations show low levels of heterozygosity at polymorphic loci. Several mechanisms may cause this. Because pulmonates are hermaphrodites this low level of heterozygosity may be due to self-fertilization. As well, neighbourhood sizes may be small, leading to localized inbreeding.

ZONEMODEL: A COMPUTER PACKAGE FOR INVESTIGATING GENETIC INTERACTIONS IN HYBRID ZONES

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Hybrid zones between parapatric taxa, maintained by balance between selection against hybrids and continual immigration from the parental populations are some of the more spectacular phenomena of natural population genetics, and are arguably important contributors to reproductive isolation between incipient species. Zonemodel is a computer package based around a program which simulates hybridization between parapatric taxa. A transect of populations is set up, each containing electronic individuals of one or other of two types. These individuals have completely defined genotypes, and during sucessive generations they migrate and hybridize, with hybrid genotypes being constructed using stochastic functions and rules of genetic To simulate natural hybrid zones under transmission. investigation, the user may define genetic systems (Mendelian and cytoplasmic genes, linkage, etc.), selection regimes (on certain genotypes, sometimes with reference to positon in the transect -"environment"), sex-determining systems, mating behaviours, and migration patterns. Gene and genotype frequencies, and patterns of linkage disequilibrium, can then be observed in successive generations.

Two phenomena which have been studied in detail with **Zonemodel** are the evolution of assortative mating (the Wallace effect), and the trailing of neutral nuclear and cytoplasmic markers by a moving hybrid zone between two subspecies of *Caledia captiva* (Orthoptera).

HYBRIDIZATION AND GENE INTROGRESSION IN TWO SPECIES OF ABALONE

Lindsay Brown

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The genetic population structures of Blacklip abalone (<u>Haliotis rubra</u>) and Greenlip abalone (<u>Haliotis laevigata</u>) are being investigated using protein electrophoresis. As well, the existence of rare individual abalone that are phenotypically intermediate between these two commonly fished species is well known to abalone divers. Electrophoresis shows that these animals are in fact genuine F, hybrids.

There is also evidence that hybrids are fertile, and may backcross to parent species, in that possible localized introgression of Blacklip genes into Greenlips and vice versa has been identified. The difficulty of distinguishing between introgression, convergent evolution, and the persistence of ancestral alleles will be emphasised, as will the implications for phylogenetic inference.

GENETIC VARIATION WITHIN AND AMONG POPULATIONS OF REEF CORALS.

David J. Ayre and Sandra Dufty

Department of Biology, University of Wollongong P.O.Box 1144, Wollongong, N.S.W. 2500

Electrophoretic surveys were carried out within populations of the reef corals *Seriatopora hystrix* and *Acropora palifera* from 9 different reefs in the central region of the Great Barrier Reef. These data have been used to assess the genetic connectedness and genotypic structure of local populations. For each reefs samples were collected from at least two depths and four habitat types.

Our preliminary analysis of the data for *S. hystrix* has revealed a population structure which is consistent with predominantly sexually generated recruitment and much stronger connectedness within than between reefs. Consistent and highly significant heterozygous deficits seem likely to reflect the effect of further subdivision of breeding groups. Interpretation of the results for *A. palifera* are confounded by the discovery that most samples include representatives of the morphologically similar *A. cunneata*. However, populations of both these species apopear to be maintained by sexually generated recruits. These species show greater genetic variation among reefs than *S. hystrix* and it is inferred that larval dispersal over tens of kilometres plays an important role in determining their population structures.

MITOCHONDRIAL DNA POLYMORPHISM IN THE DEEP SEA FISH, ORANGE ROUGHY (*HOPLOSTETHUS ATLANTICUS*).

Jennifer R. Ovenden, Adam J. Smolenski and Robert W.G. White

Fish Research Group, Zoology Department, University of Tasmania, Hobart 7001.

Ten restriction enzymes were used to survey the base sequence variation of the mitochondrial genome of 49 orange roughy (<u>Hoplostethus atlanticus</u>) collected from the east and west coasts of Tasmania. The mean diversity between all 49 genomes was a low 0.59%. Eleven different haplotypes were identified. The common haplotype was present at frequencies of 0.74 on the east coast and 0.65 on the west coast. Each of ten rare haplotypes were represented by one to three individuals only. The west coast population contained more rare haplotypes than the east coast population which suggests that the west coast. Rare haplotypes were completely sub-divided between either the east or west coast. We are currently investigating the hypothesis that the migration rate between localities is low.

MITOCHONDRIAL DNA VARIATION IN THE CRESTED NEWT SUPERSPECIES (CAUDATA: SALAMANDRIDAE): INSERTION POLYMORPHISM AND LIMITED CYTOPLASMIC GENE FLOW AMONG SPECIES.

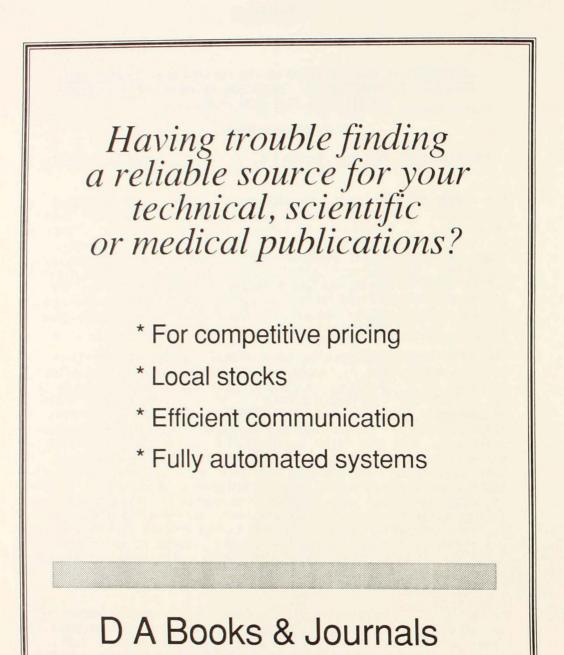
Graham Wallis

Department of Zoology, University of Otago, PO Box 56, Dunedin.

The crested newt has a widespread European distribution and encompasses four taxa recently elevated to full species: *Triturus* cristatus, *T. carnifex*, *T. dobrogicus and T. karelini*. These are distinct on morphological, chromosomal and isozymic grounds, and have fairly sharp transition zones. A widespread survey (12 countries, 49 geographic sites, 210 individuals) of mtDNA variation (20-27 restriction enzyme sites mapped per individual) was made to: (1) correlate mtDNA variation with morphological features defining the species (2) determine the degree of differentiation within and among species (3) detect any introgression among species.

The mtDNAs of each species were clearly differentiated (d = 3.9-7.1%). Additionally, geographic structuring was observed within T. carnifex and T. karelini, each displaying 2 divergent mt genome types $(\overline{d} = 3.5\%$ and 4.7% respectively). The other two (more northerly distributed) species were genetically homogeneous over most (7. cristatus) or all (T. dobrogicus) of their ranges. In the case of T. cristatus, one may infer bottlenecking as a result of Pleistocene glaciation events. This may also apply in part to T. dobrogicus, but high population connectedness and gene flow in this lowland river species may alone be sufficient for homogenization of mtDNA. Patterns of mtDNA variation were largely concordant with morphology; some interspecific mt gene flow was observed, but only very close to, or in, the transition zones. Analyses of restriction site data by UPGMA and parsimony methods (using the closely related T. marmoratus as an outgroup) produced very similar dendrograms. The levels of divergence found concur with the systematics of the group, but the differentiation within T. carnifex and T. karelini is notable.

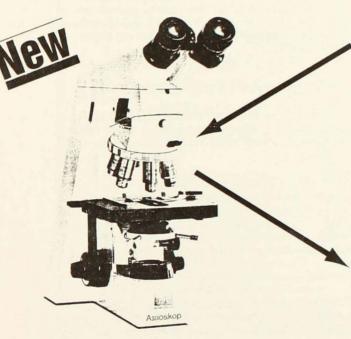
In addition to restriction site variation, several major length variants (>40bp) mapping to the control region of the molecule were found within and among populations. In one extreme case, an individual had a >50% (8500bp) reiteration of the genome. Another 2 individuals were found to be heteroplasmic for 2- and 3-fold duplications of a 1100bp sequence. Length variation has been described in the mt genomes of other poikilothermic vertebrates, contrasting with those of mammals which generally show only subtle length differences. It is important to discriminate between this and restriction site variation when analysing mtDNA, otherwise gross overestimates of nucleotide divergence may result. Length variants may present useful markers in natural populations where restriction site variation is limited.



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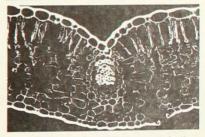
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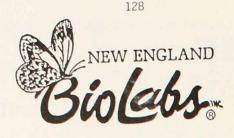
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