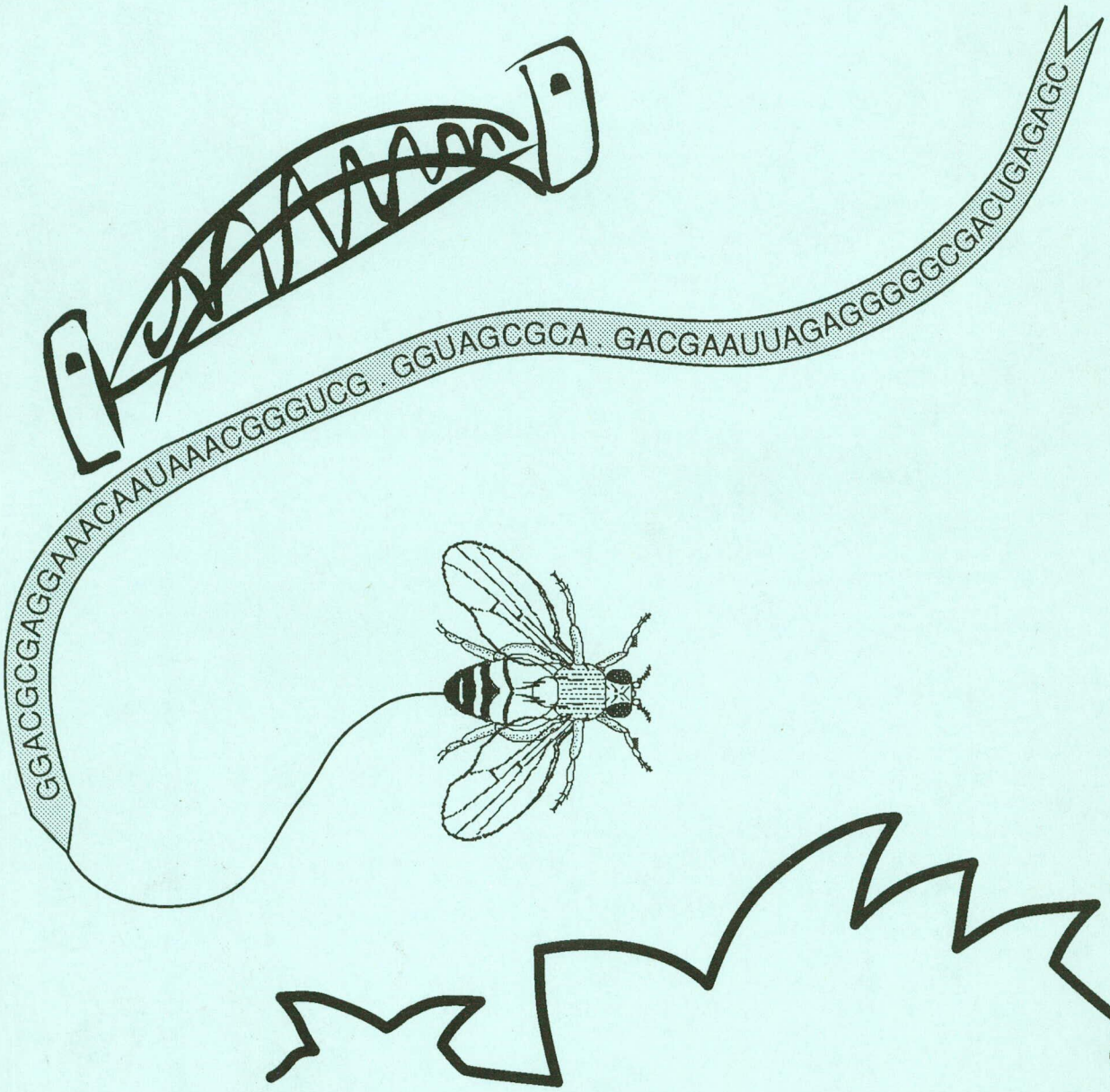
Genetics Society of Australia

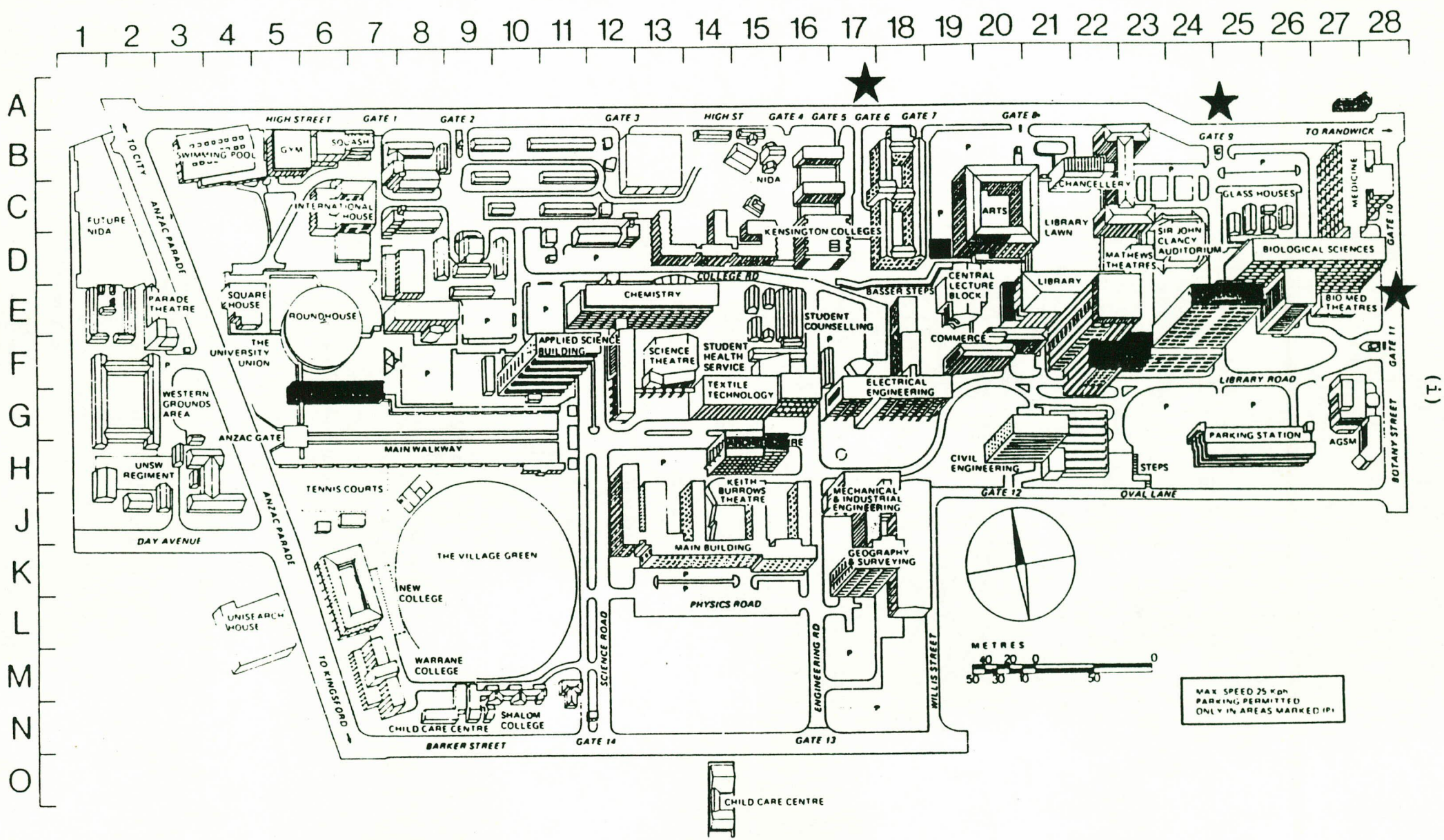
36th Annual Conference

PROGRAMME & ABSTRACTS

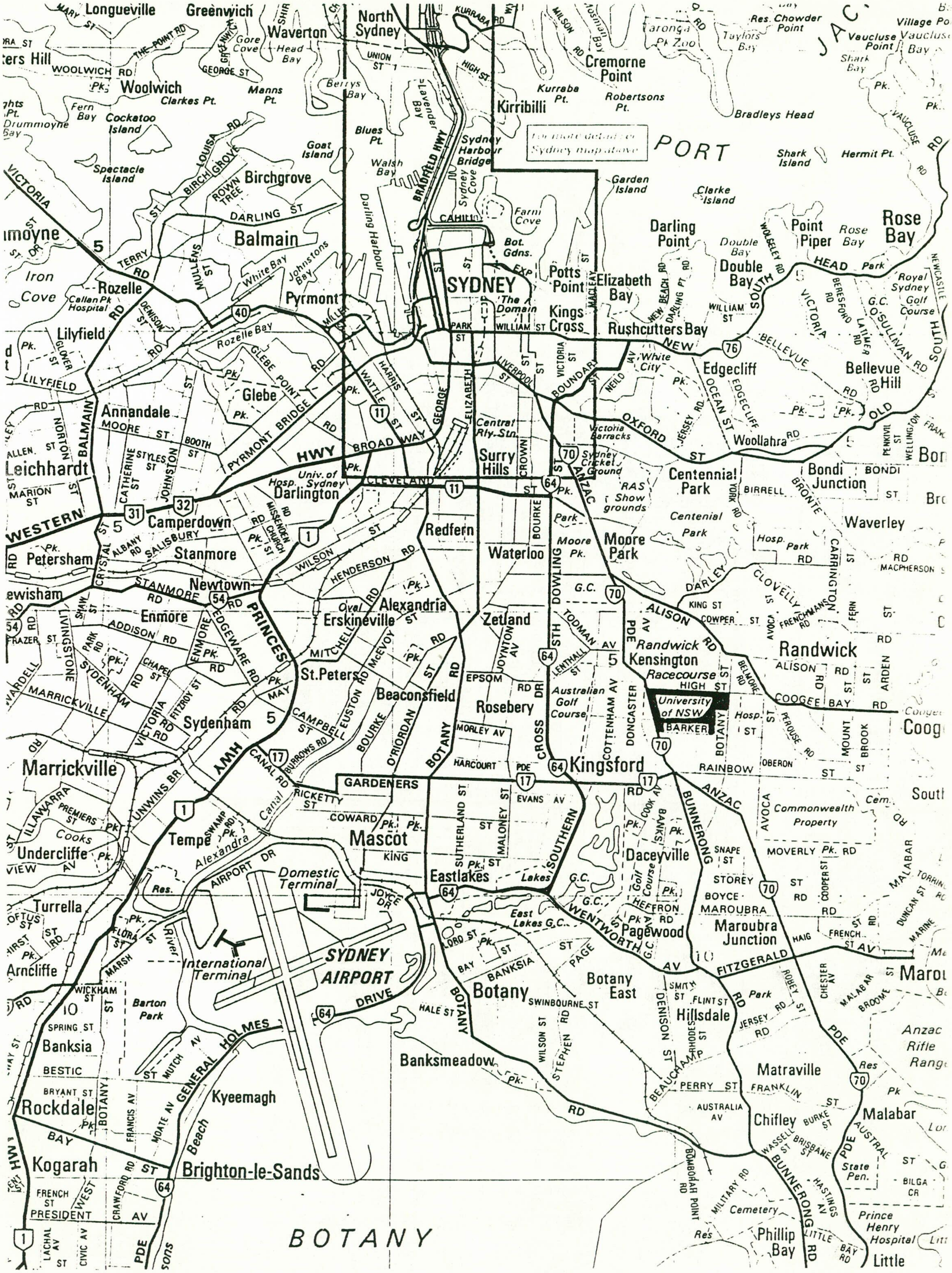


University of New South Wales, Kensington
2 - 5 July, 1989

The University of New South Wales Kensington Campus



*Biological Sciences Building D26 *Biomedical Lecture Theatres E27 *Kensington Colleges C17



Longueville
Greenwich
Waverton
North Sydney
Woolwich
Kirribilli
Cremorne Point
Taronga Pk Zoo
Bradleys Head
PORT JACKSON
Cockatoo Island
Goat Island
Spectacle Island
Birchgrove
Balmain
Garden Island
Clarke Island
Shark Island
Darling Point
Point Piper
Rose Bay
Rozelle
Pyrmont
SYDNEY
Potts Point
Elizabeth Bay
Kings Cross
Rushcutters Bay
Double Bay
Lilyfield
Glebe
Edgecliff
Bellevue Hill
Annandale
Woollahra
Leichhardt
Surry Hills
Centennial Park
Bondi Junction
Darlington
Camperdown
Petersham
Stanmore
Redfern
Waterloo
Moore Park
Waverley
Newtown
Alexandria
Erskineville
Enmore
Zetland
Randwick
Kensington
Racecourse
University of NSW
St.Peters
Beaconsfield
Rosebery
Sydenham
Marrickville
Kingsford
Tempe
Mascot
Undercliffe
Eastlakes
Daceyville
Turrella
Arncliffe
Pagewood
Maroubra Junction
Botany East
Botany
Hillsdale
SYDNEY AIRPORT
Domestic Terminal
International Terminal
Banksia
Banksmeadow
Matraville
Kyeemagh
Rockdale
Chifley
Malabar
Kogarah
Brighton-le-Sands
Phillip Bay
Little Bay
BOTANY

CONTENTS

CONFERENCE INFORMATION

WELCOME

The organising committee welcomes delegates to the 36th Annual Meeting of the Genetics Society of Australia. We wish you an enjoyable and informative meeting. If you have any questions or difficulties please contact us and we will endeavour to help. The committee comprises:

Ross Crozier
Ian Dawes
Chris Gillies
Chris Moran
John Sved

REGISTRATION

The Conference fee is $40 (Students $20). Student members of the Genetics Society of Australia who present a paper or poster have their registration fee waived, and are eligible to apply for a travel subsidy if they are from outside Sydney. The travel subsidy will be determined on a *pro rata* basis based on number of students and distance travelled.

A registration desk will be operated at the Mixer on Sunday night 2 July, and thereafter will be in the Foyer of the Biomedical Theatres before the commencement of sessions and at breaks. Members in arrears with their fees are encouraged to pay these also at the registration desk.

ACCOMMODATION

On campus accommodation for those who requested it has been allocated for the nights of 2-4 July, at the Kensington Colleges (Basser College), High Street, Gate 6 (see map). This is a short walk from the Biomedical Buildings. Single rooms are $29.50 per night, including breakfast. All payments for accommodation must be made at the Registration Desk. Please pay on arrival if you have not prepaid. Check out time on Wednesday 5 July is 10 am. If necessary, arrangements will be made for safe storage of baggage until lunch time on Wednesday.

PARKING

Parking is extremely limited in College grounds, but should be free within the University grounds proper if you mention that you are a GSA delegate. On Sunday only Gate 14 in Barker Street is open for entry to University grounds.

MEALS

Breakfast: is provided for those staying in college

Lunch: on campus

Cafeteria in basement Australian Graduate School of Management (AGSM) - near Biomed Lecture Theatres
Cafeteria in Matthews Building, also nearby
Golf House, cnr Botany & High Streets - make your own sandwiches
Staff Club, top of Electrical Engineering Building (licenced) - delegates will have honorary membership for conference
Roundhouse - various cafeterias etc

Dinner: on campus

Roundhouse and Matthews remain open

Lunch & Dinner off campus

Numerous takeways, fastfoods and restaurants are to be found adjacent to the campus, on Belmore Road, Anzac Parade and St. Pauls Road
Farther afield are unlimited opportunities.

BANKS & SHOPS

Commonwealth Bank and State Bank of NSW have branches on campus near the base of the Library Tower.
Most other banks have branches nearby off campus, particularly along Belmore Road.
Nearest local shopping is in Belmore Road.

SWIMMING POOL

A heated swimming pool is available on campus with normal entry fees for delegates.

PUBLIC TRANSPORT

Buses from Central Railway in the city which pass the UNSW are:

393 LaPerouse & 395 Maroubra Beach/Junction - along Anzac Pde, alight at High St., Kensington.

372 Cogee Beach - along Alison & Belmore Rds, alight at Prince of Wales Hospital, High St. Corner, Randwick.

TELEPHONE MESSAGES

College Number (02) 663 8111

Biological Sciences Office (02) 697 2067

EMERGENCY NUMBERS

First Aid (on campus) 2004 internal extension

Security (on campus) 2200 or 2221 internal extensions

Prince of Wales Hospital 399 0111

SCIENTIFIC SESSIONS

PAPERS

All sessions will be held in the Biomedical Lecture Theatres A & C. Contributed papers will be 15 minutes in length, with an additional 5 minutes for questions. Chairpersons will keep strictly to time to permit switching between parallel sessions. Invited papers are of 25 minutes duration plus 5 minutes for questions. Facilities will be available for the projection of 35mm slides and overhead transparencies. Slides should be given to the projectionist at least 10 minutes before the start of the session for loading into the carousels. Speakers should contact the Chairperson prior to the start of their session.

POSTERS

Posters will be displayed on Monday 3 July and Tuesday 4 July in the Foyers of the Biomedical Lecture Theatres. Authors should set up their posters by lunch time on Monday, and be in attendance during the poster sessions on both afternoons. Each poster display should fit into an area 1m X 1m, and be attached with drawing pins to the boards provided. Posters should be removed after 5pm on Tuesday 4 July.

TEA & COFFEE

These will be available at the morning and afternoon breaks in the Foyers of the Biomedical Theatres - outside Lecture Theatres A & C, and on the floor below.

JOB NOTICES ETC

A Notice board for Jobs and other notices will be provided in the Biomedical Theatres Foyer.

STUDENT TRAVEL SUBSIDY

Each student member from outside Sydney presenting a paper or poster is eligible for a travel subsidy based on the distance travelled. If you are eligible please check at the Registration Desk to ensure they you included. Cheques will be issued by the Treasurer on Tuesday afternoon. Cheques not collected will be forwarded by mail.

TRADE DISPLAYS

Trade displays will be presented in the foyers adjacent to the Tea & Coffee and Poster areas. The following companies have supported the Genetics Society by mounting Trade Displays - please remember to visit their stands.

Disposable Products Pty Lty
Representative: Ms Robyn Howson
NSW Branch Manager
PO Box 182
Botany NSW 2019

Dupont (Aust) Pty Ltd
Representative: Mr Mark Melville
Product Marketing Specialist
Paul Steet
North Ryde NSW 2113

Millipore Pty Ltd
Representative: Ms Ngaire Baker
Promotions
Private Bag 18
Lane Cove NSW 2066

Pharmacia (Australia) Pty Ltd
Representative: Mr Chris Becker
National Sales Manager
PO Box 289
Carlton South Vic 3053

Phoenix Scientific Industries Ltd
Representative: Ms Sue Laird
Marketing
PO Box 118
Mt Waverly Vic 3149

Trace Scientific Pty Ltd
Representative: Mr Nick Thliveris
Sales and Marketing
PO Box 494
Baulkham Hills NSW 2153

SOCIAL FUNCTIONS

MIXER
7.00-10.00pm Sunday 2 July, 6th Floor Biological Sciences Building. Enter by the main door facing High Street (see map). Beer, wine, soft drinks and snacks will be provided at no charge to registrants. The Registration Desk will be located at the Mixer on Sunday night.

ANNUAL DINNER
7.30pm Tuesday 4 July, Goldstein Hall, Kensington Colleges (see map). Tickets are $20 for Student Members, $30 for others, which covers predinner drinks, a hot meal with house wine, and coffee. A bush band will provide music for dancing after dinner. A limited number of unreserved tickets are available and may be purchased at the Registration desk on a first come basis.

BARBEQUE
12.30pm Wednesday 5 July. Tickets $10 to cover food and drinks. Depending on the weather, this will be held either at a campus venue, or in Centennial Park, which is close by. Members with planes to catch later in the afternoon should indicate at registration the departure times of their flights. At least 30 minutes should be allowed for transport to the airport.

ACKNOWLEDGEMENTS

The organising committte would like to thank the University of New South Wales for hosting this meeting. We would also like to acknowledge the help of many members of the staff from departments at the University of New South Wales and the University of Sydney who have been involved in various aspects of the organisation.

We would like to acknowledge the financial and material help provided by Ansett Airlines.

The support of the Sustaining Members who mounted trade displays was also very welcome.

SUSTAINING MEMBERS - 1989

GENETICS SOCIETY OF AUSTRALIA

Bio-Rad Laboratories Pty Ltd
Contact: Mr John Hewetson
Div Manager-Research Products
PO Box 371
North Ryde NSW 2113

Dupont (Aust) Pty Ltd
Contact: Mr Mark Melville
Product Marketing Specialist
Paul St
North Ryde NSW 2113

Carl Zeiss Pty Ltd
Contact: Mr Ronald Bloomberg
Manager-Scientific Instruments
PO Box 147
Camperdown NSW 2050

Millipore Pty Ltd
Contact: Ms Ngaire Baker
Promotions
Private Bag 18
Lane Cove NSW 2066

Crown Scientific
Contact: Ms Peggie Cordner
Marketing Co-ordinator
Private Mail Bag 8
Moorebank NSW 2170

Boehringer Mannheim Pty Ltd
Contact: Mr Rob Wishnowsky
Business Unit Manager
PO Box 316
North Ryde NSW 2113

The Macmillan Co of Aust
Contact: Ms Helen Jones
Promotions Manager
Locked Bag 12
South Melbourne Vic 3205

Disposable Products Pty Ltd
Contact: Ms Robyn Howson
NSW Branch Manager
PO Box 182
Botany NSW 2019

Becton Dickinson Pty Ltd
Contact: Ms Andrea Westwood
Product Manager-Diagnostic Div
100 Miller St
North Sydney NSW 2060

BDH Chemicals (Aust) Pty Ltd
Contact: Mrs Ruth Frydman
Product Manager
207 Colchester Rd
Kilsyth Vic 3137

Cambridge University Press
Contact: Mr Tony Davies
Deputy Director
PO Box 85
Oakleigh Victoria 3166

Oxford University Press (Aust)
Contact: Mr Peter Rose
Marketing Manager (Sci & Med)
GPO Box 2784Y
Melbourne Victoria 3001

Genesearch Pty Ltd
Contact: Dr Bonni Y Reichelt
Research Director
14 Technology Drive
Gold Coast Tech Park Qld 4215

Beckman Instr (Aust) Pty Ltd
Contact: Miss Karen Smith
National Sales & Marketing
24 College St,Gladesville NSW 2111

Oxoid Australia Pty Ltd
Contact: Mr Venda V Divin
Sales Director
104 Northern Road
West Heidelberg Victoria 3081

Promega Corporation
Contact: Ms Ghislaine Samways
Scientific Manager
PO Box 10
Rozelle NSW 2039

Phoenix Scientific Industries
Contact: Ms Sue Laird
Promotions
PO Box 118
Mt Waverly Vic 3149

Annual Reviews Inc
Contact: Mrs Mickey G Hamilton
Advertising/Promotion Manager
4139 El Camino Way
Palo Alto California 94306
USA

Pharmacia (Australia) Pty Ltd
Contact: Mr Chris Becker
National Sales Manager
PO Box 289
Carlton South Victoria 3053

Amersham Australia Pty Ltd
Contact: Ms Tracey Anne James
Sales Manager–Research Products
7 Lyon Park Rd
North Ryde NSW 2113

Elsevier Publications
Contact: Ms Loiuse Morse
Advertising Manager–Trends In Genetics
68 Hills Road
Cambridge CB2 1LA
United Kingdom

Trace Scientific Pty Ltd,
Contact: Mr Nick Thliveris
Sales and Marketing
PO Box 494
Baulkham Hills NSW 2153

John Morris Scientific
Contact: Ms Martine Whiteley
Advertising/Promotions Manager
PO Box 447
Willoughby NSW 2057

Selby Anax Pty Ltd
Contact: Mr Tim Fleming
Advertising Manager
Locked Bag No 65
Lidcombe NSW 2141

Gelman Sciences Pty Ltd
Contact: Mr Alex Tymson
Group Sales & Marketing Manager
PO Box 456
Lane Cove NSW 2066

List of Registrants

ADCOCK	Greg	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
ADELSON	David	CSIRO Div Animal Prod., P.O. Box 239, Blacktown 2148
AGROTIS	Alex	Genetics Dept., Monash Univ, Clayton 3168
ANDREWS	Justen	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
APPELS	Rudi	CSIRO Div. Plant Industry, G.P.O. Box 1600 Canberra 2601
ARMSTRONG	Tim	Animal Sci Dept, Univ. New England, Armidale 2351
ATKINSON	Peter	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
BABISTER	Lynda	Biological Sciences, Univ. N.S.W., Kensington 2033
BARRY	Simon	Botany Dept, ANU, P.O. Box 4, Canberra City 2601
BATTERHAM	Phil	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
BEDO	Dan	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
BLACKBURN	Liz	Mol. Biol., Univ. California Berkeley, Calif., 94720 U.S.A.
BLICK	Tony	Genetics Dept, Monash Univ, Clayton 3168
BRADLEY	Mark	Biochemistry Dept, ANU, P.O. Box 4, Canberra City 2601
BRADY	Michelle	Biological Sciences, Univ. N.S.W., Kensington 2033
BRENNAN	Francine	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
BROWN	Tony	CSIRO Div. Plant Industry, G.P.O. Box 1600 Canberra 2601
BRUZZESE	Rosalie	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
BULL	Ann	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
CALLAGHAN	M.	McMaster Lab, CSIRO An. Health, Univ. Sydney B.02, NSW 2006
CAM	Graham	CSIRO Div Animal Prod., P.O. Box 239, Blacktown 2148
CARTHEW	Susan	Biology Dept., Univ. Wollongong, P.O. 1144 , Wollongong 2500
CHANG	Andy	Biological Sciences, Macquarie Univ., NSW 2109
CHEN	Zhenzhong	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
CHRISTIAN	Pete	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
CLEGG	Mike&spouse	Botany Dept., Univ. California Riverside, Calif., 92521 U.S.A.
COBBETT	Chris	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
COLGAN	Don	Australian Museum, P.O. Box A285, Sydney South 2000
COLLET	Chris	CSIRO Div. Wildlife & Ecology, P.O. Box 84, Lyneham ACT 2602
COLLIS	Christine	CSIRO Div. Biotechnology, P.O. Box 184, North Ryde 2113
COOK	Alex	63 Beecroft Road, Beecroft 2119
COOPER	Des	Biological Sciences, Macquarie Univ., NSW 2109
COWMAN	Alan	Walter & Eliza Hall Inst., P.O. Roy.Melb.Hospital, Melbourne 3050
CROSS	Tracey	RSBS, ANU, P.O. Box 475, Canberra, ACT 2601
CROZIER	Ross	Biological Sciences, Univ. N.S.W., Kensington 2033
DAI	Kang	Biological Sciences A.12, Univ. Sydney, Sydney 2006
DARVEY	Norman	Agric. Genetics A.05, Univ. Sydney, Sydney 2006
DAVIES	Andrew	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
DAVIS	Meryl	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
DAWES	Ian	Biochemistry School, Univ. N.S.W., Kensington 2033
DAY	Kym	Botany Dept, ANU, P.O. Box 4, Canberra City 2601
DELBRIDGE	Margaret	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
DIXON	Patricia	Marine Science Centre, Univ. N.S.W., Kensington 2033
DOLEZAL	Olan	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
DOLLIN	Anne	CSIRO Div Animal Prod., P.O. Box 239, Blacktown 2148
DOYLE	Jeff&spouse	Bailey Hortorium, Cornell Univ., Ithaca, NY, 14853-4301 U.S.A.
DRINKWATER	Roger	CSIRO Div Trop Animal Prod, P.O. Box 5545 Rockhampton 4702
DRIVER	Chris	Victoria College Rusden Campus, 662 Blackburn Rd, Clayton 3168
EAST	Peter	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
FABB	Stewart	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
FARRELL	Rohan	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
FOLEY	Debra	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
FOLEY	Rhonda	McMaster Lab, CSIRO An. Health, Univ. Sydney B.02, NSW 2006
FORD	Judy	Genetics Dept, Queen Elizabeth Hospital, Woodville, SA 5011
FOSTER	Geoff	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601

FOSTER	Jamie	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
FRANKLIN	Ian	CSIRO Div Animal Prod., P.O. Box 239, Blacktown 2148
FRIPP	Yvonne	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
FROMMER	Marianne	CSIRO Lab. Molecular Biology, P.O. Box 184, North Ryde 2113
FROST	Colin	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
GAME	Anne	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
GARDINER-GARDEN	Margaret	CSIRO Lab. Molecular Biology, P.O. Box 184, North Ryde 2113
GEORGIOU	Jasmine	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
GFELLER	Astrid	Biological Sciences, Univ. N.S.W., Kensington 2033
GILLIES	Chris	Biological Sciences A.12, Univ. Sydney, Sydney 2006
GOOLEY	Andrew	Biological Sciences, Macquarie Univ., NSW 2109
GRIFFITHS	Bob	Mathematics Dept., Monash Univ, Clayton 3168
GRIGG	Geoff	CSIRO Lab. Molecular Biology, P.O. Box 184, North Ryde 2113
GRIZIOTIS	George	Biological Sciences, Univ. N.S.W., Kensington 2033
HAIG	David	Biological Sciences, Macquarie Univ., NSW 2109
HANRAHAN	Cathy	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
HARRY	Jenny	Biological Sciences, Macquarie Univ., NSW 2109
HAYMAN	David	Genetics Dept., Univ. Adelaide, Adelaide SA 5001
HOWDEN	Ross	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
HOWELLS	Tony	Biochemistry Dept., ANU, P.O. Box 4, Canberra City 2601
HULME	Dennis	McMaster Lab, CSIRO An. Health, Univ. Sydney B.02, NSW 2006
JIANG	C. Mr.	Mol Pop Genetics, RSBS, ANU, P.O. Box 475, Canberra, ACT 2601
JONES	Graham	Biological Sciences A.12, Univ. Sydney, Sydney 2006
KARAGIORGAKIS	Kristine	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
KAROTAM	Jill	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
KATZ	Margaret	Microbiology Dept., Monash Univ, Clayton 3168
KELLY	Len	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
KING	Leanne	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
KORNFELD	Geoff	Biochemistry School, Univ. N.S.W., Kensington 2033
KRISHNAPILLAI	Viji	Genetics Dept., Monash Univ, Clayton 3168
LAN	Ruiting	Animal Husbandry Dept. B.19, Univ. Sydney, Sydney 2006
LATTER	Barrie	Agric. Genetics A.05, Univ. Sydney, Sydney 2006
LEACH	Carolyn	Genetics Dept., Univ. Adelaide, Adelaide SA 5001
LEETON	Peter	Genetics Dept., Monash Univ, Clayton 3168
LE STRANGE	Kate	Plant Microbe Gp, RSBS, ANU, P.O. Box 475, Canberra, ACT 2601
LUCKETT	David	Agric. Research Institute, PMB Wagga Wagga, NSW 2650
LYON	Bruce	CSIRO Div. Plant Industry, G.P.O. Box 1600 Canberra 2601
MAHONY	Michael	Evol. Biol. Unit, South Australian Museum, Adelaide SA 5000
MARCHANT	Adam	Pop Genetics Gp, RSBS, ANU, P.O. Box 475, Canberra, ACT 2601
MARKOVIC	Boban	Centre for Safety Science, Univ. N.S.W., Kensington 2033
MARTIN	Helene	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
MARTIN	Jon	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
MATHEWS	Sarah	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
MAYO	Oliver	CSIRO Div Animal Prod., P.O. Box 239, Blacktown 2148
McKECHNIE	Steve	Genetics Dept., Monash Univ, Clayton 3168
McKENZIE	John	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
McQUADE	Leon	Biological Sciences, Macquarie Univ., NSW 2109
MERAKOVSKY	John	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
MORAN	Chris	Animal Husbandry Dept. B.19, Univ. Sydney, Sydney 2006
MORGAN	Peggy	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
MORGAN	Tony	Genetics Dept., Monash Univ, Clayton 3168
NICHOLAS	Frank	Animal Husbandry Dept. B.19, Univ. Sydney, Sydney 2006
NICHOLLS	Max	Centre for Safety Science, Univ. N.S.W., Kensington 2033
NOLCH	Guy	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
OAKESHOTT	John	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
OBEYESEKERE	Varuni	Genetics Dept., Monash Univ, Clayton 3168
O'BRIEN	Liz	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
OVENDEN	Jenny	Zoology Dept., Univ. Tasmania, G.P.O. Box 252C, Hobart 7001

PARKER	Anthony	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
PEAKALL	Rod	Biological Sciences, Macquarie Univ., NSW 2109
PERKINS	Harvey	Biochemistry Dept., ANU, P.O. Box 4, Canberra City 2601
PETERING	Jenny	Agric. Biochem. Dept., Waite Institute, Glen Osmond SA 5064
PETROVICH	Tanya	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
PHILLIPS	Marie	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
PIPER	Anita	Biol. & Biomed. Sci., UTS, Westbourne Ave, Gore Hill 2065
PITSILIS	Henrietta	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
PLAYFORD	J. Ms	Mol Pop Genetics, RSBS, ANU, P.O. Box 475, Canberra, ACT 2601
RAMSBOTHAM	Rebecca	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
REDDY	Pradnya	CSIRO Wheat Res. Institute, 51 Delhi Road, N. Ryde 2113
RICHARDSON	Imogen	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
ROBINSON	Nick	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
ROSS	Jenny	Genetics Dept., Monash Univ, Clayton 3168
ROSSETTO	M. Mr.	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
ROWELL	Dave	Botany Dept, ANU, P.O. Box 4, Canberra City 2601
SAFFERY	Richard	Genetics Dept., Monash Univ, Clayton 3168
SALEEBA	Jenny	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
SCARAMUZZI	Carol	Biological Sciences, Macquarie Univ., NSW 2109
SCHAFER	Darren	Animal Sci Dept, Univ. New England, Armidale 2351
SENTRY	John	Childrens Med. Res. Found., PO Box 61, Camperdown 2050
SHANAHAN	Cathy	CSIRO Div Animal Prod., P.O. Box 239, Blacktown 2148
SHARP	Peter	School Crop Sciences, Univ. Sydney, Sydney 2006
SHAW	Dave	Mol Pop Genetics, RSBS, ANU, P.O. Box 475, Canberra, ACT 2601
SHELDON	Bruce	CSIRO Div Animal Prod., P.O. Box 239, Blacktown 2148
SIMPSON	Paul	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
SMYTH	Kerrie-Ann	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
SRISKANTHA	A.	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
STRAFFON	Melissa	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
SUTTON	Rosemary	CSIRO Div Animal Prod., P.O. Box 239, Blacktown 2148
SVED	John	Biological Sciences A.12, Univ. Sydney, Sydney 2006
SYMONDS	Jane	Mol Pop Genetics, RSBS, ANU, P.O. Box 475, Canberra, ACT 2601
TAKIS	Helen	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
TANSEY	Bill	Biological Sciences A.12, Univ. Sydney, Sydney 2006
TAYLOR	Andrea	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
TEE	Meng-Kian	Animal Husbandry Dept. B.19, Univ. Sydney, Sydney 2006
TEN HAVE	José	Biochemistry Dept., ANU, P.O. Box 4, Canberra City 2601
THOMSON	John	Biological Sciences A.12, Univ. Sydney, Sydney 2006
THORNE	Marney	CSIRO Poultry Genetics, P.O. Box 184, North Ryde 2113
TIMMIS	Jeremy	Genetics Dept., Univ. Adelaide, Adelaide SA 5001
TORKAMANZEHI	Adam	Animal Husbandry Dept. B.19, Univ. Sydney, Sydney 2006
TOWNROW	Cathy	CSIRO Div Animal Prod., P.O. Box 239, Blacktown 2148
VIOLO	Antoniette	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
VOGRIG	Darren	Victoria College, P.O. Box 224 Malvern, Vic 3144
WALKER	Beverley	Biology Dept., Univ. Wollongong, P.O. 1144 , Wollongong 2500
WANG	Yao	Medical Mol. Biol. Group, JCSMR, ANU, Canberra ACT 2601
WARD	Kevin	CSIRO Div Animal Prod., P.O. Box 239, Blacktown 2148
WARREN	Bill	Biochemistry Dept., ANU, P.O. Box 4, Canberra City 2601
WATSON	Jacki	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083
WEBB	Penny	Genetics Dept, Univ. of Melbourne, Parkville, Vic 3052
WELLER	Gaye	CSIRO Div Entomology, G.P.O. Box 1700, Canberra 2601
WILLIAMS	Keith	Biological Sciences, Macquarie Univ., NSW 2109
WILTON	Alan	Biological Sciences, Macquarie Univ., NSW 2109
WRIGHT	Susan	Biological Sciences, Univ. N.S.W., Kensington 2033
YOO	Brian	CSIRO Poultry Genetics, P.O. Box 184, North Ryde 2113
ZELESCO	Paula	Genetics Dept, LaTrobe Univ., Bundoora Vic 3083

PROGRAMME

(Names of Presenters in bold type)

SUNDAY 2 JULY

MIXER & REGISTRATION
19.00 - 22.00 Biological Sciences Building, 6th Floor

MONDAY 3 JULY

REGISTRATION
8.30 Biomedical Theatres Foyer

WELCOME & OFFICIAL OPENING
9.00 - 9.15 Biomedical Lecture Theatre A
Chair: Ross Crozier

Professor Alan Gilbert, ProViceChancellor, UNSW

PRINCIPAL GUEST SPEAKER
9.15 - 10.30 Biomedical Lecture Theatre A
Chair: Ross Crozier

Elizabeth Blackburn, University of California, Berkley
Synthesis of telomeres by the ribonucleoprotein telomerase

MORNING TEA & TRADE DISPLAYS
10.30 - 11.00 Biomedical Theatres Foyer

SESSION 1A Biomedical Theatre A
Chair:Judy Ford

11.00 - 11.20 **D. Shaw**, N. Contreras and D. Buckle
Molecular and cytological organisation of centromeric DNA sequence in *Caledia*

11.20 - 11.40 **D. Colgan** and D. Shaw
Nucleosomes, chromosomal structure and speciation

11.40 - 12.00 **M. Nicholls**
Immunological factors and the epidemiology of some genetic diseases in man

12.00 - 12.20 **A. Smith**, F. Volpato, R. Lindeman and R.J. Trent
Cytogenetic and molecular studies in the Prader Willi syndrome

SESSION 1B — Biomedical Theatre C
Chair: Ian Franklin

11.00 - 11.20 **R.C. Griffiths**
A probability distribution on genealogical trees

11.20 - 11.40 **R.H. Crozier**, Y.C. Crozier and A.G. Mackinlay
The CO-I and CO-II region of honeybee mitochondrial DNA: evidence for variation in insect mitochondrial evolutionary rates

11.40 - 12.00 **D. Haig** and M. Westoby
Of mice and maize: chromosomal imprinting and intragenomic conflict

12.00 - 12.20 **A.D. Marchant**
Phylogenetic relationships in the context of geographical distribution: comparison of sibling species in the genus *Caledia*

LUNCH
12.20 - 13.30

SESSION 2A — Biomedical Theatre A
Chair: Len Kelly

13.30 - 13.50 **P. Batterham**, J. Pollock and S. Benzer
What is the role of the *lozenge* gene in *Drosophila* eye development

13.50 - 14.10 S. Hardy, A. Davies and **P. Batterham**
Cloning the *lozenge* gene of *Drosophila melanogaster*

14.10 - 14.30 **T.Z. Petrovich** and L.E. Kelly
Characterization of a suppressor of behavioural mutants of the *stoned* locus in *Drosophila melanogaster*

14.30 - 14.50 **A.L. Bull**, R. Ramsbotham and L. Kelly
Cloning and characterisation of genes encoding calmodulin-binding proteins from *Drosophila melanogaster*

SESSION 2B — Biomedical Theatre C
Chair: Viji Krishnapillai

13.30 - 13.50 **M.E. Katz**, I.B. Richardson and M.J. Hynes
Molecular analysis of the divergently transcribed *lamA* and *lamB* genes of *Aspergillus nidulans*

13.50 - 14.10 **M. Davis** and M.J. Hynes
Nitrogen control of *amdS* expression in *Aspergillus nidulans*

14.10 - 14.30 **M. Mahony**, P. Baverstock and M. Smith
The relative roles of chromosomes and genes in species isolation in mammals: the Australian *Rattus* as a model

14.30 - 14.50 **K. Li**, C. Crowther, H. Pynor and D. Walsh
Regulation and control of stress genes in mammalian embryonic development

AFTERNOON TEA, POSTERS & TRADE DISPLAYS
14.50 - 16.00 Biomedical Theatre Foyers

SESSION 3 INVITED SPEAKERS - Biomedical Theatre A
Chair: Oliver Mayo

16.00 - 16.30 **Jeff J. Doyle** and Jane L. Doyle, Bailey Hortorium, Cornell University
Multiple origins of polyploidy in the wild perennial relatives of soybean
16.30 - 17.00 **Michael T. Clegg**, University of California, Riverside
Evolution of chloroplast encoded genes

MONDAY EVENING FREE

TUESDAY 4 JULY

SESSION 4 *SYMPOSIUM - Genetic Engineering and Transgenesis*
INVITED SPEAKERS - Biomedical Theatre A
Chair: Chris Moran

9.00 - 9.30 **Alan F. Cowman**, Walter & Eliza Hall Institute
Molecular mechanisms of drug resistance in malaria
9.30 - 10.00 **Kevin Ward**, C.R. Byrne, C.D. Nancarrow, J.D. Murray, Z. Leish and B.W. Wilson, C.S.I.R.O. Div. Animal Production, Prospect
The transfer of new metabolic pathways to domestic animals
10.00 - 10.30 **Bruce R. Lyon**, C.S.I.R.O. Div. Plant Industry, Canberra
Engineering herbicide resistance in plants

MORNING TEA & TRADE DISPLAYS
10.30 - 11.00 Biomedical Theatres Foyer

SESSION 5A Biomedical Theatre A
Chair: John McKenzie

11.00 - 11.20 **N. Darvey**
Bioethics and biotechnology
11.20 - 11.40 **A. Taylor**, S. Ramus, N. Murray, J. Graves, W. Sherwin, R. Martin and K. Handasyde
Molecular genetic studies of koala populations
11.40 - 12.00 **C. Jiang** and J.B. Gibson
Restriction endonuclease variation in the region of the *Adh* locus in Australian and Chinese populations of *D. melanogaster*
12.00 - 12.20 **A. Agrotis** and S. W. McKechnie
Haplotype variation at an enzyme locus in a natural population of *Drosophila melanogaster*

SESSION 5B — Biomedical Theatre C
Chair: Ian Dawes

11.00 - 11.20 **Y. Wang**, H.D. Campbell and I.G. Young
Transcriptional regulation of *interleukin -5* gene expression

11.20 - 11.40 **W.P. Tansey** and D.F. Catanzaro
The role of distal promoters elements in the control of human growth hormone gene expression

11.40 - 12.00 **G.J. Jones** and D.F. Catanzaro
Transcriptional control imparted by regulatory elements in the 5' flanking DNA of the rat prolactin gene

12.00 - 12.20 **M. Gardiner-Garden a**nd M. Frommer
CpG islands: the pro-opiomelanocortin gene is an unusual case

LUNCH
12.20 - 13.30

SESSION 6A — Biomedical Theatre A
Chair: Yvonne Fripp

13.30 - 13.50 **P. Matthews**
Ribosomal DNA variation in wild and cultivated taro (*Colocasia esculenta*): implications for prehistory

13.50 - 14.10 S. Chao, **P.J. Sharp**, A.J. Worland, R.M.D. Koebner and M.D. Gale
Genetic maps of wheat homoeologous group 7 chromosomes

14.10 - 14.30 **R. Peakall**
Ecological constraints to outcrossing in orchids

14.30 - 14.50 **B.A. Walker** and S.H. James
Genetic variation, plasticity and population structure in a native legume *Viminara juncea* (Schrad & Wendl.) Hoffmanns

SESSION 6B — Biomedical Theatre C
Chair: Peter East

13.30 - 13.50 **A.J. Howells**
Structure and function of the protein products of the *white*, *scarlet* and *brown* genes of *D. melanogaster*

13.50 - 14.10 **A. Torkamanzehi** and C. Moran
Analysis of P element induced quantitative variation in *Drosophila melanogaster*

14.10 - 14.30 J.P. Stoye, S. Fenner, G.E. Greenoak, **C. Moran** and J.M. Coffin
Insertion of a retrovirus has caused the hairless mutation of the mouse

14.30 - 14.50 **J. Sved**, L. Blackman and D. Verlin
Inducing recombination in *Drosophila* with P factors

AFTERNOON TEA, POSTERS & TRADE DISPLAYS
14.50 - 16.00 Biomedical Theatre Foyers

SESSION 7A Biomedical Theatre A
Chair: Keith Williams

16.00 - 16.20 **J.N. Timmis**, D.L. Whisson and A.M. Binns
Deletion as a means of isolating avirulence genes from rust fungi
16.20 - 16.40 S. Davies and **V. Krishnapillai**
Molecular and genetic organization of the origin of replication of a bacterial plasmid
16.40 - 17.00 E. Ratnaningsih, A. Kearney, R. Saffery and **A. Morgan**
Restriction mapping of *Pseudomonas* chromosomes

SESSION 7B Biomedical Theatre C
Chair: David Hayman

16.00 - 16.20 **J. Ford** and A. Correll
Mechanisms of aneuploidy: differences in behaviour of X chromosomes and autosomes
16.20 - 16.40 **J. Watson**, J. Spencer and J.A.M. Graves
Mappimg human X-linked genes in marsupials and monotremes
16.40 - 17.00 **M.P. Bradley** and U.H. Wiberg
Biochemical approaches towards the identification of sex-specific proteins

ANNUAL BUSINESS MEETING
17.00 - 18.00 Biomedical Theatre A

ANNUAL SOCIETY DINNER
19.30 Goldstein Hall, Kensington Colleges

WEDNESDAY 5 JULY

SESSION 8 INVITED SPEAKERS - Biomedical Theatre A
Chair: John Sved

9.00 - 9.30 **Keith Williams** and N.A. Farrar, Macquarie University
Replication and maintenance of plasmids in simple eukaryotes
9.30 - 10.00 **John McKenzie**, University of Melbourne
The evolution of insecticide resistance
10.00 - 10.30 **Rudi Appels**, C.S.I.R.O. Div. Plant Industry, Canberra
Evolution at the *Nor* and *5SDna* loci in grasses of the Triticeae

MORNING TEA
10.30 - 11.00 Biomedical Theatres Foyer

M.J.D. WHITE ADDRESS
11.00 - 12.00 Biomedical Lecture Theatre A
Chair: Des Cooper

Professor Ian W. Dawes, School of Biochemistry, UNSW
Complex regulation of metabolic gene expression in the yeast *Saccharomyces cerevisae*

CLOSE
12.00 Biomedical Theatre A

BARBEQUE
12.30 UNSW Campus or Centennial Park

POSTERS

1. **M. Morgan** and P. East
   Cloning genes from the 14A region of the *Drosophila melanogaster* X-chromosome

2. **K. LeStrange**, G. Bender, M. Djordjevic, J. Weinman, J. Redmond and B. Rolfe
   Gene regulation in *Rhizobium* strain NGR234 is controlled by a bacterial gene and a plant signal

3. **I.B. Richardson** , A. Adrianopoulos, T.G. Littlejohn, M. Katz, R. van Heeswyck and M.J. Hynes
   Regulation by the *amdR* gene of *Aspergillus nidulans*

4. **H. Perkins** and A. Howells
   Repeated sequences in the Australian sheep blowfly, *Lucilia cuprina*

5. **C.M. Collis** and G.W. Grigg
   A mutant of *Escherichia coli* showing resistance to phleomycin and to heat inactivation is defective in ubiquinone synthesis

6. **C. Shanahan**, N. Rigby, J. Murray, and K. Ward
   Insertion of a fusion gene into the mouse Y-chromosome; effects of expression in transgenic mice

7. **G. Weller** and G. Foster
   Revised genetic map of *Lucilia cuprina* and evolutionary correlations

8. **P. Batterham**, A. Davies and M. Green
   Enhancers and suppressors of the *lozenge* phenotype in *Drosophila melanogaster*

9. P. Batterham, **A. Davies**, G. Pasquini and R. Stocker
   Genetic and phenotypic analysis of mutations at the *lozenge* locus in *Drosophila melanogaster*

10. **M. Phillips**, L .Salkoff and L.E. Kelly
    Characterization of *Drosophila* genes with possible involvement in GABA metabolism

11. **R. Howden**, M. Phillips, H.M. Dahl and J.F.B. Mercer
    The isolation and characterization of Drosophila genes with homology to a mammalian aromatic amino acid hydroxylase gene

12. **J. Ovenden**, R. White and M. Adams
    Mitochondrial DNA lineage selection in derived lake Galaxiid species

13. **P.R.J. Leeton** and D.R. Smyth
    A highly repeated sequence found within the large genome of *Lilium speciosum*

14. **B. Markovic** and M. Nicholls
    The application of DNA *in-situ* hybridization to the study of skin diseases

15. **J. Symonds** and J. Gibson
    Restriction map variation in the region of the *αGpdh* gene and effects on GPDH activity in *D. melanogaster*

16. **N. Robinson**, N. Murray, W. Sherwin, and J. Graves
    Modelling genetic changes in a bandicoot colony

17. **J. Saleeba** and M. Hynes
    Homology studies reveal the function and regulation of *aciA* in *Aspergillus nidulans*

18. **T. Cross**, E. Creaser and J. Pateman
    Molecular analysis of the *alc* gene region of *Aspergillus nidulans*

19. B. Dickson and **C. Cobbett**
    Regulation of the *aroF tyrA* operon of *Escherichia coli* : interaction between operator sites

20. **G. Foster** and G. Weller
    Inversions, translocations and genetic control of *Lucilia cuprina*

21. **M. Delbridge** and L. Kelly
    The identification and cloning of the gene for a heat-stable calcium binding protein from *Drosophila melanogaster*

22. **C.J.I. Driver** and W. Mullins
    Long lived strains of *Drosophila melanogaster* with reduced body fat

23. **D. Luckett** and R. Smithard
The production of doubled-haploids by anther culture from Australian barley and wheat germplasm

24. **G. Adcock**, P. Batterham, L.E. Kelly and J.A. McKenzie
Mutagenesis produced insecticide resistance in *Drosophila melanogaster*

25. **R. Drinkwater**, S. Moore and J. Hetzel
An analysis of heterologous *vntr* probes for linkage studies in cattle and sheep.

26. **D.G. Bedo**
In situ hybridisation studies of nucleoli in the Mediterranean fruit fly, *Ceratitis capitata*

27. **C. Collet**, R. Joseph and K. Nicholas
Cloning and cDNA sequence of a novel marsupial milk protein gene

28. **C. Collet**, R. Joseph and K. Nicholas
Cloning, cDNA sequence and prolactin-dependent expression of a marsupial β-lactoglobulin

29. **K. Dai** and C. Gillies
Synaptonemal complex analysis of Robertsonian translocation in sheep

30. **J. Petering**, P. Henschke and P. Langridge
Genetic engineering of wine yeasts

31. **P. Reddy**, H. Carpenter and G. Donovan
Cloning and characyerization of HMW glutenin genes in wheat

32. M. Healy, M. Dumancic, **J. Karotam**, M. Myers, R. Russell, J. Brady and J. Oakeshott
Regulation of expression of the duplicated genes *esterase-6* and *esterase-p*

33. **A.C.M. Chan**, K.L. Williams, J.G. Williams and A. Ceccarelli
Complementation of a *Dictyolstelium discoideum* thymidylate synthase mutation with the mouse gene provides a new selectable marker for transformation

34. **A.A. Gooley**, A. Champion and K.L. Williams
Genetic analysis of post-translational modification of cell-surface and ECM glycoprotein in *Dictyolstelium discoideum*

ABSTRACTS

SYNTHESIS OF TELOMERES BY THE RIBONUCLEOPROTEIN TELOMERASE

Elizabeth H. Blackburn, Carol Greider, Dorothy Shippen-Lentz, Margaret Lee, Guo-Liang Yu and Dan Romero.
Department of Molecular Biology, University of California, Berkeley, CA 94720, USA.

Telomeres stabilize chromosomal ends and allow their complete replication *in vivo.* In diverse eukaryotes, the essential telomeric DNA sequence consists of variable numbers of tandem repeats of simple, G+C-rich sequences, with a strong strand bias of G residues on the strand oriented 5' to 3' toward the chromosomal terminus. This strand has been shown to form a 3' overhang at the chromosomal terminus in 3 different groups of eukaryotes.

We identified a ribonucleoprotein enzyme, telomerase, from the ciliates *Tetrahymena and Euplotes.* This activity adds repeats of the species-specific telomeric sequence, TTGGGG in the case of the *Tetrahymena* telomerase or TTTTGGGG for the *Euplotes* enzyme, onto the 3' end of a single-stranded DNA primer consisting of a few repeats of the G-rich strand of any of the known eukaryotic telomeric sequences[1,2]. Addition of repeats occurs, one nucleotide at a time, by *de novo* synthesis, which is not templated by the DNA primer. Structural analysis of synthetic DNA oligonucleotides that are active as primers has shown that they form discrete conformers at temperatures below 40° [3]. Telomerase kinetics showed the enzyme has a high affinity for such primers.

The telomerase RNAs from 4 *Tetrahymena* and one *Euplotes* species were identified and sequenced. Although they have diverged rapidly between species, each RNA contains a short stretch of nucleotides complementary to the telomeric repeats synthesised by that telomerase. We showed that these nucleotides act as a template which directs the synthesis of G-rich repeats. Conserved structural properties of the different telomerase RNAs suggest the RNA may have additional roles in telomerase function.

The *Tetrahymena* telomerase RNA gene on a high copy number vector was overexpressed in *Tetrahymena* cells. While no phenotype was seen with a wild-type overexpressed gene, a mutant overexpressed telomerase RNA gene with an altered template sequence gave a long telomere phenotype.

[1] Greider and Blackburn, Nature, **337**, 331-337, 1989. [2] Shippen-Lentz and Blackburn, Molec. Cell Biol, in press, 1989. [3.] Henderson et al, Cell, **51**, 899-908, 1987

SESSION 1A

MOLECULAR AND CYTOLOGICAL ORGANISATION OF A CENTROMERIC DNA SEQUENCE IN CALEDIA.

Dave Shaw, Nelida Contreras and Dave Buckle.

Molecular and Population Genetics Group, R.S.B.S.,
Australian National University, A.C.T. 2601

A prominent feature of evolution in the genus Caledia has involved changes in the position of the centromere along each of its 12 chromosomes.

In the MORETON subspecies, centromeric variation reaches extreme levels along a 1500 km latitudinal cline where the position of the centromere moves gradually from distal to more medial locations on every member of the genome giving rise to concerted genomic change along the cline.

At the southern limit of its distribution the MORETON genome is always composed of telo- and acrocentric chromosomes whereas in the north metacentric chromosomes predominate. Intermediate populations are characterised by chromosomes in which the centromere occupies different positions on the same chromosome giving rise to extreme levels of polymorphism along the cline. Over 400 different chromosomal morphs have so far been identified. The gradual changes in genomic structure (centromere position) are correlated with major changes in developmental strategies (voltinism) which also show similar patterns of clinal variation.

To investigate the mechanics of chromosomal change in this genus, and the structure of the eukaryote chromosome in general, we have recently initiated a search for DNA sequences involved in the organisation of centromeres. An 880 bp Eco R1 restriction fragment has been isolated, sequenced and cytologically mapped to the Caledia genome.

In situ hybridisation of cRNA copies of the sequence to both mitotic and meiotic chromosomes has revealed that the 880 bp sequence is located extremely close to the active centromere. The sequence was isolated from a genome in which all the chromosomes are telocentric (terminal centromeres) and do not have the large c-banding regions that usually encompass the centromeric regions of other higher eukaryotic organisms.

It is envisaged that this sequence will allow us to walk along the chromosome and facilitate the isolation of those DNA sequences that are directly involved in kinetochore organisation and attachment to the mitotic and meiotic spindle fibres during cell division.

SESSION 1A

NUCLEOSOMES, CHROMOSOMAL STRUCTURE AND SPECIATION

Donald Colgan[1] and David Shaw[2]

[1]Ken and Yasuko Myer Molecular Evolutionary Biology Unit, Australian Museum, P.O. Box A285 Sydney South, N.S.W. 2000.

[2]Population Genetics Group, R.S.B.S., A.N.U., G.P.O. Box 475, Canberra City, A.C.T. 2601.

The possible roles of chromatin structure in the origin and implementation of reproductive isolation between taxa will be examined. Most attention will be paid to how differences in the characteristics of nucleosomes packaging homologous DNA sequences affect the viability and fertility of F_1 and F_2 hybrids. The biology of nucleosomes may differ between taxa in four main properties. These are: 1, the length of DNA in each nucleosome; 2, the position of specific nucleosomes with respect to important regulatory genes; 3, the strength of the association of nucleosomes with particular sites; and 4, the presence or absence of biochemical modifications (such as the acetylation of histones or their conjugation with ubiquitin). Examples will be given of model systems where such differences may be involved in the processes of population divergence and speciation. These include the grasshopper Caledia captiva where F_2 and backcross breakdown between subspecies has been shown to be due to the disruption by recombination between chromosomes of blocks of genes which are internally co-adapted. The evolution of chromatin structure is the prime candidate for generating this co-adaptation. We will discuss the possible origins of differences between taxa in chromatin characteristics and will present a programme for the correlation of such differences with speciation events.

IMMUNOLOGICAL FACTORS AND THE EPIDEMIOLOGY OF SOME GENETIC DISEASES IN MAN

Max Nicholls

Centre for Safety Science, University of NSW,
Box 1, Kensington, NSW Australia 2033

The possibility of certain virus diseases masquerading in appropriate circumstances as genetic diseases has been recognized for a long time; Caffey's disease, Leber's optic atrophy, incontinentia pigmenti and Creutzfeld-Jacob disease (kuru) fall into this category.

Segregations in some of the diseases fit neatly into X-linked or autosomal dominant patterns. Two other diseases will be considered in some detail: Huntington's disease (HD) and Fragile-X (F-X, Martin Bell) syndrome. There is no problem with the segregation of HD, but there is the strange but irrefutable anticipation of age of onset in the affected children of males with the gene. F-X is even stranger: tracing cases back to a common ancestor shows that in many cases a normal man has produced a family with all carrier daughters and no affected sons, compatibile with X-linkage; his carrier daughters then produce mildly affected daughters and severely affected sons, with a 50% probability in each case.

There have been many hypotheses to explain these facts, the most recent being hypotheses concerned with methylation of parts of the genome at gametogenesis. Our work on the effect of the maternal immune system in mammalian pregnancies can provide an alternative interpretation of the data in each disease.

CYTOGENETIC AND MOLECULAR STUDIES IN THE PRADER WILLI SYNDROME

[1]A. Smith, [2]F. Volpato, [2]R. Lindeman, [2]R.J. Trent

[1]Oliver Latham Laboratory, Dept. of Health, N.S.W.
[2]Clinical Immunology Research Centre, University of Sydney.

Molecular and cytogenetic studies (with high resolution banding) have been performed on 22 patients with the Prader Willi Syndrome (PWS). Two of the patients have abnormalities detectable by DNA mapping with chromosome 15q11-12 specific probes. These two patients have a normal karyotype and classical PWS clinically. With probe p3.21 the abnormal DNA restriction fragment was inherited from their mother while the normal allele from the father is missing. DNA rearrangements in these two unrelated families are not identical since probe 85(2.4) fails to detect an abnormality in one of the two. Increased intensity of the abnormal band would suggest that repetitive DNA elements are present.

* DNA probes were kindly donated by the following: p3.21 and pML34, the late Professor S. Latt, Boston MA; 85(2.4), Professor Fa-Ten Kao, Denver CO.

A PROBABILITY DISTRIBUTION ON GENEALOGICAL TREES

Robert C. Griffiths

Monash University, Clayton, 3168

In the neutral-infinitely-many-site model of population genetics a gene is thought of as an infinitely long sequence of completely linked sites where mutations occur at sites never before mutated. Over the generations loss of allele types by random drift and introduction of new types by mutation maintains equlibrium in the population.

If a sample of n genes is taken from the population, a tree can be constructed from the segregating sites. This tree displays the mutational history of the sample back to a common ancestor. The probability distribution of these trees can be found. Such trees are easily simulated.

THE CO-I AND CO-II REGION OF HONEYBEE MITOCHONDRIAL DNA: EVIDENCE FOR VARIATION IN INSECT MITOCHONDRIAL EVOLUTIONARY RATES.

Ross H. Crozier[1], Y. Ching Crozier[1], and Antony G. Mackinlay[2].

Schools of Biological Science[1] and Biochemistry[2], University of NSW, Kensington, 2033

The sequence of a region of honeybee (*Apis mellifera ligustica*) mitochondrial DNA, which contains the genes for cytochrome c oxidase subunits I and II (CO-I and CO-II), ATPase subunits 6 and 8, and the inferred genes for $tRNA^{Asp}$, $tRNA^{Leu}_{UUR}$, $tRNA^{Lys}$, and $tRNA^{Trp}$ has been sequenced. The region contains the segment previously identified as incurring a length increase in some other bee strains, including Africanized bees. The sequence information shows that several shifts of tRNA genes have occurred between *Apis* and *Drosophila*, but shifts of other kinds of genes have yet to be demonstrated. The sequence is generally more A+T rich than is the corresponding *Drosophila* sequence. Parsimony analyses of the CO-I and CO-II sequences from mouse and *Xenopus* as outgroups show significantly more amino acid substitutions on the branch to *Apis* (120) than on that to *Drosophila* (44), indicating a difference in the long-term evolutionary rates of hymenopteran and dipteran mtDNA.

OF MICE AND MAIZE: CHROMOSOMAL IMPRINTING AND INTRAGENOMIC CONFLICT

David Haig and Mark Westoby

School of Biological Sciences, Macquarie University, 2109.

The embryo of viviparous species acquires resources from its mother, at some cost to the mother's future reproduction. For any given locus in the embryo, the maternally-derived allele is present in half of the embryo's maternal half-sibs, but the paternally-derived allele is absent from these half-sibs. Therefore, the paternally-derived allele suffers less from costs to the mother's future reproduction than does the maternally-derived allele. If an allele can have different expression depending on its parent of origin, natural selection should cause alleles to be more active in resource-acquisition from the mother when paternally-derived than when maternally-derived. "Chromosome imprinting" in mouse embryos and maize endosperm is interpreted from this perspective.

SESSION 1B

Phylogenetic relationships in the context of geographical distribution: comparison of sibling species in the genus *Caledia*.

A.D. Marchant
Molecular and Population Genetics
Research School of Biological Sciences
The Australian National University.

The "Torresian" subspecies of *Caledia captiva* (Orthoptera: Acrididae: Acridinae) is distributed over a large area of northern Australia, extending from the Kimberlies to South-east Queensland, as well as occurring in an area of Papua. Karyotypically, this subspecies shows very little variation over its whole range.

Another subspecies ("Southern Group") of *C. captiva* occurs along the NSW coast, extending also into eastern Victoria and South-east Queensland. This subspecies has been divided into three taxa, on the basis of extensive karyotypic differences displayed. In south-east Queensland, the "Moreton" taxon has a karyotype containing mostly metacentric elements; in eastern Victoria the "Lake's Entrance" taxon has a karyotype entirely made up of acro-& telo-centric elements, while on the N.S.W. coast, the "South East Australian" taxon shows a cline for [illegible] of metacentric and sub-metacentric chromosomes, increasing northwards, and thus displaying all karyotypes intermediate between "Lake's Entrance" and "Moreton".

Analysis of mitochondrial DNA in these two subspecies has revealed a very different pattern. There is very little differentiation of the mtDNA in the "Southern Group", while there is extensive differentiation in mtDNA from different areas of the Torresian range (central Qld., northern Qld., Northern Territory and Papua).

If mtDNA is assumed to provide a reasonable "molecular clock", then these contrasting patterns suggest that the karyotypic differentiation shown by the "Southern Group" has happened suddenly and recently - with implications for the mechanisms and effects of chromosomal change.

Alternatively, the patterns could indicate differential survivability of mtDNA linkages, a possibility that I consider less likely.

WHAT IS THE ROLE OF THE *lozenge* GENE IN *Drosophila* eye development ?

Philip Batterham[1], John Pollock[2] and Seymour Benzer[2]

[1] Dept. of Genetics, University of Melbourne, Parkville 3052

[2] Division of Biology, California Institute of Technology, Pasadena , U. S. A.

Previous studies divided the *lozenge* (*lz*) locus into four recombinationally separable sub- loci spanning 0. 14 map units around position 27. 7 on the X chromosome of *D. melanogaster.* Complementation analysis separated *lz* mutations into two functional units. Cistron A is defined by four mutations mapping to the proximal (*spectacle*) sub- locus which are eye specific in their effect. Cistron B is defined by mutations mapping to each of the four sub- loci, which affect the development of the eyes, antennal sensillae, tarsal claws, pulvilli, spermathecae and the crystal cells of the hemolymph.

The present study focuses on the role of the *lz* gene in eye development. Initial experiments addressed the nature of the eye defect in mutants of both cistrons. Eye tissue sections were analysed using transmission electron microscopy or various staining techniques, including tissue specific monoclonal antibodies. These experiments indicated that extreme *lz* mutants lack the normal ommatidial structure in which clusters of eight photoreceptor cells are flanked by pigment cells. Rather in *lz* mutants these cell types form a bilayer beneath the lens cells with photoreceptor cells laying beneath a pigment cell layer. The morphology of the photoreceptor cells is highly abnormal and in extreme mutants distinct clusters cannot be recognized. Differences were observed between mutants representative of Cistron A and B in the distribution and thickness of the pigment cell layer.

The second line of investigation has been the analysis of eye development in *lz* mutants. An examination of eye imaginal discs from third instar larvae revealed that *lz* mutants establish a matrix of photoreceptor cells indistinguishable from the wild type pattern. However the *lz* eye defect is clearly present by mid pupal development. These data indicate that the *lz* gene product is required in the first half of pupal life for normal eye development. This investigation continues and is being complemented by the molecular analysis of the *lz* gene and its product.

CLONING THE *lozenge* GENE OF *Drosophila melanogaster*

Stephen Hardy, Andrew Davies, and Philip Batterham

Department of Genetics, University of Melbourne, Parkville 3052

In previous studies a genomic library was constucted using DNA from the *lz'* mutant which has a copy of the retrotransposon gypsy inserted at the *lz* locus (cytological location 8D8-9). Two clones isolated from this library by gypsy transposon tagging *in situ* hybridized to regions 8D and 10B on wild type chromosomes. These clones contained (non gypsy) sequences which are highly re-iterated in the *Drosophila* genome and were therefore not useful in the isolation of further genomic clones spanning the *lz* locus. Given the size and complexity of the *lz* locus we have pursued a number of alternative approaches to the cloning of the *lz* gene(s) .

1. P Transposon Tagging

Two mutations *lz* ^{PM2} and *lz* ^{MR2} have been shown by reversion studies and *in situ* hybridization analysis to have a P element at the *lz* locus. The *MR2* strain contains a single P element, while the *PM2* strain has two copies. Genomic libraries of these mutants have been prepared.

2. Tp (1:1) L2

The transposition mutation Tp (1:1) L2 is caused by the insertion of a chromosomal segment from 8D - 10B in the *giant* (3A) region. Using clones supplied by Professor V. Pirrotta (Baylor) , the transposition insertion point has been localized to a single, small Bam H1 fragment. A genomic library is currently being screened for breakpoint clones i. e. 3A-8D and 10B-3A.

3. Clones from an eye specific library

Two clones isolated by Professor C. Zuker (U. C. San Diego) from a library produced using adult eye specific mRNA *in situ* hybridize to the 8D and 10B regions. When used as probes these clones both reveal restriction differences between a putative deletion mutant and wild type on Southern blots.

CHARACTERIZATION OF A SUPPRESSOR OF BEHAVIOURAL MUTANTS OF THE *STONED* LOCUS IN *DROSOPHILA MELANOGASTER*.

Tanya Z. Petrovich and Leonard E. Kelly.
Dept. of Genetics, University of Melbourne, Parkville 3052.

A suppressor of the neurological mutant *stoned* (*stn*) has been isolated in *Drosophila melanogaster*. Genetic characterization of this suppressor, *su(stn)*, has been achieved. It has been shown that the suppressor allele *su(stn)* is semi-dominant with the *su(stn)*$^{+}$ wildtype allele. The suppressor appears to suppress both the behavioural component of the *stn* phenotype and the reduced viability of the *stn* alleles. The suppressor locus maps to the X-chromosome between the markers *singed* and *ocelliless* (approx. map position 22.0). Cytological examination of this region (7F) in *su(stn)* mutants has shown that there exists a chromosomal rearrangement. Molecular studies of this region are currently in progress, with the aim of cloning the *su(stn)* locus.

CLONING AND CHARACTERISATION OF GENES ENCODING CALMODULIN-BINDING PROTEINS FROM *Drosophila melanogaster*.

Ann Bull, Rebecca Ramsbotham and Len Kelly,
Dept. of Genetics,
University of Melbourne.

Calmodulin is a small highly conserved calcium-binding protein found at high levels in the nervous system. It is thought to act as a modulator of calcium action by activating various enzymes in response to intracellular calcium levels. Calmodulin-binding proteins were detected in *Drosophila* heads by running head extracts on polyacrylamide gels and probing Western blots with labelled calmodulin. To identify genes encoding calmodulin-binding proteins a head cDNA library in lambda gt11 was probed with radioiodinated calmodulin. From fifty original isolates, three non-homologous clones have been identified. These clones have been localised to regions of polytene chromosomes and are currently being characterised.

Molecular analysis of the divergently transcribed *lamA* and *lamB* genes of *Aspergillus nidulans*

Margaret E. Katz, Imogen B. Richardson and Michael J. Hynes

Dept. of Genetics, University of Melbourne, Parkville Vic. 3052

Four *Aspergillus nidulans* genes are known to be under the control of the *trans*-acting regulatory gene *amdR*. Mutations in the *amdR*-controlled *lamA* gene block utilization of 2-pyrrolidinone as a nitrogen or carbon source. We have cloned the *Aspergillus nidulans lamA* gene from a cosmid library by complementation of the *lamA5* mutation. A plasmid, pLAM7, recovered from a lam+ *A. nidulans* transformant was found to contain the *lamA* gene and a new gene under *amdR* control which we have designated *lamB*. *Aspergillus terreus*, a species of Aspergillus that cannot utilize 2-pyrrolidinone, was used to investigate the function of the *lamB* gene. Interspecific transformation experiments with *A. terreus* showed that both the *lamA* and *lamB* genes are essential for the utilization of a new nitrogen source, 2-pyrrolidinone, in that species. Recombinant DNA techniques were then used to construct a strain a of *A. nidulans* lacking a functional *lamB* gene. Studies with this strain revealed that *lamB*, like *lamA* is involved in 2-pyrrolidinone utilization in *A. nidulans* and conversion of exogenous 2-pyrrolidinone to endogenous γ-amino butyric acid (GABA) requires a functional *lamB* gene.

The expression of *lamA* and *lamB* is coordinately controlled. Both genes are subject to carbon and nitrogen metabolite repression in addition to *amdR*-mediated induction by ω-amino acids. A probable *amdR* binding site has been localized to a 200 bp fragment in the *lamA/lamB* intergenic region. When multiple copies of this fragment are introduced via transformation, utilization of 2-pyrrolidinone is reduced, presumably due to titration of the *amdR* gene product. Sequence analysis of the 200bp fragment revealed a region of strong homology to the *amdR* binding site of two other genes in the *amdR* regulon, *amdS* and *gatA*.

SESSION 2B

NITROGEN CONTROL OF *amdS* EXPRESSION IN *Aspergillus nidulans*

Meryl Davis and Michael Hynes

Dept. of Genetics, University of Melbourne, Parkville 3052

The synthesis of the acetamidase (*amdS*) enzyme in *A. nidulans* is controlled by a number of different regulatory proteins. Regulatory sequences 5' to the *amdS* gene have been identified which define the binding sites for the *amdR*, *amdA* and *facB* proteins. Studies of *in vitro* generated deletions of the *amdS* 5' region linked to *amdS-lacZ* fusions are being used to localize sequences involved in *areA* control of *amdS* expression.

areA encodes a positively acting regulatory protein which mediates nitrogen metabolite repression in *A. nidulans*. The *areA* protein activates the transcription of a large number of catabolic enzymes, including *amdS*, in response to nitrogen limitation. The *areA* gene has been cloned by complementation of an *areA*$^-$ mutant. Transformation of the cloned gene back into *A. nidulans* allows gene dosage studies to be used to explore the activation/repression properties of this control system.

THE RELATIVE ROLES OF CHROMOSOMES AND GENES IN SPECIES ISOLATION IN MAMMALS: THE AUSTRALIAN *RATTUS* AS A MODEL

Michael Mahony[1], Peter Baverstock[2] and Meredith Smith[1]

1 Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide.
2 Northern Rivers C.A.E., PO Box 157, Lismore, NSW. 2480.

The process of speciation in mammals is being investigated using the Australian *Rattus* as a model. The eight Australian *Rattus* species, possess a suite of characteristics that makes this group extremely valuable as a model of speciation. In particular, it will be possible to resolve the relative roles of genes and chromosomes as barriers to gene flow between species. This is possible because different pairwise crosses have different and uncorrelated levels of genetic and chromosomal divergence. Thus the study involves an analysis of the fertility of hybrids between various combinations of species. The first cross involving *R. colletti* and *R. villosissimus* has been completed. Reproduction in these species and their hybrids have been extensively investigated. These species differ by six chromosomal rearrangements but show no electrophoretic differences at 55 loci. Moreover, the nature of the rearrangements are such that "chains" are formed at first meiosis, which can result in a variety of unbalanced and balanced products after segregation. Thus, this cross emphasises the role of chromosomes in the reproductive isolation of these species and minimises the influence of genetic differences. The ability of hybrids to transmit genetic material hinges on two features - their ability to produce gametes, and the viability of the gametes produced. These features will be presented for the cross between R.C. and R.v.

SESSION 2B

REGULATION AND CONTROL OF STRESS GENES IN MAMMALIAN EMBRYONIC DEVELOPMENT.

Karen Li, Carol Crowther, Helen Pynor and David Walsh.

Department of Veterinary Clinical Sciences, University of Sydney, NSW.

The heat shock response and heat shock gene transcription of 27, 71/73 and 88 kD heat shock proteins (hsp) in 9.5-11.5 day cultured rat embryos have been studied.
A heat shock of 43°C for 7.5 min caused developmental defects of the brain and head as a result of massive cell death 2-3 hr after the initial exposure. A mild heat shock of 42°C for 10 min is not teratogenic and induced acquired thermotolerance whereby embryos were protected from a lethal heat shock.
At the molecular level, heat shock results in the immediate inhibition of total protein synthesis and the selective synthesis of the hsps. In this study, the response of the hsp genes to the teratogenic and thermotolerant heat shock was studied at the transcriptional level. mRNAs for hsps were identified by Northern analysis and the rate and time of induction were determined by dot blot analysis. Results show an immediate synthesis and rapid transport of the mRNA to the cytoplasm where it was degraded 2-3 hr after the initial response. Heat shock genes for hsp 27, 71, 73 and 88 showed specific responses to the various heating regimes indicating regulation at the transcriptional level. Using *in situ* hybridization techniques, we have shown that expression of the 71 hs gene is tissue specific, being actively induced in both the neuroectoderm and mesoderm of the neural plate but not in the endoderm.
Using cell flow cytometry and the inhibitor ICRF 159, we have studied the effects of heat shock on the cell cycle of the neuroectoderm. A lethal heat shock initially stopped cells from cycling through S phase but not G2-M causing a depletion of cells in S phase. The thermoprotective heat shock arrested cells at entry to S phase and delayed the cell cycle by lengthening S phase. We propose that this delay and subsequent synchrony may function to protect cells from stress and regulate recovery.
The rapid heat shock response shown in the presomite embryo suggests a critical regulatory role for these genes in embryonic development and adaptation.

MULTIPLE ORIGINS OF POLYPLOIDY IN THE WILD PERENNIAL RELATIVES OF SOYBEAN

Jeff J. Doyle and Jane L. Doyle

L. H. Bailey Hortorium, Cornell University, Ithaca, NY 14853 USA

While the majority of the more than a dozen currently recognized species of wild perennial soybean relatives (*Glycine* subgenus *Glycine*) are diploid at $2n = 40$, and are confined to Australia, *G. tabacina* comprises a polyploid complex whose $2n = 80$ cytotypes are widespread in eastern Australia and the islands of the Pacific. These tetraploids comprise at least two morphological groups that have been shown to be reproductively isolated from one another, and it has been hypothesized that these types had different origins, sharing only one of their two diploid progenitor genomes. This hypothesis is supported by our studies of variation in nuclearly encoded ribosomal RNA genes (rDNA), for which two types of plants are observed. Both types are fixed hybrids for two rDNA repeat classes referable to diploid members of the subgenus, and the two types share one repeat class. Numerous chloroplast DNA (cpDNA) restriction site markers distinguish the same two classes of plant. The molecular data are concordant both with morphological markers and with studies of pairing in artificial hybrids. Phylogenetic relationships of diploid progenitors indicate that the genomic constitutions of the two polyploids are AAB_2B_2 and BBB_2B_2, referred to as A and B types, respectively. Studies of cpDNA variation in the B type polyploid reveal the existence of considerable polymorphism. The various cpDNA types were all referable to plastomes found among several different B genome diploid species confined to Australia, suggesting that the B polyploid has itself had numerous independent origins. The apparent lack of cpDNA differentiation between diploids and polyploid pairs suggests recent origin of the highly successful polyploid. Furthermore, the lack of divergence between plastomes of geographically widely separated polyploids suggests that dispersal has been recent. B type polyploids with different plastomes are interfertile and genetic diversity due to contributions from diverged diploid progenitors is therefore capable of being transferred throughout the B polyploid complex. This could account in part for the success of this cytotype as judged by its wide geographic and ecological range.

CLEGG, MICHAEL T. Department of Botany & Plant Sciences, University of California, Riverside, CA 92521 USA. Evolution of chloroplast encoded genes.

The cholorplast genome (cpDNA) has been the object of intensive study in plant molecular biology. Owing to this rich context of molecular information, and owing to the relative ease with which cpDNA can be characterized, the chloroplast genome has also become a principle focus for work in plant molecular evolution. Recent methodological advances have made it possible to obtain sequences of particular genes from many different plant taxa. The analysis of complete DNA sequence data, obtained from different plant lineages, provides information on rates of molecular evolution, on the kinds of mutational processes responsible for evolutionary change and on the relation between structure-function and mutational change. Detailed analyses of mutational change in intergenic regions, and in introns, has revealed heterogeneous patterns of evolutionary change, presumably resulting from selective constraints. The rate of synonymous evolution for the gene rbcL (large subunit of ribulose-,5-bisphosphate carboxylase) is approximately 1.5×10^{-9} substitutions per site per year for annual plant species, and preliminary evidence suggests a much slower rate among long-lived Palm taxa. These slow rates of molecular evolution mean that cpDNA sequence data can be used to investigate genetic relationships at the deepest levels of plant evolution.

Molecular Mechanisms of Drug Resistance in Malaria

ALAN F. COWMAN

The Walter and Eliza Hall Institute of Medical Research
P O Royal Melbourne Hospital
Victoria 3050 Australia

Chloroquine and the antifolate drug pyrimethanine are two of the most important chemotherapeutic reagents used against the causative agent of human malaria, **Plasmodium falciparum**. The multiple drug resistance (**mdr**) gene has been implicated in resistance to chloroquine and also a number of other drugs such as mefloquine. We have isolated the **mdr** gene from **P.falciparum** and shown that it is amplified in some chloroquine isolates. The basis of this amplification has been analysed by pulse field gel electrophoresis of chromosomes and these results suggest that the **mdr** gene mediates chloroquine resistance. The antifolate drug pyrimethanine binds very tightly to the dihydrofolate reductase enzyme of the parasite which is the reason it is such an effective antimalarial drug. A key residue has been identified that encodes resistance to this drug. This amino acid change appears to be the major mechanism of resistance throughout the world.

SESSION 4

THE TRANSFER OF NEW METABOLIC PATHWAYS TO DOMESTIC ANIMALS

K.A. Ward, C.R. Byrne, C.D. Nancarrow, J.D. Murray, Z. Leish and B.W. Wilson.

CSIRO Division of Animal Production,
PO Box 239, Blacktown, 2148

The productive capacity of domestic animals is limited by the dietary availability of specific nutrients and by the metabolic efficiency with which such nutrients are utilised. These limitations are the result of the loss during evolution of genes encoding the enzymes for various metabolic pathways. Wool growth provides a clear example of this. It is limited in the first instance by the supply to the wool follicle of the sulphur amino acid cysteine and in addition the follicle's highly-active glycolytic and pentose phosphate pathways elicit a substantial demand for glucose. The ruminant digestion of sheep results in dietary situations where both of these nutrients are in short supply. With the advent of new techniques for the isolation and modification of specific gene sequences and their efficient transfer to sheep by embryo microinjection, it is now possible to consider the replacement of metabolic pathways in this species by isolating from other sources gene sequences encoding the required functional enzymes. We are attempting to utilise this approach to overcome the cysteine limitation to wool growth by introducing a functional cysteine biosynthetic pathway, and to increase the supply of glucose to the wool follicle by introducing a functional glyoxalate cycle.

In order to provide a cysteine biosynthetic pathway in sheep, we have isolated the cysE and cysK genes from the bacterium Eschericia coli. These genes encode the enzymes serine transacetylase and O-acetylserine sulfhydrylase, which catalyze the biosynthesis of cysteine from serine and H_2S. To provide the necessary eukaryotic promoter and polyadenylation signal sequences for the bacterial genes, we have utilised the sheep metallothionein-Ia promoter sequence and a promoter-less sheep growth hormone gene. Initial evaluation of this method for obtaining expression of bacterial genes in eukaryotes was made using the cysK gene. By Northern blotting and the assay of O-acetyl sulfhydrylase, the expression of four different MT-Ia promoter\growth hormone\cysK fusion genes was measured in tissue culture cells, and one of the most suitable combinations, MTCK11, was used for microinjection into mice. Four lines of transgenic mice containing MTCK11 have been created, and expression of the gene has been examined in progeny from two of these lines. The cysE coding sequence has been fused to the MT-Ia promoter\growth hormone sequences to produce MTCE11, a similar fusion gene to MTCK11. Evaluation of this gene in transgenic mice is currently in progress. Both genes have been microinjected into sheep embryos, and the lambs from this experiment are due to be born during July, 1989.

In order to introduce a functional glyoxalate cycle in sheep, we have utilised the aceA and aceB genes from E. coli, and modified them for expression in similar fashion to that used for the cysE and cysK genes. The aceA and aceB genes encode the enzymes isocitrate lyase and malate synthase, which provide a shunt through the tricarboxylic acid cycle such that the two decarboxylation steps of the cycle are bypassed. Two-carbon units such as acetate can be used as a source of glucose by this means. Since acetate is a major source of dietary energy in ruminants, the ability to convert the volatile acid to glucose is predicted to increase the efficiency with which sheep grow wool. Currently, these genes have been examined in cell culture and are being examined in transgenic mice.

ENGINEERING HERBICIDE RESISTANCE IN PLANTS

Bruce R. Lyon

CSIRO Division of Plant Industry, Canberra, ACT

The introduction of novel traits into agronomically important crop plants is one of the most promising aspects of the application of genetic engineering to plant breeding. Genes from plants and micro-organisms have already been incorporated into tobacco, potato and tomato plants to provide herbicide, virus and insect resistance, and advances in tissue culture and regeneration protocols are now making this technology applicable to other crops.

The genetic engineering of plants for herbicide resistance has generally utilised one of three distinct strategies: 1) overproduction of the herbicide target enzyme by cloning multiple copies of the relevant gene, 2) substitution with a gene from another organism which encodes a resistant enzyme, or 3) introduction of a detoxification pathway derived from another organism. Examples of these strategies will be presented with emphasis on the methods used to introduce a herbicide detoxification gene derived from a bacterium into tobacco plants and have it expressed.

SESSION 5A

BIOETHICS AND BIOTECHNOLOGY

Norman Darvey

School of Crop Sciences, The University of Sydney, N.S.W., 2006.

Biotechnology over the coming decades will answer many important questions relevant to genetics and cell differentiation. Its benefits will include the development of new vaccines, markers for plant or animal diseases and improved agricultural production. The cost factor however raises questions of the how, when and where of research funding for biotechnology, especially at a time when economic and environmental constraints, as well as the quality of life are key considerations in the lives of most Australians. In this presentation I will not deal with the "hotbed" issues of eugenics but rather emphasise reservations to agricultural biotechnology in both developed and developing nations, as listed below:

Developing Countries: 1) Most progress has been the result of traditional plant breeding and improved agronomic practice. Will biotechnology change this, and if so, when? 2) Funding for biotechnology is usally at the expense of plant breeding and other traditional sciences (pathology, cytogenetics). The consequences of this could be catastrophic on third world agriculture. Biotechnology should be funded apart from, and in addition to, traditional agriculture - at least until the benefits of biotechnology are realised. 3) Local expertise in developing countries is often inadequate - Bandwaggon Syndrome - International advice is often spurious in that the rules for developed countries are inappropriate to survival agriculture - fear of international aid for nebulous zero-possibility projects. 4) The lie - Biotechnology is the political solution to all problems - drought, disease, famine, economic woes - It is the means of liberating society! 5) Products of biotechnology from developed countries are too expensive. Plant variety rights and/or patents limit distribution. 6) Research in developed countries focusses on the major international food crops (not on the locally adapted food crops in developing countries).

Developed Countries: 1) False hopes and promises - Scientists not learning from past mistakes associated with colchicine, mutation breeding, somaclones, etc. 2) Biotechnology is often expensive Cf. Alternatives - Grant bodies are often prejudiced and only support projects with a strong biotech. base - irrespective of likelihood of success. 3) Promises of biotechnology are often nebulous and there is no time scale - Promises of traditional methodology are time specific (often long term), but usually guarantee the end product. 4) Teachers of plant breeding/cytogenetics are being replaced by biotechnologists. Who will teach plant breeding? Who will convert products of biotechnology into something agronomically useful? 5) Biotechnologists are often biochemists with no background or understanding of field work or of the total interaction of Plant-Genotype-Environment. There are problems of respect between the biotechnologist and plant breeder - The battle of control - Who services who? 6) Cytogenetic substitutions/alien translocations usually maintain genetic equivalence/balance. However effect of insertions (additions), the types of genes that can be inserted and their interaction with existing genes as well as position effects require elucidation. 7) Who can afford the products of biotechnology? What are the motives behind private biotechnology? Profit or control.

SESSION 5A

MOLECULAR GENETIC STUDIES OF KOALA POPULATIONS

Andrea Taylor[1], Susan Ramus[1], Neil Murray[1], Jennifer Graves[1], Bill Sherwin[1], Roger Martin[2] and Kath Handasyde[2].

[1]Dept. of Genetics and Human Variation, LaTrobe University, Bundoora, 3083
[2]Dept. of Zoology, Monash University, Clayton, 3168.

Although the koala (Phascolarctos cinereus) is not generally considered endangered, its recent history in South-East Australia suggests there may be genetic problems. Since European settlement koala populations have fluctuated dramatically in size, and had artificial reintroductions from a limited stock. Significant habitat destruction has also occurred. We expect that Victorian koala populations have different amounts of genetic variation, with some being quite monomorphic. One aim of our research is to use protein and DNA polymorphisms to test this hypothesis. We will also assess any correlations between levels of genetic variation and the incidence of characteristics which may affect individual fitness.

So far, DNA and protein studies have detected genetic variation in the six populations surveyed, although levels of variation are low, even in the "hypervariable" regions of DNA used for individual identification (fingerprinting) in other species. The effects of artificial relocations may be seen in the data: migration rates calculated from the protein data reflect the history of relocation. The protein data also showed elevated levels of homozygosity which may result from a high frequency of inbreeding. This may be a natural consequence of koala demography and dispersal, but its combination with artificially lowered levels of variation between individuals could lead to genetic problems.

If these results are confirmed by further work, it would be prudent to alter the present pattern of the koala relocation program.

SESSION 5A

Restriction endonuclease variation in the region of the *Adh* locus in Australian and Chinese populations of *Drosophila melanogaster*.

C. Jiang and J.B. Gibson.

Molecular and Population Genetics

Research School of Biological Sciences

The Australian National University.

Restriction endonuclease variation in a 12 kb region encompassing the *Adh* gene has been assessed in Australian populations from different points along the Adh^S cline and compared with that in Chinese populations from similar latitudes. Although most of the variants were found in populations from both continents there was less nucleotide substitution variation and haplotype diversity in the Chinese populations. The majority of insertions or deletions detected had relatively slight effects on ADH activity or CRM. A number of exceptions were found in which null or low activity alleles had insertions in the *Adh* gene. Some of the insertions in the *Adh* region were cloned and probed with a variety of known transposable elements; sequences homologous to B104 elements were detected.

HAPLOTYPE VARIATION AT AN ENZYME LOCUS IN A NATURAL POPULATION OF *Drosophila melanogaster*.

Alex Agrotis and Stephen W. McKechnie

Department of Genetics and Developmental Biology,
Monash University, Clayton, Victoria, 3168.

Over the course of a five year period the population size of adult *Drosophila melanogaster* in the cellar of the Chateau Tahbilk winery underwent seasonal fluctuations, experiencing an extreme crash in numbers during the winter months (McKenzie, 1980). The potential for random processes to act within the cellar due to founder effects, population sub-structuring, and isolation were considerable. However, when the *Adh* protein polymorphism (2 alleles) was examined, the population appeared to remain in a state of dynamic equilibrium, with stable gene frequencies and genotypic distributions over the course of the study.

This study reports on the molecular variation present in a 2.7 kb *Adh* region of a large sample of second chromosome lines derived from three separate pre-vintage collections from the Tahbilk cellar, taken in 1986, 1987, and 1988. Variation was detected by restricting genomic DNA with 4-base cutting enzymes, separating the fragments on denaturing polyacrylamide gels, electroblotting and probing to detect polymorphic restriction sites and size variation. The sensitivity of this technique is such that size differences of as little as 1 bp between fragments can be discerned, and its application by other workers has revealed a large number of alleles, or haplotypes, at the *Adh* locus in natural populations of *Drosophila melanogaster*.

The detection of such variation provides a more sensitive assay to assess the population genetic effects of the bottlenecks in size, and it will enable us to see if particular haplotypes are associated with the winery habitat. Haplotype distributions, levels of haplotype diversity, and levels of nucleotide heterozygosity are compared among the cellar collections over the three consecutive years and to a local outside non-bottleneck population collected in 1988.

McKenzie, J.A. (1980). *Australian Journal of Zoology* 28, 709-716

TRANSCRIPTIONAL REGULATION OF INTERLEUKIN-5 GENE EXPRESSION

YAO WANG, HUGH D. CAMPBELL AND IAN G. YOUNG

Medical Molecular Biology Group, John Curtin School of
Medical Research, The Australian National University
Canberra, ACT 2601

Interleukin 5 (IL-5), or eosinophil differentiation factor (EDF) is a glycoprotein originally characterized in mice which is a growth and a differentiation factor for the eosinophil lineage and is also active on mouse B cells. Its specificity for the eosinophil lineage in myeloid haemopoiesis suggests that IL-5 may play a significant role in the regulation of eosinophilia which is characteristic of allergic responses and infections by helminths. IL-5 is expressed by T lymphocytes only after stimulation with antigens, mitogens or chemical activators such as phorbol esters. Our cloning and characterization of the murine IL-5 cDNA and the IL-5 gene has provided an opportunity to investigate the regulation of IL-5 gene expression.

Our studies to date have mainly used the T cell hybrid NIMP-TH1 which does not express detectable IL-5 mRNA unless the cells are stimulated by phorbol myristate acetate (PMA) or phytohemagglutinin (PHA). The IL-5 gene in this T cell hybrid shows similar regulation to that found in normal T lymphocytes. Expression of IL-5 gene in NIMP-TH1 after induction with PMA is completely blocked by the immunosuppressive drug cyclosporin A and the anti-inflammatory steroid dexamethasone.

Induction of IL-5 mRNA synthesis is also blocked by cycloheximide suggesting that gene expression requires the production of other protein factors. Direct measurements of transcriptional activity in isolated nuclei indicate that the effects of both induction and inhibition are primarily at the level of transcription.

Further work is aimed at exploring the effects of other steroid hormones on the transcription of the IL-5 gene and at defining the molecular mechanisms involved in IL-5 regulation in T lymphocytes.

THE ROLE OF DISTAL PROMOTER ELEMENTS IN THE CONTROL OF HUMAN GROWTH HORMONE GENE EXPRESSION

William P. Tansey and Daniel F. Catanzaro
School of Biological Sciences, University of Sydney, NSW 2006

The human growth hormone (hGH) gene family provides an excellent system in which to study the molecular basis of differential tissue-specific gene control. Despite extensive DNA sequence homology throughout both coding and non-coding regions, the members of the GH gene family - encoding both GH and chorionic somatomammotropin (CS) - are expressed in a highly tissue- and cell-type specific fashion. The principal GH gene, hGH-N, is expressed only in the somatotrophs of the anterior pituitary, whilst expression of two CS genes, CS-A and -B, and a GH variant (hGH-V) is confined to the syncytiotrophoblast of the chorion.

Promoter elements associated with the controlled expression of the GH genes have been identified. Transient expression in a rat pituitary tumour cell line, GC, has shown that in both hGH-N and hCS-A DNA sequences located within the first 500 base-pairs (bp) 5' to the transcription start site are required for efficient transcription initiation. Binding of a tissue-specific factor, GHF-1, to at least one site within the GH-N or CS-A proximal promoters (sequences located to -150) appears essential for transcriptional control.

Our work has centred on the further analysis of GH promoter architecture, with particular emphasis on the involvement of sequences other than those associated with GHF-1, the so-called 'distal promoter' elements (upstream of -150). To this end, the distal regions of hGH-N and hCS-A have been 'switched' between the two promoters and the ability of the chimaeras to drive the transient expression of a reporter gene determined by transfection into GC cells.

Our investigation has shown that deletion of distal sequences in hGH-N, or the switching with similar regions from hCS-A, leads to a four-fold reduction in transient expression over the native promoter. Interestingly, deletion of distal sequences within the hCS-A promoter, or switching with corresponding hGH-N regions, leads to a significant increase in transient expression, above the level of the native hCS-A or hGH-N promoters.

These data suggest significant differences between the distal promoters of hGH-N and hCS-A. The hGH-N distal region appears to contain an element(s) capable of potentiating transcription initiation, whilst the hCS-A distal promoter exerts a significant repressor effect (in cells of pituitary origin). The adenovirus major late transcription factor (MLTF) has been shown to bind within the distal promoter of hGH-N but not hCS-A, suggesting a role for the MLTF in the control of hGH-N promoter function. This hypothesis is currently under investigation, as is the precise nature of the repressor effect displayed within the hCS-A distal region.

TRANSCRIPTIONAL CONTROL IMPARTED BY REGULATORY ELEMENTS IN THE 5' FLANKING DNA OF THE RAT PROLACTIN GENE

Graham J. Jones and Daniel F. Catanzaro
School of Biological Sciences, University of Sydney, NSW, 2006

Prolactin (Prl) is a member of the evolutionarily related growth hormone (GH) family. The Prl gene is transcribed from lactotrophic cells within the anterior pituitary gland and is positively regulated by the steroid hormone oestrogen. This pattern of cell specific expression and hormonal regulation is controlled through the binding of *trans*-acting factors to *cis*-acting sequences in the non-transcribed 5' flanking DNA of the Prl gene.

Functionally, this region of the gene can be divided into proximal promoter and distal enhancer regions which act synergistically in directing transcription initiation. Both the promoter and the enhancer include multiple binding sequences for the cell specific factor, Pit-1. Oestrogen induction is modulated by the binding of the oestrogen receptor to the oestrogen responsive element (ORE) within the enhancer. In addition to these positive regulatory sequences, there is evidence for a negative regulatory sequence in the promoter, distinct from the proximal Pit-1 sequences.

We have investigated further the role of the ORE and Pit-1 sequences in the enhancer region. The ORE does not act as an enhancer, rather enhancer activity is dependant upon the presence of distal Pit-1 sequences interacting in a non-additive manner with the ORE. This interaction is specific, as enhancer-like activity cannot be mimicked when proximal Pit-1 sequences are substituted into the enhancer. Our current work continues with this functional analysis, evaluating several pituitary tumour cell lines, 235-1, GH_3 and GC, each exhibiting differences in the expression of Prl.

The regulatory sequences in the Prl 5' flanking DNA function in an interdependant manner and this would seem to preclude the presence of a single determinant of cell-specific transcription. Our work is directed at understanding how multiple regulatory sequences combine to properly direct Prl transcription.

CpG ISLANDS: THE PRO-OPIOMELANOCORTIN GENE IS AN UNUSUAL CASE

Margaret Gardiner-Garden [1,2] and Marianne Frommer [1,3]

[1]Kanematsu Laboratories, Royal Prince Alfred Hospital, Camperdown 2050
[2]Macquarie University, North Ryde 2113
[3]CSIRO Division of Biotechnology, North Ryde 2113

Associated with a large number of vertebrate genes are regions known as CpG islands. These regions have an unusually high CpG dinucleotide frequency compared to bulk DNA due to
a) a high G+C content, and
b) a lack of the normal CpG-depletion found in vertebrate genomes.

Most CpG islands are found at the 5' end of "housekeeping" genes. However, a number of tissue-specific genes are also associated with CpG islands. For instance, we have observed that the vast majority of genes that are transcribed in the germline and/or neural tissues are associated with CpG islands. The mammalian pro-opiomelanocortin (POMC) genes are in this class. CpG islands associated with tissue specific genes can be located at the 5'end (5' CpG island) or downstream of the translation start site (3' CpG island). The mammalian POMC genes are an unusual case. Each has both a 5' CpG island and a 3' CpG island (Gardiner-Garden and Frommer J. Mol. Biol. **196**, 261-282, 1987).

In the case of the mouse POMC gene, we have shown that the 3' CpG island is a 5' CpG island relative to short RNA transcripts found in the testes. Primer extension and S1 nuclease mapping of these transcripts shows that they have different start sites, none of which has a corresponding TATA box. A similar lack of a TATA box has been reported for several housekeeping genes with CpG islands, but no other tissue-specific genes to date.

Preliminary Northerns indicate that in numerous mouse tissues short RNA transcripts exist which may arise from the 5' CpG island of the POMC gene. These transcripts hybridize to a probe of the region of 5' CpG island upstream of the first exon, but not the first exon itself. This raises the possibility that the 5' CpG island is not associated with the tissue-specific POMC transcripts but with these more widespread short transcripts.

RIBOSOMAL DNA VARIATION IN WILD AND CULTIVATED TARO (*COLOCASIA ESCULENTA*): IMPLICATIONS FOR PREHISTORY.

Peter Matthews
Department of Prehistory, Research School of Pacific Studies, ANU.
Population and Molecular Genetics Group, Research School of Biological Sciences, ANU.

The primary aim of the study was to determine the historical status (indigenous and/or introduced) of taro varieties in Australia and Papua New Guinea. Ribosomal DNA (rDNA) cloned from taro was used for hybridisation probe analysis of DNA extracts cut with restriction enzymes. In taro, as in other species, fragments from the large inter-genic spacers of rDNA repeat units are highly variable. Assays of wild and cultivated taro from Asia and the Pacific were made using *Taq* I and *Hinf* I. The size-frequency distributions of the resulting spacer fragments are highly correlated. The distribution for *Taq* I peaks at 2.7-2.8 kbp and has extremes of 1.9-2.1 kbp and 4.5-4.7 kbp (260 fragments in 168 assays). The distribution for *Hinf* I peaks at 2.9-3.1 kbp and has extremes of 2.3-2.5 kbp and 4.5-4.7 kbp (241 fragments in 127 assays). Fragments in or close to the modal size range were found in cultivated taro from Asia and the Pacific, and in Pacific wild taro. Some Australian and Papua New Guinean wild varieties are distinct because they have extremely small or large spacer fragments, or have a distinct combination of *Taq* I and *Hinf* I fragments within the usual size range. Within Australia, different rDNA patterns are distributed homogeneously within different regions. In Papua New Guinea, a range of patterns was found among wild taro within a relatively small area.

The historical status of taro in Australia and Papua New Guinea cannot yet be determined. Wild taro in these countries may be indigenous, feral after introduction by humans, or both. The extensive distribution of wild taro in Australia and Papua New Guinea has been verified and described in some detail. As far as can be seen with the present survey, there has been endemic differentiation of wild populations in Australia and Papua New Guinea (indicated by rDNA patterns found only in these countries). Future observations of taro from outside Australia and Papua New Giuinea may verify or disprove that endemic differentiation has occurred. Other characters must be studied to determine the degree and nature of genetic differentiation in wild populations. Whether indigenous or introduced, it appears that taro varieties have not been moved between different regions in northern Australia, despite the traditional use of taro as a food, by Aborigines.

GENETIC MAPS OF WHEAT HOMOEOLOGOUS GROUP 7 CHROMOSOMES

S. Chao, P.J. Sharp*, A.J. Worland, R.M.D. Koebner and M.D. Gale

Institute of Plant Science Research, Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2JB, U.K.

* Present address: School of Crop Sciences, University of Sydney N.S.W. 2006.

Eighteen cDNA clones, fourteen anonymous and four of known function, have been identified as having homologous DNA sequences on the group 7 chromosomes of wheat. The loci determined by these probes have been mapped on one or more chromosomes in this homoeologous group with linkage data derived from various F_2, random inbred, doubled haploid and single chromosome recombinant populations. The maps also include three genes controlling isozyme production, five disease resistance genes, two anthocyanin pigment genes and a vernalization response gene.

The data have been used to examine the degree of RFLP variability in the three wheat genomes and the relative efficiency of various restriction enzymes in detecting RFLPs in wheat. The mapping data have been used to determine the extent of map co-linearity over the A, B and D genomes of wheat, to identify genetic linkages between marker genes and genes of agronomic significance, and to examine the structure of a chromosomal segment transferred from Aegilops ventricosa to wheat.

ECOLOGICAL CONSTRAINTS TO OUTCROSSING IN ORCHIDS

Rod Peakall

School of Biological Sciences, Macquarie University, NSW 2109

Several features of the orchids may place constraints on outcrossing rates and other population genetic characteristics. Firstly, the pollen is packaged as a mass of many thousands of grains-the pollinium. Pollination may therefore involve the receipt of a single genotype and pollen carry over schedules may be markedly different to other plant groups. Secondly, more than 10,000 orchid species secure pollination by food, sexual and other forms of deceit. Consequently, patterns of pollen flow may be very different to food rewarding and wind pollinated plants. Thirdly, clonality is wide spread and in association with pollinator movements may place constraints on outcrossing. Most orchid seeds are difficult to germinate because they require mycorrhizal infection for germination. Consequently, outcrossing rates cannot be estimated by the normal electrophoretic analysis of progeny arrays. Two promising techniques have been developed to estimate outcrossing. The first method utilizes coloured pollinia labels to track pollen flow in natural populations. The second method compares the electrophoretic band patterns of bulked seed from a single fruit with maternal banding patterns. Studies using these techniques are in progress.

GENETIC VARIATION, PLASTICITY AND POPULATION STRUCTURE IN A NATIVE LEGUME *VIMINARIA JUNCEA* (SHRAD. & WENDL.) HOFFMANNS.

Beverley A. Walker[1] and Sidney H. James. [2]
1. Department of Biology, University of Wollongong
2. Department of Botany, University of Western Australia

The shrub legume Viminaria juncea is a common and widespread species of moist habitats of Southern Australia. The species appears to have developed a high degree of plasticity in response to different environmental conditions, including phenotypic variation of its juvenile habit. The levels and distribution of genetic diversity at 9 polymorphic allozyme loci in 17 populations in the south-west of Western Australia were estimated. Population structure and environmental correlates with polymorphism, allozymic and morphological variation are discussed.

STRUCTURE AND FUNCTION OF THE PROTEIN PRODUCTS OF THE *WHITE, SCARLET* AND *BROWN* GENES OF *D. MELANOGASTER*

Antony J. Howells

Department of Biochemistry, Faculty of Science, Australian National University, Canberra, A.C.T., 2601.

The eye colour of wild-type adults of *D. melanogaster* is red-brown, due to the presence of red (drosopterins) and brown (xanthommatin) pigments within the pigment cells of the compound eyes. Mutations at the *white* locus can completely abolish production of both types of pigments and caused pure white eyes, while *scarlet* mutations affect the production of the brown pigment and *brown* mutations the production of red pigments giving red and brown eye colours respectively. The functions of the protein products of these genes in pigment biosynthesis, and how mutations at *white* can simultaneously affect the production of both types of pigment are long-standing problems of Drosophila molecular genetics. The cloning and sequencing of these genes (*white:* O'Hare *et al*, J. Mol. Biol. 180: 437-455, 1984; *brown:* Dressen *et al*, Mol. Cell. Biol. 8: 5206-5215, 1988; *scarlet:* Tearle *et al*, Genetics, in press, 1989) has provided new insights into the problems.

The amino acid sequences of the protein products of these genes, derived by translating the nucleotide sequences, suggest that they are strongly related; the overall homology between the *white* and *brown* proteins is 29%, while that between the *white* and *scarlet* proteins is 37%. However there are highly conserved regions in all three proteins where the homology is greater than 60%. Even more striking is the comparison of hydropathy profiles, which shows that all three proteins have very similar distributions of hydrophobic and hydrophilic regions along the polypeptide chains. Two conserved features of the amino acid sequence are compatible with the hypothesis that these are membrane proteins with permease function. Firstly, towards the N-terminal end there are highly conserved sequences showing strong homology to the ATP-binding domain found in a number of well-characterized bacterial proteins involved in membrane transport (eg, the *his*P protein of *E. coli*). Secondly, the C-terminal halves of the proteins are quite hydrophobic and contain several highly hydrophobic potential trans-membrane segments.

These observations suggest the following model for integrating the functions of the three genes in pigment biosynthesis. It is proposed that all three code for proteins that span the plasma membrane of pigment cells, being involved in the active transport of precursors of the pigments into these cells. Since *white*$^+$is necessary for the production of both types of pigment, it seems likely that the active permease complexes in the membrane must consist (minimally) of dimers involving the interaction of the *white* and *brown* proteins to permit entry of red pigment precursors and the *white* and *scarlet* proteins for the precursors of the brown pigment.

Analysis of P element induced quantitative variation in *Drosophila melanogaster*

Adam Torkamanzehi and Chris Moran
Dept of Animal Husbandry, University of Sydney, NSW 2006.

In *Drosophila melanogaster*, it is possible to use the P-M system of hybrid dysgenesis to generate mutations at many loci at well above the spontaneous rate. Early experiments to assess the importance of transposition mediated mutations affecting quantitative loci have yielded ambiguous and/or unrepeatable results. The two main experimental approaches, namely crossing P and M strains and subsequent selection, and chromosome contamination experiments, have been confounded with problems such as the influence of background variation and inappropriateness of non-dysgenic (P♀♀xM♂♂) populations to serve as genuine transposition negative controls for dysgenic populations, where high levels of transposition and mutation are expected. In three independent selection experiments, we have found no evidence of consistently enhanced response in dysgenic lines relative to non-dysgenic controls contra Mackay's 1984 and 1985 results. Further we have found independent evidence of high levels of transposition in our non-dysgenic lines, consistent with our results but not those of Mackay.

Here we discuss results obtained using a new experimental design in which the negative controls definitely lack transposition and furthermore any selection responses due to transposition in the experimental lines can be seen in the absence of background response. This approach has utilised an M stock and a co-isogenic P stock derivative obtained by microinjection of P elements into the M stock. The P stock has about 50 P elements like a natural P stock. Selection lines were initiated from the M parental stock (negative control) and from a dysgenic cross of the M and P stocks. Substantially enhanced responses were obtained in the experimental lines which had been mutagenised by the original insertion of P elements as a result of microinjection and following the dysgenic cross. Clearly transposable element mediated mutation can be a substantial source of quantitative genetic variation.

Insertion of a retrovirus has caused the hairless mutation of the mouse.

J.P. Stoye[1], S. Fenner[1], G.E. Greenoak[2], C. Moran[3] and J.M. Coffin[1].

[1]Dept of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston MA 02111, USA.
[2]Dept of Veterinary Pathology, University of Sydney, NSW 2006.
[3]Dept of Animal Husbandry, University of Sydney, NSW 2006.

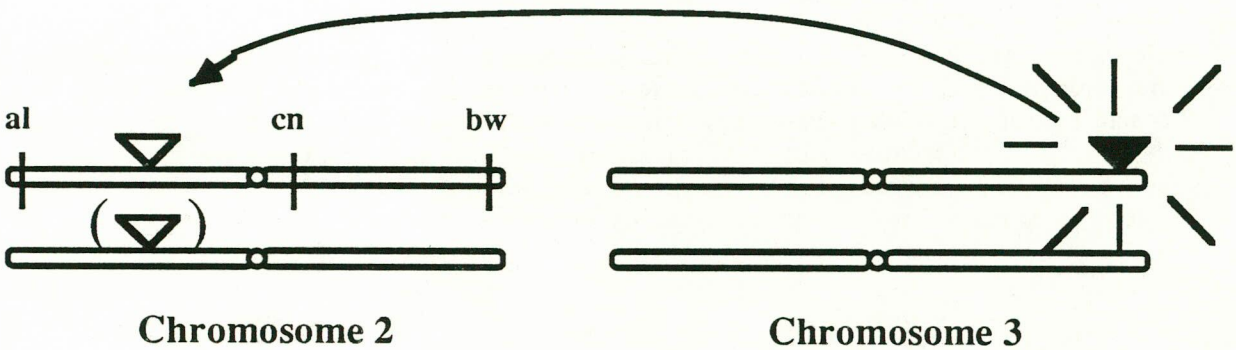
Initially by use of an oligonucleotide probe specific for polytropic Murine Leukemia Virus (MuLV), it has been established that the hairless mutation is caused by a retroviral insertion. 60 backcross progeny showed perfect co-segregation of a 3.1 kb Eco R1 fragment with the *hr* allele and a 5.5 kb fragment with the wildtype allele. Proof of the insertion was provided by a revertant (hr^+) stock produced at the University of Sydney. Both the oligo and flanking DNA probes detected a 6.3 kb Eco R1 fragment in the revertant. Sequence analysis has proved that the retrovirus is essentially deleted from the revertant. The extra 800 bp of DNA relative to wildtype consists of a long terminal repeat (LTR) left behind when the virus excised, probably by homologous recombination between the flanking LTRs.

Preliminary data indicate that the insertion is within a large intron and attempts are continuing at Tufts to identify and clone the coding sequences. As studies within the Department of Veterinary Pathology have shown that the hr^+ reversion dramatically reduces the predisposition to UV radiation induced skin cancers (relative to *hr* mutants and independent of hair loss), we are keen to determine the nature of the *hr* gene product, as well as to examine its ontogeny and tissue specificity.

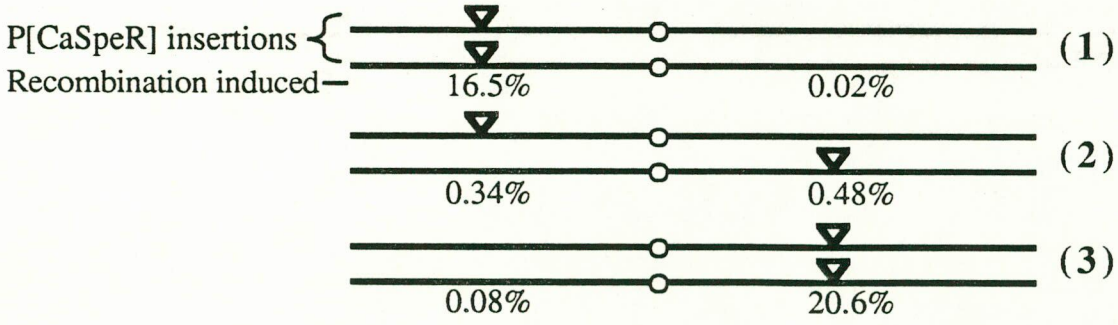
INDUCING RECOMBINATION IN *DROSOPHILA* WITH P FACTORS.

John Sved, Leila Blackman and **Dawn Verlin**, Biological Sciences, Sydney University.

P factors in *D. melanogaster* have been shown to cause male recombination, as well as other dysgenic traits. Normally this recombination occurs when strains containing many P factors are crossed to strains containing none. We have shown that recombination of a similar nature can be induced by a system composed of just two genetically-engineered P elements. One element, denoted P[Δ2-3](99B), is a nearly complete element inserted near to the right-hand end of chromosome 3 (see figure below). This element supplies the 'transposase' which is capable of mobilising other incomplete P elements in the genome. The second element can be of almost any constitution provided that it contains P element ends, and we have used the element P[*CaSpeR*], which contains a copy of the white gene, and which has been transformed to a number of sites in the genome.



Two experimental designs have been used which differ in whether the Ca element is present in single dose, or in double dose at homologous sites. The results for chromosome 2 recombination in one experiment given below show that both conditions induce recombination, but that there is a dramatic increase in frequency when elements occupy homologous sites. The levels found in case (2) below, where there is a single element on each arm, are comparable to those of experiments in which there is only a single P[*CaSpeR*] element.



The recombination appears to be exact, as determined by the lack of phenotypic effects in recombinant products. We have cloned the regions containing the two elements shown above. At the level detectable by Southern hybridisation, there are no differences in the restriction patterns for the parental chromosomes and for recombinant chromosomes.

DELETION AS A MEANS OF ISOLATING AVIRULENCE GENES FROM RUST FUNGI.

J.N. Timmis, D.L. Whisson and A.M. Binns

Department of Genetics, University of Adelaide,
Adelaide, South Australia 5000, Australia.

The genetics of flax rust (*Melampsora lini*) and its host-flax (*Linum usitatissimum*) is well understood (Shepherd and Mayo, 1972) and has been utilized here in an attempt to isolate rust avirulence genes. DNA from a strain of flax rust (228090) homozygous for four linked avirulence genes (*A-L5, A-L6, A-L7, A-Lx*) was prepared. A deletion mutant involving *A-L7* and *A-Lx* was produced by gamma irradiation of strain Aec 52 which is genotypically:

$$\frac{A\text{-}L5}{A\text{-}L5}\ \frac{A\text{-}L6}{A\text{-}L6}\ \frac{A\text{-}L7}{a\text{-}L7}\ \frac{A\text{-}Lx}{a\text{-}Lx}$$

but otherwise isogenic with 228090. A strain (68.1), homozygous for the deletion was then isolated by selfing, and tested against 31 differentials to verify that it arose from Aec 52. These tests indicated that *A-L5* and *A-L6* had also been deleted confirming the origin of the mutation by deletion rather than by somatic recombination. The new mutant was able to infect four different host varieties specifically resistant to rust strains containing *A-L5*, *A-L6*, *A-L7* and *A-Lx* avirulence genes. Using a method (Kunkel *et al.*, 1985) which allows the cloning of the specific DNA sequences deleted from the mutant genome, called the Phenol Emulsion Reassociation Technique (PERT) a 250bp DNA fragment was isolated. This clone hybridizes DNA from the 228090 strain but not DNA from the deletion mutant. Experiments are now underway to isolate additional differentially hybridizing clones and to isolate genomic regions flanking the 250bp clone.

Kunkel, L.M., Monaco, A.P., Middlesworth, W., Ochs, H.D. and Latt, S.A. (1985) *PNAS*, 82: 4778-4782.

Shepherd, K.W. and Mayo, G.M.E. (1972) *Science*, 175: 375-380.

MOLECULAR AND GENETIC ORGANIZATION OF THE ORIGIN OF REPLICATION OF A BACTERIAL PLASMID

Sean Davies and Viji Krishnapillai

Department of Genetics and Developmental Biology, Monash University, Clayton, Vic. 3168

Current models of the control of bacterial plasmid DNA replication are based on controlling initiation either by RNA countertranscript (antisense) inhibition of an essential initiation factor or by the negative/positive interaction of an initiator protein with tandem DNA repeats (iterons).

In order to extend these to the Pseudomonas bacterial plasmid R91-5, the DNA sequence of its origin of replication has been determined. Analysis of the 2815 base pair (bp) sequence revealed two open reading frames (ORF) of 888 bp and 276 bp separated by 1066 bp. Promoters showing partial homology to consensus E. coli promoters recognized by Sigma-70 or Sigma-54 of RNA polymerase and a Shine-Delgarno sequence have been identified for the ORFs. Northern Analysis confirms the synthesis of a 900 and a 300 nucleotide transcript corresponding to the ORFs. Amino acid analysis of the DNA sequence of the larger ORF indicates that the 296 amino acid protein is basic and has the characteristics of a DNA-binding protein in terms of the conservation of critical amino acids forming a helix-turn-helix structure. These properties suggest that it is the replication initiation protein, whose locus is repA. The 276 bp ORF has been confirmed to correspond to the incompatibility locus incA.

Comparision with other plasmids from E. coli, Bacillus subtilis and Staphylococcus aureus indicates that the control of replication of R91-5 does not conform to the current models and suggests some exceptional features. These include the lack of overlap of incA with repA, absence of countertranscripts and well-defined iterons.

SESSION 7A

RESTRICTION MAPPING OF PSEUDOMONAS CHROMOSOMES

E. Ratnaningsih, A. Kearney, R. Saffery and A. Morgan

Department of Genetics and Developmental Biology,
Monash University, Clayton, Victoria, 3168

Pulsed field agarose gel electrophoresis has been used to separate SpeI generated fragments of Pseudomonas aeruginosa PAO and P. putida PPN genomic DNA, yielding chromosomal sizes of 5800 kb and 3850 kb respectively. Southern blot analysis of these gels using as probes either clones from a cosmid library carrying genes of known map position, or clones from a SpeI junction fragment library, has allowed the construction of a genetic/physical map of the P. aeruginosa PAO chromosome.

MECHANISMS OF ANEUPLOIDY: DIFFERENCES IN BEHAVIOUR OF X CHROMOSOMES AND AUTOSOMES

Judith Ford and Anthony Correll

Genetics Department, The Queen Elizabeth Hospital, Woodville, South Australia 5011

Karyotypic studies of human chromosomes at metaphase have suggested that sex chromosomes undergo increased errors of division in older subjects. Previous studies from this laboratory suggested that autosomes might also exhibit changes in division with age. Our results suggested that autosomes undergo different types of error in older subjects but that the rate of error does not markedly change. This paper reports a study which was performed on anaphase cells to further these observations and test the concepts underlying them. Whole blood cultures were harvested without the use of microtubule inhibitors. Prior to fixation, cultures were exposed to warm hypotonic KCl solution for 5 minutes to induce anaphase. The cells were fixed rapidly in methanol/acetic acid in an ice bucket. Cells were then spread carefully onto slides. Tritium labelled alpha satellite sequences specific to chromosomes 17 and X were hybrized to the slides of 6 subjects of different ages. The positions of the chromosomes were noted in metaphase and anaphase cells.

X Chromosomes: At metaphase, X chromosomes were found to be either in the metaphase ring, displaced within the ring or displaced into the cytoplasm outside the spindle. Displacement rates varied between subjects. There seemed to be no obvious correlation of inner displacement with age but some correlation with outer displacement with age. At anaphase 6 different types of behaviour were recognized. These were normal segregation, chromatid malsegregation, nondisjunction, central lagging, peripheral lagging and monopolar segregation. Monopolar segregation was only seen in younger subjects whilst in older subjects, both chromatids and sometimes both chromosomes were likely to be involved in lagging. Two subjects of age 64 showed both 1:0 and 0:0 segregations.

Chromosome 17: At metaphase, chromosomes 17 were displaced within the ring at a high frequency in all subjects. This was found to correlate with the frequency of segregation of chromatids within the rings at anaphase although younger subjects were more likely than older to have one chromatid within the ring and one on the ring. Outer displacements were rare for this chromosome and no monopolar segregations were observed. All 5 other types of behaviour were observed for chromosome 17 but changes in the types of error occured with age. Subjects over 50 years of age showed only nondisjunction. There was no evidence of lagging nor chromatid malsegregation following lagging in these subjects.

The results for the 2 chromosomes are similar to the predictions made in earlier studies. The data show that there are both differences between chromosomes and between subjects for the same chromosome. It appears that younger subjects have more capacity to retrieve chromatids which lie in inappropriate positions at metaphase.

SESSION 7B

Mapping human X-linked genes in Marsupials and Monotremes

Jacki Watson, James Spencer and Jennifer A. Marshall Graves

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

Marsupials and monotremes provide the only variants in mammalian sex chromosome gene content and function. Thus they are of particular significance in the understanding of the evolution of mammalian sex chromosomes and X-chromosome inactivation.

We have used in situ hybridization to investigate the location in marsupials and monotremes of several sequences derived from the human X chromosome. The results are tabulated below.

There is a group of genes which are X-linked and subject to X-chromosome inactiviation in all three mammalian groups. The more closely related therian mammals have a further group of X-linked, X-inactivated genes which are autosomal in monotremes, and there is a further group of genes which are X-linked and subject to X-chromosome inactivation in the Eutheria only.

TABLE:

GENE SYMBOL	HUMAN MAP LOCATION	MARSUPIAL MAP LOCATION	MONOTREME MAP LOCATION
G6PD	Xq28	X	Xq
GDX	Xq28	X	Xq
P3	Xq28	X,3	Xq
MCF2	X	?	Xq,2
RCP	Xq28	X,5,3	Xp,1,2
PLP	Xq21-q22	X,5	Xp,1,2
MAOA	Xp21-p11	5	1,2
TBG	X	1	3
CGD	Xpter-p12	3,5,6	1,4
PKS	Xp	X,3	2

BIOCHEMICAL APPROACHES TOWARDS THE IDENTIFICATION OF SEX-SPECIFIC PROTEINS

Mark P. Bradley[1] & Ulf H. Wiberg[2]

[1]A.B. Technology, Pty, Ltd, C/- Department of Biochemistry, Faculty of Science, Australian National University, Canberra 2601
[2]Institute for Human Genetics, Albertstrasse 11, Freiburg i Br.,D-7800 FRG

Sex-specific proteins are of interest to both geneticists and reproductive biologists, as such proteins may have unique functions in the complex processes of sex differentiation; gamete development; and/or differential growth. Our interests have been primarily orientated towards the study of male-specific proteins, expressed in mammalian testis. Male-specific antibodies raised in female HS rats, by intrasplenic immunisation were used to identify a male-specific protein of molecular weight 20,000, in cellular extracts from spermatozoa and testis. In an attempt to purify these proteins, ovine testicular extracts were subjected to several different chromatography steps; ion-exchange, gel filtration and finally immunoaffinity chromatography on a Sepharose-4B to which male -specific antibodies had previously been bound. With this protocol we were able to isolate a protein with a molecular weight of 20,000 and a pI close to 4.2. Preliminary amino acid analyses of this preparation revealed striking similarities to a male-specific protein previously isolated from cultured Daudi cell supernatant, by Ohno (personal communication) and thought to be the H-Y antigen. Immunohistochemical analysis on the distribution of this male-specific protein show that it is expressed on germ cells shortly after the onset of puberty, and mature spermatozoa express the antigen over the posterior part of the head and midpiece of the flagellum. These observations agree well with the reported expression of the MEA gene (male-enhanced antigen) within the testis, as recently described by Lau. It may well turn out that the male-specific antigen we have described and MEA are one and the same.

REPLICATION AND MAINTENANCE OF PLASMIDS IN SIMPLE EUKARYOTES

Keith L. Williams and Nigel A. Farrar

School of Biological Sciences, Macquarie University, Sydney NSW 2109

Several small high copy number nuclear plasmids have been characterised in the simple eukaryotes *Saccharomyces cerevisiae* and *Dictyostelium discoideum* . The DNA of these plasmids is organised in the same way as chromosomal DNA. Since the plasmids are autonomously replicating DNA circles, replication and copy number controls represent attractive models for eukaryote gene regulation. The *S. cerevisiae* two micron plasmid encodes four genes, includes two *cis* - acting regions and contains an inverted repeat of 599 bp. The plasmid is highly transcribed. An elaborate system for maintaining plasmid copy number will be reviewed. The *Dictyostelium* plasmids are less well characterised, but DNA sequencing and functional analysis indicate that their study will illuminate aspects of developmental gene regulation. In addition to their interest for fundamental studies, these plasmids offer considerable utility for the development of vectors for the expression of homologous or heterologous genes.

SESSION 8

THE EVOLUTION OF INSECTICIDE RESISTANCE

John A McKenzie

Dept. of Genetics, University of Melbourne, Parkville 3052

The development of resistance to insecticides is discussed within an evolutionary framework in which the selective agent is unambiguously defined. The discussion will suggest why laboratory selection programmes usually produce resistance with a polygenic basis while natural selection favours allelic substitution at a single locus.

In the latter case the relevance of subtle interactions between genotypes to relative fitness will be considered as will the selection, in appropriate evolutionary circumstances, of modifiers that ameliorate the initial deleterious effects of resistant alleles.

The interface between developmental, population and molecular genetics in resistance studies will be emphasised while attempting to identify areas of future research. In this context the continuum between "applied and yet to be applied research" will be apparent.

"Evolution at the *Nor* and *5SDna* loci in grasses of the Triticeae"

R.Appels CSIRO, Division of Plant Industry, PO box 1600, Canberra ACT

The grasses related to wheat form a group of economically important species which are found in the Triticeae. The study of repetitive sequences such the ribosomal RNA genes at the *Nor* loci and the 5SDNA units at the *5SDna* loci has shown that the repetitive units at a given locus evolve in concert and provide;
(1) useful taxonomic characters for studying relationships between species and,
(2) valuable probes for detecting "alien" chromosome segments introduced into wheat.

The DNA sequence data-base now available for *Nor* loci will be discussed in detail to illustrate the pattern of sequence change and, in the case of sequence comparisons between a unit from the D genome of wheat and a unit from the progenitor of the D genome (*Triticum tauschii*), a possible time-scale over which changes can occur. The study of the wheat D genome and *T.tauschii Nor* units is in collaboration with I.Vinizky and J.Dvorak (Davis). The spacer region between the rRNA genes is evolving at a much faster rate than the gene regions but the comparison between the wheat D genome and *T.tauschii Nor* units has shown that within the time that has elapsed since the "fixation" of a *T.tauschii* chromosome set in hexaploid wheat, this spacer has not accumulated more mutations than the gene regions. On the broader time-scale which separates the *Nor* loci of different diploid species in the Triticeae, the faster rate of evolution of the spacer region is clearly evident but even in this situation certain regions are more conserved than others and a model of gene expression at the *Nor* loci which takes into account this observation will be discussed.

Examples of the application of genome specific probes from the spacer regions in *Nor* loci include the characterization recombinant chromosomes, the determination of the origin of a chromosome segment conferring BYDV resistance to wheat and the analysis of polyploid species in the Triticeae.

COMPLEX REGULATION OF METABOLIC GENE EXPRESSION IN THE YEAST *Saccharomyces cerevisiae*

Ian W. Dawes

School of Biochemistry, University of N.S.W., Kensington 2033

Promoters of yeast genes transcribed by RNA polymerase II are often surprisingly complex with multiple control systems acting to modulate the rate of transcription of the gene. This control is mediated via DNA-binding proteins which recognise specific short sequences in the 5'-untranslated region of the gene, and in some cases these motifs overlap making regulation quite complicated. For several yeast genes examined in sufficient detail it is also clear that there are sequences within the coding region which can influence the rate of transcription, and these downstream activation sites can pose problems in trying to achieve maximal expression from a yeast promoter. The regulation mechanism for metabolic genes is usually much less specific than in prokaryotic systems: rather than there being specific activator or repressor proteins for a particular biosynthetic pathway, there appear to be combinations of more general control systems which modulate transcription according to the nutritional status of the cell.

These aspects of control will be discussed with reference to results obtained with the yeast *LPD1* gene encoding lipoamide dehydrogenase, which functions in two complexes required for the respiration of pyruvate via the citric acid cycle. This gene has multiple, and overlapping elements involved in its regulation, and has a downstream activation site required for maximal gene expression.

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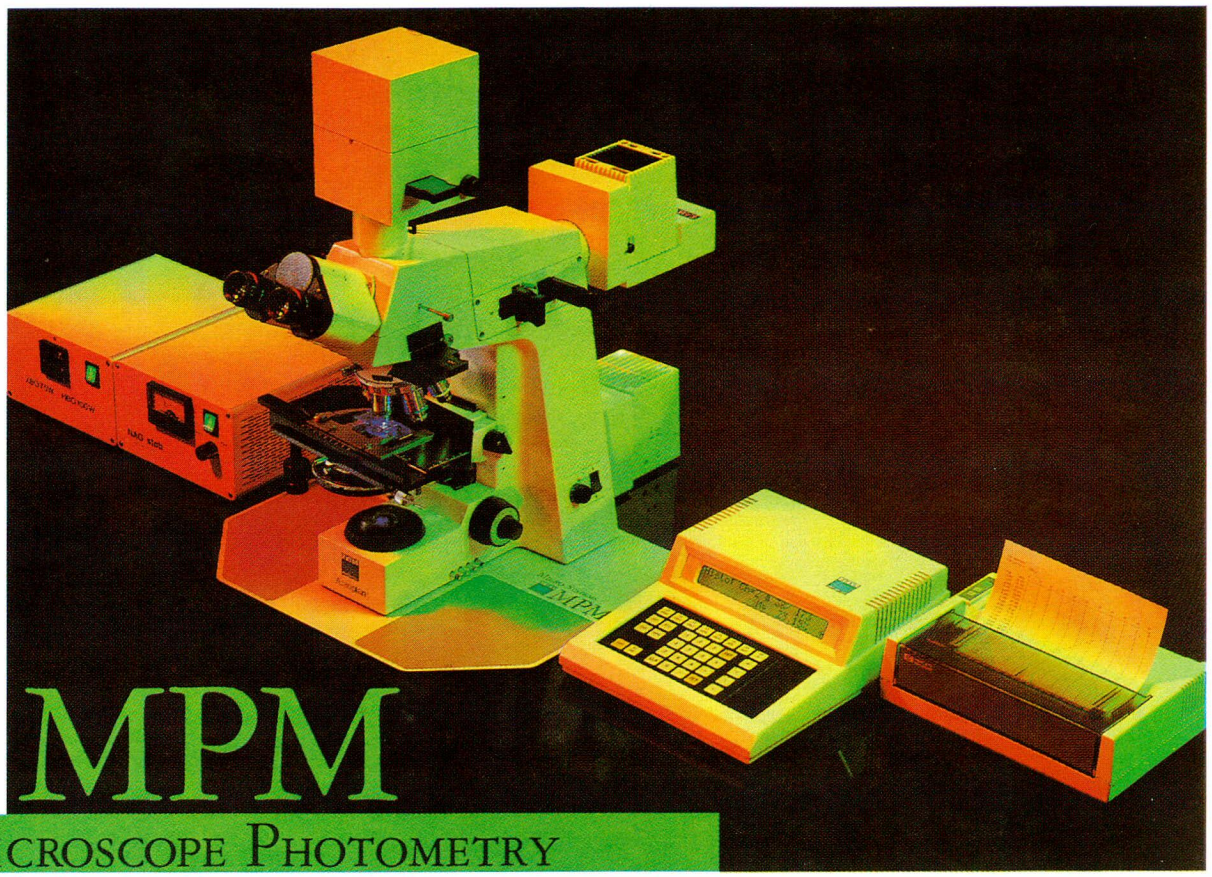

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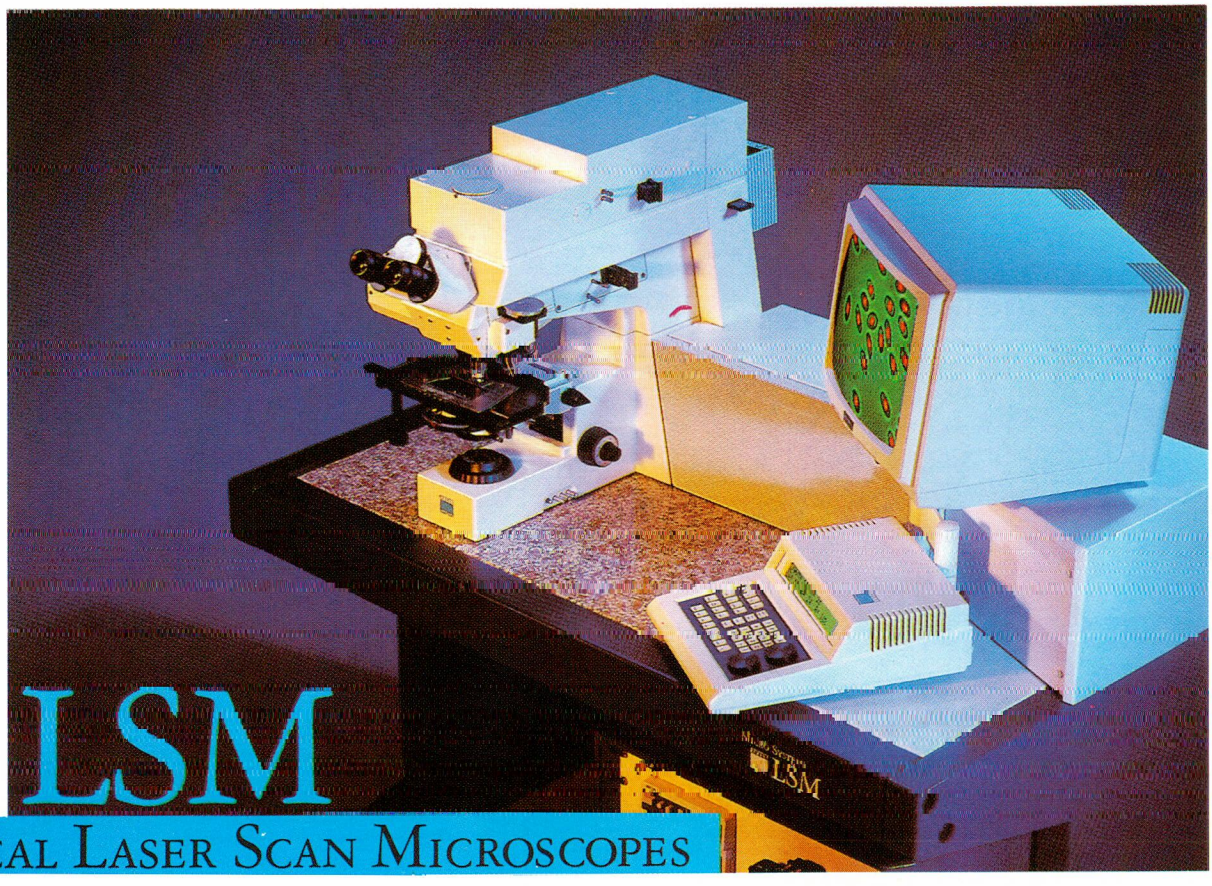

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POSTER 1

Cloning genes from the 14A region of the *Drosophila melanogaster* X-chromosome.

M. Morgan[1] and P. East[2]
1. Department of Genetics, Universityof Melbourne.
2. CSIRO Division of Entomology, Canberra.

The *scalloped* (*sd*) and *shibire* (*shi*) loci of *Drosophila melanogaster* are closely linked mutations on the X-chromosome mapping to positions 51.6 and 52.2 respectively. Both genes are located within the deficiency $Df(1)sd^{72b}$, which deletes polytene bands 13F1 to 14B1.

The *sd* locus affects the development of the wing and haltere imaginal disks. Mutant alleles vary in their phenotypic severity from minor nicks in the wing margin to almost complete abolition of the wing blade, similar to the phenotype of *vestigial* (*vg*). The *shi* gene is required both for neural transmission in the adult and the correct differentiation of a variety of tissues throughout development. Pleiotropic temperature sensitive (ts) alleles at the *shi* locus can induce rapid adult paralysis at $30^{0}C$, morphological defects in diverse adult structures, and are ts maternal effect, embryonic and pupal lethal.

In order to facilitate the cloning and molecular analysis of the *sd* and *shi* loci, the genes were screened for new mutations inducible by EMS, ionizing radiation and P-elements. New alleles were induced at the *sd* locus by all mutagens used and vary in their phenotypic expression. Unconditional recessive lethal *shi* alleles were induced by EMS, X-rays and ^{60}Co but no P-induced *shi* mutations were isolated using P-element mutagenesis.

The cytological characterization of the newly induced mutations places the *sd* locus in 13F and the *shi* locus in 14A between 14A7-B1. A putative clone of the *sd* locus, λsd, has been isolated from a genomic library constructed from the P-induced mutant sd^{87a2}. The *in situ* hybridization of this clone shows it to be located at or close to the position of the left hand breakpoint of $Df(1)sd^{72b}$ at 13F1. Homologous clones which have been isolated from a Canton-S genomic library are presently being used to probe Southern blots of the deficiency and the P-induced *sd* alleles.

In order to clone the *shi* locus a bi-directional chromosome walk has been initiated with a lambda clone, λG2, which *in situ* hybridizes at 13F16-18, within the $Df(1)sd72^{b}$ deletion and was kindly provided by David Sullivan (Syracuse University). Overlapping λ clones covering 80kb have been isolated and the two directions of the walk oriented by the *in situ* hybridization of various restriction fragments. The left end of the walk towards the *sd* locus hybridizes at 13F7-8, while the right end hybridizes at 14A4-6, close to the cytological location of the *shi* locus. Further steps in the walk are proceeding in this direction.

GENE REGULATION IN *Rhizobium* strain NGR234 IS CONTROLLED BY A BACTERIAL GENE AND A PLANT SIGNAL.

Kate Le Strange [1], Greg Bender [1], Michael Djordjevic [1], Jeremy Weinman [1], John Redmond [2] and Barry Rolfe [1].

[1] Plant Microbe Interactions Group, Research School of Biological Sciences, Australian National University, Canberra 2601.

[2] School of Chemistry, Macquarie University, North Ryde 2113.

The *Rhizobium* nodulation gene *nodD* activates the genes responsible for nodule formation (nodulation or *nod* genes) in a wide variety of *Rhizobium*:legume interactions. NodD is a transcriptional activating protein, binding to promoters of inducible *nod* genes.

Plant derived compounds interact with NodD to induce these *nod* genes, and so initiate the nodulation process. The plant compounds responsible in legume species are multi-ring phenolic compounds of the flavonoid type - see figure 1. Anti-inducers in the same systems are also flavonoid compounds which competitively inhibit NodD dependent activation of other *nod* genes.

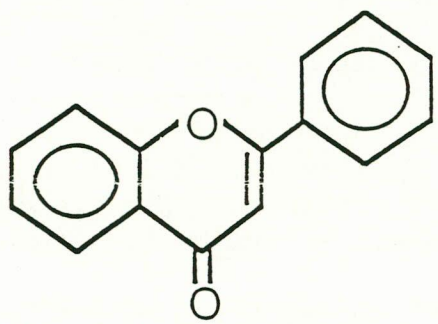

Figure 1 - flavonoid type multi-ring compound

Rhizobium strain NGR234 is one of the few *Rhizobium* strains capable of forming nodules with a non-legume. Molecular characterisation of *nodD* in this strain shows it to be highly conserved at a genetic level to other *Rhizobium* *nodD* genes, however at a functional level it is capable of interacting with a much broader range of plant phenolic compounds.

The interaction of the NGR234 *nodD* and this broad range of plant compounds is currently being investigated by the generation of chimaeric *nodD*s. Attention is focussed on a region of the gene thought to confer the broad plant compound interaction capacity.

REGULATION BY THE *amdR* GENE OF *Aspergillus nidulans*

I.B.Richardson[1] A. Andrianopoulos[1] T.G. Littlejohn[2]
M.Katz[2] R. van Heeswyck[3] and M.J.Hynes[1]

1. Department of Genetics, University of Melbourne
2. Department of Microbiology, Monash University
3. Department of Biochemistry, La Trobe University

The positively acting regulatory gene *amdR* of *Aspergillus nidulans* is involved in regulating the expression of at least five structural genes:*amdS*, *gatA*, *gabA*, *lamA* and *lamB*.

Binding sites for the amdR protein in the 5′ region of the *amdS*, *gatA*, *lamA*, and *lamB* have been determined *in vivo* using titration studies. Transformants containing multiple copies of subclones from the 5′ regions of these genes have been shown to have reduced growth on 2-pyrrolidinone media due to titration of amdR away from the *lamA* and/or *lamB* structural genes. Studies with oligonucleotides from within the titrating region of *amdS* has confirmed that the amdR binding site is contained within a 21 base pair sequence. Homology to this sequence can be found in the 5′ regions of *gatA*, *lamA* and *lamB*.

In vitro studies on amdR binding are being carried out using crude nuclear extracts and mobility shift and DNaseI footprinting assays. A binding activity has been identified within the 21 base pair region of the 5′ of *amdS* already identified as essential for amdR binding *in vivo*.

The *amdR* gene has been cloned and sequenced. The protein sequence encodes a domain in the N-terminal portion with significant homology to the putative zinc finger DNA binding domains of other fungal regulatory proteins such as GAL4 and qa-1F.

REPEATED SEQUENCES IN THE AUSTRALIAN SHEEP BLOWFLY, *LUCILIA CUPRINA*

Harvey Perkins & Tony Howells
Department of Biochemistry, The Faculties
Australian National University
Canberra, 2601

Various studies into the molecular biology of the Australian sheep blowfly, *Lucilia cuprina* , have revealed the prevalence of repeated sequences in the DNA of this species.

Analyses of the *white* and *topaz* loci have indicated that various short (<20 bp) sequences are present, either in tandem arrays or as dispersed elements, in and around these two genes, and are thought to be dispersed throughout the genome. *Lucilia* , therefore, apparently fits the "short periodicity interspersion" pattern of sequence organization rather than the somewhat atypical "long periodicity interspersion" pattern found in some organisms, including *Drosophila* .

In the course of current work aimed at the isolation of blowfly transposable elements (another form of repeated sequence DNA), a tandemly arranged repeat sequence of approximately 180bp has been discovered and subsequently cloned and is now being characterized.

The preponderance of dispersed repeated DNA in the blowfly genome presents problems which make molecular biological studies of this species, although interesting, extremely difficult and exasperatingly frustrating.

A MUTANT OF ESCHERICHIA COLI SHOWING RESISTANCE TO PHLEOMYCIN AND TO HEAT INACTIVATION IS DEFECTIVE IN UBIQUINONE SYNTHESIS.

Christina M. Collis and Geoffrey W. Grigg

CSIRO Division of Biotechnology, Laboratory for Molecular Biology, P.O. Box 184, North Ryde, N.S.W. 2113, Australia.

A mutant of Escherichia coli, selected for resistance to the antibiotic and antitumour agent phleomycin, has been characterized and the phleomycin-resistance determinant identified. The mutation also confers resistance to the lethal effects of heating at 52 degrees C. Other characteristics of the phleomycin-resistant strain include slow growth rate and inability to grow on succinate as sole carbon source (Suc^- phenotype), cross-resistance to aminoglycoside antibiotics, and slight sensitivity to hydrogen peroxide, methyl methane sulphonate and gamma-irradiation. Some of these characteristics, together with mapping data, suggested that the phleomycin-resistance and Suc^- determinant probably lay within the ubiF gene coding for an enzyme effecting a step in the biosynthesis of ubiquinone. The phenotypes of known mutants defective in this and other steps of the ubiquinone pathway were found to be closely similar to those of the original phleomycin-resistant strain.

POSTER 6

INSERTION OF A FUSION GENE INTO THE MOUSE Y-CHROMOSOME; EFFECTS ON EXPRESSION IN TRANSGENIC MICE.

Catherine Shanahan, Nola Rigby, James Murray and Kevin Ward.

C.S.I.R.O. Division of Animal Production, P.O. Box 239, Blacktown, N.S.W. 2148.

Five lines of transgenic mice carrying a sheep metallothionein 1a - sheep growth hormone fusion gene have been established. In four of these lines the fusion gene has inserted as a tandem array into autosomal loci while in one line, 754, insertion into the Y chromosome has occurred. Animals with an autosomal insertion site express transgene mRNA in the brain and testes constituitively and in a range of other tissues including liver, lung, kidney and intestine after zinc stimulation (ie. 25mM zinc sulphate in the drinking water). Mice from line 754 express transgene mRNA only in the testis, even with zinc stimulation. In situ hybridisation has been used to map the site of insertion of the transgene onto the Y chromosome. The results support a highly specialized role for the Y chromosome.

POSTER 7

REVISED GENETIC MAP OF *LUCILIA CUPRINA* AND EVOLUTIONARY CORRELATIONS

Gaye Weller and Geoff Foster

CSIRO Division of Entomology, Canberra 2601

Revised autosomal genetic maps of *Lucilia cuprina* are presented. Since the last map published (Foster *et al* Genetical Research 37:55-69 (1981)), at least 30 new morphological mutants, a number of electrophoretic loci and two physiological mutations have been described and added to the map.

Foster *et al* 1981 noted homologies between the five autosomal linkage groups of *Lucilia cuprina*, *Musca domestica* and the five major linkage elements of *Drosophila*, and proposed that these linkage groups had remained essentially intact during evolution. So far we have found no exceptions to these correlations and several new mutations which lend support to the linkage-group conservation hypothesis.

POSTER 8

ENHANCERS AND SUPPRESSORS OF THE *lozenge* PHENOTYPE in *Drosophila melanogaster*.

Philip Batterham, Andrew Davies and Mel Green*

Department of Genetics, University of Melbourne, Parkville 3052
*Department of Genetics, U. C. Davis, California, U. S. A.

This poster presents preliminary data on the analysis of four mutations which modify the phenotype associated with mutations at the *lozenge* (*lz*) locus.

1. *E(lz)* is a dominant mutation which enhances the phenotype associated with the gypsy insertion mutant, lz^{1}. *E(lz)* has been localized to the region around map position 75 on the third chromosome. *E(lz)* does not affect the phenotype of other *lz* mutants, but may affect the phenotype of other gypsy mutants.

2. Three mutations at the *zeste* locus (z^{1}, z^{a} and z^{v77h}) enhance the eye phenotype associated with seven *lz* alleles tested. This enhancement may be explained by either the level of eye pigment in *zeste* mutants or a role for *zeste* as a "trans acting regulator" of *lz*.

3. Two suppressor mutations have been mapped to the third chromosome. One of these suppresses the phenotype associated with lz^{1} and the other suppresses lz^{34k}.

POSTER 9

GENETIC AND PHENOTYPIC ANALYSIS OF MUTATIONS AT THE *LOZENGE* LOCUS IN *DROSOPHILA MELANOGASTER*

Philip Batterham, Andrew Davies, Gabriel Pasquini and Reinhard Stocker*

Dept. of Genetics, University of Melbourne, Parkville 3052
*Zoologisches Institut, Universität Freiburg, Freiburg, Switzerland

In the context of an ongoing molecular study of the *lozenge* (*lz*) locus fifteen recently isolated alleles (along with eight previously characterized alleles) have been subjected to genetic and phenotypic analysis. On the basis of these studies the mutations were placed into two groups:

A. Four mutations mapping to the proximal (*spectacle*) sub-locus affect only eye phenotype. These mutations fail to complement each other, but complement all other mutations mapping to the *lz* locus.

B. All other mutations (with the exception of lz^K) fail to complement each other and typically affect the development of eyes, antennal sensillae, tarsal claws, pulvilli, spermathecae and the crystal cells of the hemolymph.

It is therefore concluded that these mutations define two cistrons which can be aligned with the genetic map. Cistron A is confined to the *spectacle* sub-locus and affects only *lz* eye function. Cistron B spans the entire locus and affects eyes, antennae, tarsal claws, pulvilli, spermathecae and the crystal cells. lz^K is considered to be an exceptional mutation analagous to those found at other complex loci.

Seven of the mutants analyzed in this study were generated by either PM or MR mutagenesis. Each of the mutations maps to the *spectacle* sub-locus; one mutation belongs to Cistron A, while the remainder belong to Cistron B. Evidence of instability in PM or MR crosses in combination with *in situ* hybridization data indicate that four of the mutants have a P element inserted in the region of the *lz* locus. These mutants are being used in the cloning of the *lz* gene and thus should be useful in understanding the genetic complexity associated with the *spectacle* sub-locus at a molecular level.

POSTER 10

CHARACTERIZATION OF *DROSOPHILA* GENES WITH POSSIBLE INVOLVEMENT IN GABA METABOLISM.

Marie Phillips[1], Lawrence Salkoff [2], and Leonard. E. Kelly[1].

1. Dept. of Genetics, The University of Melbourne, Parkville, Victoria, Australia. 3052.
2. Dept. of Anatomy and Neurobiology and Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri, U.S.A. 63110.

The biosynthetic pathway for GABA metabolism is of considerable interest to Dipteran geneticists studying seizure mutants and insecticide resistance. We have used cross-species hybridization studies in an attempt to isolate *Drosophila* genes involved in this pathway. A putative gene for *Drosophila* glutamate decarboxylase (GAD) has been isolated by F. R. Jackson and K. Elliott (Society of Neuroscience . Abstracts 1987).

We have obtained four other genomic clones with homology to the feline GAD cDNA. *In situ* hybridisation to *Drosophila* polytene chromosomes has shown that none of these clones is the putative GAD gene isolated by Jackson and Elliott. The genomic clone (G-2) with strongest homology to the feline gene was used to screen *Drosophila* cDNA libraries. Four clones of differing lengths were obtained by screening the Itoh λGt11 head cDNA library. All have homology to the 3' Eco R1/ Sal1 (1.1kb) fragment of the feline gene. The largest clone (~ 2kb) has been sequenced. This clone has extensive nucleotide homology to the feline gene. The deduced amino acid sequence suggests significant conservation at the protein level. The detailed sequence analysis and its significance will be discussed.

Cross species hybridization studies have also been used in an attempt to isolate the *Drosophila* gene for α-oxo-glutarate transaminase (GABA-T), the catabolic enzyme involved in GABA metabolism. The molecular characterisation of putative clones for this gene will be presented.

THE ISOLATION AND CHARACTERIZATION OF DROSOPHILA GENES WITH HOMOLOGY TO A MAMMALIAN AROMATIC AMINO ACID HYDROXYLASE GENE

Ross Howden[1], Marie Phillips[1], Hans-Henrick M. Dahl[2] and Julian F.B. Mercer[2]

[1] Dept. of Genetics, University of Melbourne, Parkville, Vic. 3052.
[2] Murdoch Institute, Royal Children's Hospital, Parkville, Vic. 3052.

The hydroxylation of aromatic amino acids is an integral part of amino acid metabolism in both vertebrates and invertebrates. Phenylalanine hydroxylase(PH) and tyrosine hydroxylase(TH) belong to a multi-gene family with significant homology at the nucleotide and protein level, both within and across species. Aromatic hydroxylases catalyze the synthesis of catecholamines and defects in genes coding for these enzymes may lead to behavioural and nervous system abnormalities. We are using cross species hybridisation and immunological studies to identify Drosophila aromatic hydroxylases for future genetic studies.

Western Blots of Drosophila head, thorax and abdomen protein probed with a mammalian monoclonal antibody to phenylalanine hydroxylase, PH8 [Jennings et al. Biochemistry 24: 556-561(1985)] showed cross-reactivity between the antibody and a protein of approximately 45 kd. Other fainter signals were obtained and these may represent other hydroxylases and/or protein breakdown products. A 2.4 kb cDNA probe (HD67) coding for human phenylalanine hydroxylase [Kwok et al. Biochem. J. 235:133-138 (1986)] and the PH8 antibody were used to isolate Drosophila cDNA clones from a Drosophila head cDNA expression library. Seven positive clones have been isolated from 30,000 plaques with the largest cDNA clone being approximately 1.5kb. This clone is being sequenced for comparison to known aromatic hydroxylases in other species. Polytene chromosome in situ hybridisation studies are in progress to determine the position of the Drosophila putative PH gene. The molecular characteristics of this clone will be discussed.

POSTER 12

MITOCHONDRIAL DNA LINEAGE SELECTION IN DERIVED LAKE GALAXIID SPECIES

Jennifer Ovenden[1], Robert White[1] and Mark Adams[2]

[1]Department of Zoology, University of Tasmania, G.P.O. Box 252C, Hobart 7001
[2]Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide 5000

The fish genus *Galaxias* includes freshwater species which are thought to be derived from populations of diadromous, widely distributed, abundant species which became trapped in inland lakes by geological or climatic changes. In Tasmania, *G. auratus* and *G. tanycephalus* have a restricted distribution in inland lakes and are genetically and morphologically similar to the widespread *G. truttaceus*. We have analysed mtDNA and allozyme diversity of these two land-locked species. Mitochondrial, but not nuclear, DNA diversity in *G. auratus* and *G. tanycephalus* sampled was reduced, implying that the species have experienced at least one severe, but transitory bottleneck event. Four *G. auratus* and three *G. tanycephalus* mitochondrial DNA haplotypes were found. They were most closely related to the same *G. truttaceus* haplotype clade; one of many identified in a previous study. As we have previously shown that extant *G. truttaceus* clades are not geographically patterned, we suspect that either selection is responsible for the survival of the same set of mitochondrial genomes in the lacustrine species or that one lacustrine species is derived from the other.

POSTER 13

A HIGHLY REPEATED SEQUENCE FOUND WITHIN THE LARGE GENOME OF *LILIUM SPECIOSUM*

Peter R.J. Leeton and David R. Smyth

Department of Genetics and Developmental Biology,
Monash University, Vic. 3168

The genomes of *Lilium* species are very large, around 30-40 million kbp. Much of this is middle to highly repetitive DNA. This study concerns an investigation of a highly repetitive sequence dispersed in the genome of *Lilium speciosum*. Initially, an abundant 3.5 kbp band was detected in genomic DNA digested with the restriction endonuclease *Bam*HI. When this sequence was radiolabelled and probed back to *Bam*HI digested genomic DNA, it also hybridized to several other smaller bands. The hybridization intensities varied between bands, suggesting possible sequence diversity and/or copy number variation.

The 3.5 kbp genomic fragment was also used to probe an EMBL3 library of *L. speciosum* DNA. Fifteen percent of plaques hybridized to this probe. Isolation and restriction mapping of fourteen of these has clarified the results of the genomic hybridization experiments: the element exists predominantly as a 3.5 kbp *Bam*HI-bound sequence, as well as in other smaller repeated entities that could have arisen through deletion or truncation of the larger sequence. Cross-hybridization experiments have revealed that regardless of the size of the fragment, homology to the repeated sequence does not extend far from the *Bam*HI sites, indicating that the size of the largest full element is close to 4 kbp. The sequence does not cross-hybridize with the *del* retrotransposon (recently described from *Lilium* species) and it does not have long terminal repeats (LTRs) associated with viral-like retrotransposons.

The *L. speciosum* element occupies approximately 4 percent of this species' genome, and occurs in about 30,000 copies. Sequencing of a full element is being undertaken to determine its exact size, the nature of the terminal sequences, and any internal structure it may possess. The presence of this repeated sequence within other *Lilium* species and related plant genera is also being investigated.

POSTER 14

THE APPLICATION OF DNA IN-SITU HYBRIDIZATION TO THE STUDY OF SKIN DISEASES.

Boban Markovic and Max Nicholls.
Centre for Safety Science,
University of New South Wales,
P.O. Box 1, Kensington. 2033.

cDNA probes for a variety of lymphokine mediators and protein mRNAs (IL-1α, IL-1β, IFN-α, IL-2, IL-2r, IL-4, IL-6, IL-6r, TGF-α, procollagens,...) were labelled using the Photobiotin protocol of Bresatec (University of Adelaide, South Australia). The procedure is rapid and large amounts of DNA probe can be labelled (in our case 20μg per reaction).

A protocol of in-situ hybridization was developed using the biotinylated probes on lymphocytes isolated from whole blood by the ficoll-paque gradient centrifugation method, from lymphocytes stimulated with PHA (Phytohaemagglutinin), and from lymphocytes cultured and prepared by the standard protocol used for cytogenetic examination of chromosomes.

After hybridization of probes with native mRNAs, specific visualization was by avidin-alkaline phosphatase and the BCIP/NBT detection system. Data* is presented from the examination of lymphocytes and studies of sections of skin from a number of disorders.

Levels of cDNA probe binding in skin sections*.

Probe	Normal	Lymphocytic Infiltrate	Granuloma Annulare	Chronic Dermatitis
Control NP	-			-
pBR322	-	-/+	-	-
Total mRNA	++++	+++	++	+++
IL-2	+	++/+++	+/-	+++
IL-2r	+	++	+	+
IFN-α	+	+	+/++	+/-
1204	++	+	+	+
HuLT	+	+	++	+/-
KK4	+/++	++/+++	++	++
IL-1α	+	+	-	+/-
IL-1β	+++	+++	++	++/+++
1301	+++	+++	++	++/+++
MCGF	+++	++/+++	+++	++/+++

POSTER 15

RESTRICTION MAP VARIATION IN THE REGION OF THE αGpdh GENE AND EFFECTS ON αGPDH ACTIVITY IN *DROSOPHILA MELANOGASTER*.

Jane Symonds and John Gibson.
Molecular and Population genetics Group, R.S.B.S., The A.N.U., Canberra. A.C.T. 2601.

Restriction map variation in a 20kb region encompassing the αGpdh locus has been investigated in second chromosome lines of *Drosophila melanogaster* isolated from natural populations in Australia. Eight hexanucleotide restriction enzymes were used to digest DNA extracted from five αGPDH low activity lines, in addition to 29 lines with normal levels of activity from the Cardwell population (Queensland).

The restriction map data showed that in 38% of the lines at least part of the αGpdh locus was duplicated - the duplication does not appear to encompass the whole of the αGpdh transcription unit as 50-100bp at the 5′ end of the gene are not duplicated. All the duplications found seem to have similar structures at the resolution obtained by the hexanucleotide enzymes. In all cases homozygotes for a duplication were electrophoretically either fast or slow which suggests, if the duplicated gene is transcribed, that the two loci encode electrophorectically indistinguishable proteins. The detailed molecular structure of the duplication is being investigated.

Two restriction enzymes, *Sac*I and *Pst*I, have been used to detect the presence of the duplication in the progeny of single female lines, as both digests give a characteristic extra band if the duplication is present. Using this method we have screened populations from elsewhere in Australia and from China and Africa. These data show that the distribution of the duplication is widespread.

Of the five low activity lines tested, only Cb62, was found to have the duplication. The relationship between the restriction map variation and αGPDH activity variation in Australian populations will be illustrated.

POSTER 16

MODELLING GENETIC CHANGES IN A BANDICOOT COLONY

Nick Robinson, Neil Murray, Bill Sherwin, and Jenny Graves.

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

Concern over declining numbers of Victorian Eastern Barred Bandicoot (Perameles gunnii) is such that in 1987 the Department of Conservation, Forests and Lands initiated a captive breeding program at Gellibrand Hill Park (GBH) near Melbourne. Previous work suggests that genetic variation may have already been lost from Victorian P. gunnii.

Deterministic and stochastic models for predicting further changes in levels of inbreeding, heterozygosity and allelic variation in the GBH colony under various proposed management regimes are presented here.

In order to check the reliability of such models, pedigrees are being constructed with the aid of "fingerprint" variants, and other RFLPs will be used to monitor the results of the chosen management regime. The information derived from ongoing monitoring and predictions will be of vital importance in the management of this and other endangered populations.

POSTER 17

HOMOLOGY STUDIES REVEAL THE FUNCTION AND REGULATION OF *aciA* IN ASPERGILLUS NIDULANS.

Jenny Saleeba and Michael Hynes.

Dept. of Genetics, University of Melbourne, Parkville 3052.

aciA is an acetate inducible gene in *Aspergillus nidulans*. The gene has been fully nucleotide sequenced and it's protein sequence inferred. Attempts to define the function of the *aciA* gene product have followed two strategies. The first was to construct an *aciA*$^-$ strain by molecular methods and screen the strain on a variety of growth media in an attempt to identify a difference in phenotype between the *aciA*$^-$ strain and wild-type. Any difference in growth would presumably be due to the lack of a functional *aciA* gene product.

The second strategy was to compare the protein sequence of *aciA* to those in protein sequence databases. The aim of this strategy was to identify areas of homology between the *aciA* protein and database proteins that may reveal a functional similarity between the two proteins. Results of this work will be discussed.

POSTER 18

MOLECULAR ANALYSIS OF THE *alc* GENE REGION OF *Aspergillus nidulans*.

Tracey Cross, Ernest Creaser and John Pateman.

Plant Molecular Biology, Research School of Biological Sciences, Australian National University, Canberra 2601.

Recognition and subsequent control of structural genes by the binding of regulatory proteins to the DNA is fundamental to gene regulation. In prokaryotes many of the aspects of this interaction have been resolved, but the same does not apply for eukaryotes. It has been determined that one important motif utilised by some eukaryote regulatory proteins in this binding is the 'zinc finger'.

In *Aspergillus nidulans* the utilisation of ethanol is controlled by the positively acting gene *alcR*. The protein product of this gene, AlcR, activates expression of the genes *alcA* (alcohol dehydrogenase) and *aldA* (aldehyde dehydrogenase) in the presence of inducers. All three genes have been cloned and sequenced.

Current work is focussed on the investigation of a zinc finger motif located in the N-terminal end of the *alcR* gene. This motif shows a high degree of homology to other zinc finger motifs from fungal regulatory genes. Also a series of *alcR* mutants show partial rescue of their phenotype in the presence of zinc. Both *alcR* and *alcA* are located close together on the same chromosome. Another interesting aspect of this regulon is a gene located (by Northern analysis) between these two genes which has an as yet undetermined phenotype.

POSTER 19

REGULATION OF THE AROF TYRA OPERON OF ESCHERICHIA COLI : INTERACTION BETWEEN OPERATOR SITES

Barry Dickson and Chris Cobbett

Dept. of Genetics, University of Melbourne, Parkville 3052

The aroF tyrA operon of E. coli codes for two enzymes involved in the biosynthesis of the aromatic amino acids. This operon, along with seven other transcription units, is regulated by the $tyrR^+$ gene product. A comparison of the nucleotide sequences of a number of these $tyrR^+$-regulated promoters has identified a 20-base pair conserved sequence which appears in one or more copies in each of these promoter regions. Three such sequences, known as TYR R Boxes, have been identified in the aroF promoter region.

This poster describes the construction and characterization of a series of mutants in which the distances between the three TYR R boxes have been changed. The effects of these mutations on repression in vivo have been assayed using gene fusions. The results indicate that boxes 1 and 2 must be aligned on the same side of the DNA helix in order to interact but that the interaction of box 3 with boxes 1 and 2 is not dependent on their helical alignment.

INVERSIONS, TRANSLOCATIONS AND GENETIC CONTROL OF *LUCILIA CUPRINA*

Geoff Foster and Gaye Weller

CSIRO Division of Entomology, Canberra 2601

Female-killing systems based on sex-linked translocations and deleterious mutations are currently being developed and evaluated for genetic control of sheep blowfly. The combined effects of semisterility for male-linked translocations and homozygosis in the field of these mutations give a maximum of 94% genetic death per generation for currently available strains. Recent field trials have demonstrated the effectiveness of such strains in suppressing natural populations to undetectable levels. However, genetic recombination in males, leading to breakdown of the strains in mass-rearing colonies, poses a serious obstacle to large-scale implementation of this method of control.

One potential solution to the problem of strain instability is to suppress male recombination through the use of chromosomal inversions. We are presently constructing strains containing both sex-linked translocations and third-chromosomes homozygous for a large viable pericentric inversion plus the eye colour mutations *white* and *yellowish*. Females are homozygous for the inversion and these mutations, plus *topaz* on chromosome 5, while males are heterozygous for the translocation, the inversion and the mutations. In mass-rearing colonies, male recombination between the translocation and the chromosome-3 mutations should be suppressed. Released females are unable to survive in the field because of the effects of the mutations. Males are able to mate with field females and transmit both the mutations and inversion to their daughters. These daughters are heterozygous for the inversion, and are semisterile because crossing over within the inversion generates inviable duplication/deficiency zygotes. Thus the inclusion of the inversion should both stabilize the strain and increase the maximum rate of genetic death to approximately 97% per generation.

POSTER 21

THE IDENTIFICATION AND CLONING OF THE GENE FOR A HEAT-STABLE CALCIUM BINDING PROTEIN FROM DROSOPHILA MELANOGASTER

Margaret Delbridge and Leonard Kelly

Dept. of Genetics, University of Melbourne, Parkville 3052

Cell-cell communication plays an important role in the functioning of the nervous system. One way in which cells may respond to external stimuli, is by increasing the level of intracellular free Ca^{2+}, and the subsequent activation of Ca^{2+}-dependent processes.

Recent reports indicate the presence, in mammalian brain, of a number of heat-stable calcium-binding proteins (CaBP's) that are distinct from calmodulin (McDonald and Walsh, Biochem. J. 232: 559-567, 1985). We have attempted to characterise equivalent CaBP's from Drosophila with the intention of using the genetics of this organism to understand the physiological and biochemical roles of these proteins.

A heat-stable Ca^{2+}-binding protein of M_r 23 kd (DCABP-23) has been purified from Drosophila and partially characterized. Polyclonal antibodies have been raised against this protein and they have been used to probe Western blots of extracts from various Drosophila tissues as well as mammalian brain. The protein is present in many tissues including neural tissue, ovaries and gut. The antibodies also cross react with a 23 kd protein in mammalian brain.

The antibodies have been used to screen a Drosophila head cDNA library made in the expression vector λgt11 and clones positive for a β-galactosidase/DCABP-23 fusion protein were selected. These partial cDNA clones have then been used to screen for other full length cDNA's. Analysis of the sequence of these clones may provide some clues as to the function of this CaBP.

POSTER 22

LONG LIVED STRAINS OF *DROSOPHILA MELANOGASTER* WITH REDUCED BODY FAT.

C J I DRIVER and W MULLINS

Victoria College, Rusden Campus, 662 Blackburn Rd, Clayton, VIC 3168

Strains of *Drososphila melanogaster* were derived from single females captured at Gembrook. Nine strains out of 18 had lifespans greater than or equal to that of Canton-S. A second group of strains was generated from F2 females of a cross of W with the longest lived of these strains . Three strains were found that were even longer lived than either parent strain.

Food intake and body fat were both found to correlate with longevity (R=+0.53 and -0.94 respectively). Analysis of partial correlation coefficients showed that the effect of food intake disappeared after correcting for body fat, whereas the effect of body fat was independent of food intake. Number of offspring, sex ratio of offspring, dry body weight and activity showed no correlation with lifespan.

In a wider survey, most strains were close to the regression line for body fat and longevity, derived from the first survey or had a lifespan shorter than predicted . It is suggested that this shortfall reflects inbreeding depression .

The Production of Doubled-haploids by Anther Culture from Australian Barley and Wheat Germplasm

David Luckett and Ross Smithard

NSW Agriculture & Fisheries
Agricultural Research Institute
Wagga Wagga NSW 2650

Anther culture is widely recognized as a powerful technology for rapidly producing homozygous doubled-haploid (DH) lines. Cultivar improvement using DH can bring commercial release forward by up to four years, resulting in research cost savings and increased farmer profits. In addition, the breeder is able to increase throughput and can conduct early-generation yield trials and quality tests with increased precision and reliability.

The attainment of efficient anther culture systems in cereals has been time-consuming and difficult. Recent developments overseas in media composition have resulted in vast improvements for the various components of anther culture efficiency:

(i) the proportion of anthers responding in culture;
(ii) the number of embryos produced per responding anther;
(iii) the proportion of embryos which germinate;
(iv) the ratio of green to albino regenerants; and
(v) the proportion of green regenerants which are spontaneously doubled.

For barley and wheat anther culture a number of media modifications have been employed. Maltose has wholely replaced sucrose as the carbon source and the determinant of osmotic potential. Agarose is now the preferred gelling agent in all cases while glutamine is used as the nitrogen source. Hormone use has been reduced, although 2,4-D and BAP are still required to direct microspores through embryogenesis in wheat and barley, respectively.

At Wagga we have produced fertile green regenerants from a range of barley and wheat cultivars (foreign and local) and also from breeders' conventional F_1 and F_2 hybrids. Genotype specificity certainly exists but all hybrids can be successfully cultured to some extent. The rate of spontaneous doubling is about 65% in barley and 30% in wheat. Colchicine doubling of barley haploids and wheat triploids has been performed but it is by no means an easy task. The first DH have not yet been field tested.

POSTER 24

MUTAGENESIS PRODUCED INSECTICIDE RESISTANCE IN *DROSOPHILA MELANOGASTER*

Adcock G.J., Batterham P., Kelly L.E., and McKenzie J.A.

Dept. of Genetics, University of Melbourne, Parkville 3052

Drosophila melanogaster may have an important role as a model organism for insecticide resistance studies. It offers superior genetical and biochemical techniques, over those available for pest species. Selection for monogenic resistance has been attempted in *Drosophila* to the insecticides dieldrin, diazinon and cyromazine. In the sheep blowfly, *Lucilia cuprina*, resistance has developed to dieldrin and diazinon, but not cyromazine.

Dosage mortality curves were constructed for susceptible *Drosophila* strains. Doses above the LD100 were used to screen for resistant variants, using EMS mutagenesis, to increase variation.

Two cyromazine resistant strains have been isolated, and the genes conferring resistance in each, mapped. Dosage mortality data and fitness tests have been used to further define the phenotypes of each strain.

For insecticides to which pests have not developed resistance, *Drosophila* can be used to study the mode of their resistance inheritance. The results will be discussed with respect to their relevance in predicting the evolution of resistance in *Lucilia*.

POSTER 25

AN ANALYSIS OF HETEROLOGOUS VNTR PROBES FOR LINKAGE STUDIES IN CATTLE AND SHEEP

Roger Drinkwater, Stephen Moore and Jay Hetzel

Molecular Genetics Group,
Division of Tropical Animal Production,
CSIRO, Rockhampton, Qld.

One method of obtaining DNA markers for genes with undefined products in livestock is to perform linkage studies using polymorphic variable number tandem repeat (VNTR) DNA sequences on families in which the desired gene is segregating. Many of the VNTR probes that have been isolated are conserved among different species, although not all are effective for linkage analysis and each must be assessed in each species.

In sheep, the VNTR sequences Zetaglobin, M13, Per, pYNZ22 and poly-CA are effective, polymorphic, multilocus probes. In cattle, the probes Zetaglobin, Per and poly-CA will be useful in linkage studies, in addition to M13, PUCJ and alphaglobin p3'HVR64 previously analysed by Georges et. al. (Cytogenet. Cell Genetics, 47: 127, 1988). The VNTR probes insulin, apolipoprotein and BKM are ineffective in sheep and cattle. There was no close linkage of polymorphic bands within individual sheep or cattle patterns, indicating a wide genomic distribution of the VNTR loci, although the possibility that the loci tend to be telomeric or centromeric has yet to be investigated.

Maximum resolution and sensitivity of VNTR banding patterns using the heterologous VNTR probes were obtained using DNA hybridization in dried agarose gels; a method that eliminates the need to transfer target DNA to support membranes.

In situ hybridisation studies of nucleoli in the Mediterranean fruit fly, *Ceratitis capitata*

D. G. Bedo

Division of Entomology, CSIRO, P. O. Box 1700, Canberra, ACT 2601, Australia

Nucleolar structure was studied in mitotic and three polytene tissues of the Mediterranean fruit fly, *C. capitata* using in situ hybridisation with a tritium labelled rDNA probe and silver staining. In mitotic metaphase chromosomes nucleolar organiser regions were localised in the short arms of both sex chromosomes.

In polytene nuclei of trichogen cells, salivary glands and fat body rDNA was only detected within nucleoli. Nucleoli in these tissues have similar structure with rDNA labelling concentrated in a fibrillar core. Silver staining resulted in very heavy staining of polytene nucleoli and interphase nucleoli in diploid cells. Silver staining of nucleolar organisers in metaphase chromosomes is weak or absent although the X chromosome has numerous heavy silver bands in other locations.

The results suggest nucleolar structure is conserved in polytene tissues contrasting with the variability of autosomal banding patterns and sex chromosome structure. They also indicate that silver staining in not necessarily specific for nucleolar regions.

CLONING AND cDNA SEQUENCE OF A NOVEL MARSUPIAL MILK PROTEIN GENE

Chris Collet, Roslyn Joseph and Kevin Nicholas

CSIRO Wildlife and Ecology, P.O. Box 84, Lyneham, A.C.T., 2602.

Marsupial milk changes in composition during lactation to meet the changing nutritional requirements of the pouch young as it begins to leave the pouch. In the tammar wallaby (*Macropus eugenii*), two kinds of milk are secreted: "early" milk for about 180 days and "late" milk for the remainder of lactation. These milks differ in all their main constituents of lipid, carbohydrate, protein and electrolytes (1).

Milk protein concentration increases during the transition from early to late milk and continues to rise throughout the remainder of lactation. This increase in protein concentration is correlated with the appearance of a major whey protein, Late-Lactation Protein (LLP) and also with the increased synthesis of proteins already present in the early milk. During the transition, LLP concentration increases dramatically to a level where this protein accounts for approximately 25% of the total milk protein (2).

To study the modulation of gene expression in the tammar mammary gland during the transition from early to late-phase milk, we are attempting to clone several milk protein genes which are differentially expressed during the two phases of lactation. This paper reports the molecular cloning and characterization of the LLP gene and its expression in late-lactation.

To clone LLP, the partial amino acid sequence previously derived for the amino terminus of the protein (2) was used to construct a 35-mer oligonucleotide with 8192 degeneracies. End-labelled oligo was used to screen 200 pfu of a late-phase mammary gland cDNA library and three clones hybridized positive. The nucleotide sequence of a 780bp insert corresponding to LLP is presented. Northern analysis of mammary gland RNA revealed the presence of a single transcript of approximately 800 bases during late phase but not during early phase lactation. In contrast, *a*-lactalbumin mRNA is present in both phases of lactation.

Future experiments will concentrate on the role of different factors (hormonal, growth and intrinsic) in the induction of LLP gene expression in tammar mammary glands during the transition from early to late phase of lactation.

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CLONING, cDNA SEQUENCE AND PROLACTIN-DEPENDENT EXPRESSION OF A MARSUPIAL *B*-LACTOGLOBULIN

Chris Collet, Roslyn Joseph and Kevin Nicholas

CSIRO Wildlife and Ecology, P.O. Box 84, Lyneham, A.C.T., 2602.

B-lactoglobulin is a major milk protein of many eutherians and all marsupials (1,2) and may serve to transport vitamin A to the young (3). This paper reports the cloning, partial cDNA sequence and expression of the *B*-lactoglobulin gene from the tammar wallaby *Macropus eugenii*.

A 35-mer oligonucleotide probe of 2048 redundancies was constructed from the protein sequence of a related species, the grey kangaroo (*M. giganteus*; 2) and used to screen 200 pfu of a lactating mammary gland cDNA library. Ten clones hybridized positive to the end-labelled oligo and seven of these had identical inserts. Sequencing demonstrated these clones to be partial cDNAs comprising the carboxy-terminal 128 residues of tammar *B*-lactoglobulin. Northern analysis of total RNA revealed the presence of two transcripts of approximately 850 and 1000 bases throughout lactation. The relative intensities of the bands suggests differential utilization of the polyadenylation signals detected by sequencing.

Tissue culture of mammary gland explants from late pregnant tammars showed that the hormone-dependent induction of *B*-lactoglobulin gene expression was maximal in the presence of prolactin alone. The prolactin-induced accumulation of *B*-lactoglobulin mRNA was not modulated by either cortisol or insulin.

The simple prolactin-dependent induction of *B*-lactoglobulin gene expression contrasts the more complex requirements for milk protein gene expression in many eutherian mammals (4) and thus, provides a potentially superior model system to study the mechanism of prolactin signal transduction.

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SYNAPTONEMAL COMPLEX ANALYSIS OF ROBERTSONIAN TRANSLOCATION IN SHEEP

Kang Dai and Chris Gillies

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

The domestic sheep (*Ovis aries*) has a chromosome number of 2n = 54. Autosomes consist of three large metacentric pairs, and 23 pairs of acrocentrics of progressively decreasing length. The X chromosome is acrocentric and larger than any autosomal acrocentric, the Y is metacentric and the smallest chromosome. Bruére and co-workers from Massey University, N.Z. have described three Robertsonian translocations in sheep, and studied the light microscopic meiotic behaviour and breeding characteristics of these. We have begun a study of pachytene pairing in male meiosis from these translocation heterozygotes, using synaptonemal complex spreading techniques.

A synaptonemal complex karyotype has been constructed for sheep, and the translocation chromosomes in one heterozygote have been identified by somatic banding and synaptonemal complex measurements. Using XY chromosome pairing behaviour, substages of pachytene can be defined. Chromosome pairing in the translocation heterozygote results in formation of a trivalent at pachytene. In early stage cells the lateral elements are unpaired at the centromere regions of the trivalent, but complete pairing is possible by mid-pachytene. Pairing interaction is sometimes seen between the trivalent and the XY bivalent.

POSTER 30

GENETIC ENGINEERING OF WINE YEASTS

Jenny Petering[1], Paul Henschke[2] and Peter Langridge[1]

[1] Dept. of Agricultural Biochemistry, Waite Agricultural Research Institute, Glen Osmond, 5064

[2] Australian Wine Research Institute, Glen Osmond, 5064

Recombinant DNA technology offers the potential to introduce or delete specific biochemical functions in wine yeasts. Initial studies have been aimed at establishing an appropriate system for introducing recombinant DNA molecules into polyploid yeast strains.

The transformation system utilizes a mutant *Saccharomyces cerevisiae* acetolactate synthase gene. This gene confers herbicide resistance upon transformed cells and acts as a dominant selectable marker.

Results presented here demonstrate that satisfactory transformation efficiencies can be achieved with both a self-replicating plasmid and an integrating vector in several wine yeast strains. Analysis of the transformed yeast has shown that:

i) both types of vector are maintained in the yeast population throughout a fermentation trial

ii) the introduction of foreign DNA does not seem to have an adverse effect on fermentation performance; and

iii) the integration event can be targeted to a specific site on the yeast genome.

POSTER 31

CLONING AND CHARACTERIZATION OF HMW GLUTENIN GENES IN WHEAT

Pradnya Reddy, Helen Carpenter and Gregory Donovan

CSIRO Division of Plant Industry, Wheat Research Unit,
North Ryde 2113

The wheat endosperm contains a highly aggregating fraction of proteins known as the glutenins. The glutenins interact with other endosperm proteins to confer to bread wheat their particular baking quality. Because of the importance of the glutenins we have made a detailed study of the molecular structure and composition of their encoding genes. The high-molecular-weight (HMW) subunits, which form a major fraction of glutenins, are encoded by genes present on chromosomes 1A, 1B and 1D. Analysis of a number of genotypes of bread wheat showed that certain glutenin subunits are not expressed in some wheats (Lawrence *et al.* 1988). Restriction analysis of these null lines has shown that "deletion" of DNA sequences has occurred at the glutenin gene loci. We have isolated genomic clones from these null subunit wheat lines using bacteriophage lambda as the cloning vector. The clones will be used to prepare "genome specific" DNA probes. These probes will in turn be used to analyze the qualitative and quantitative variation present at individual HMW glutenin gene loci in different wheat cultivars.

Reference:
Lawrence, G.J., MacRitchie, F., and Wrigley, C.W. (1988). Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the Glu-A1, Glu-B1 and Glu-D1 loci. J. Cereal Sci. 7, 109-112.

REGULATION OF EXPRESSION OF THE DUPLICATED GENES ESTERASE-6 AND ESTERASE-P.

M. Healy,[1], M. Dumancic[1], J. Karotam [1], M. Myers[1], R. Russell[1], J. Brady[2] and J. Oakeshott[1].

[1] CSIRO Division of Entomology, Canberra, A.C.T., 2601.
[2] Department of Biology, Indiana University, Bloomington, U.S.A.

Esterase-6 (EST6), the major β-carboyxlesterase of *D.melanogaster*, is produced primarily in the ejaculatory duct of the adult male reproductive system. EST6 is transferred from the male to the female during mating and is rapidly translocated to the female's haemolymph. The male-donated EST6, through mechanisms not yet understood, acts to stimulates utilisation of stored sperm and delays receptivity of the female to remating.

Molecular analysis of the genomic DNA surrounding the *Est-6* gene revealed a second esterase gene, *Est-P*, beginning 197bp downstream of the *Est-6* termination codon. The *Est-6/Est-P* genes form a tandem duplication that appears homologous to the *Est-4/Est-5* duplication of *D.mojavensis* and the *Est-1/Est-J* duplication of *D.buzzatii*.

The *Est-6/Est-P* genes and their protein products are stucturally very similar. Exons 1 and 2 of the *Est-6* and *Est-P* genes show 66% and 57% similarity respectively, and the EST6 and ESTP proteins exhibit about 60% amino acid similarity.

However, the regulation of expression of the *Est-6* and *Est-P* genes is very different. Studies on the pattern of expression of each gene product at the mRNA and protein levels reveal significant differences, both spatial and temporal. As well, comparison of the flanking sequences 5' and 3' of the *Est-6* and *Est-P* genes reveals no siginifcant sequence similarity. These observations suggest functional differences have arisen between the two proteins through the divergence of the regulatory sequences.

Studies to identify the *cis*-acting elements regulating expression of the *Est-6* gene have revealed two candidate regulatory sequences 5' of the translation start site that are perfectly conserved in *D.melanogaster, D.simulans* and *D.mauritiana*. In addition, P-element mediated germline transformation of *Est-6* genes carrying deletions of the 5' flanking sequences demonstrate that 1.1kb 5' and 0.9kb 3' of the gene are sufficient, and that at least 270bp 5' are necessary, for the major aspects of adult expression.

POSTER 33

Complementation of a Dictyostelium discoideum thymidylate synthase mutation with the mouse gene provides a new selectable marker for transformation

Andy C.M. Chang[+], *Keith L. Williams*[+], *J.G. Williams*[*] *and Adriano Ceccarelli*[*]

+ School of Biological Sciences, Macquarie University, Sydney, N.S.W., Australia 2109

* Imperial Cancer Research Fund, South Mimms, Herts EN6 3LD, U.K.

ABSTRACT

A cDNA encoding mouse thymidylate synthase has been inserted 3' to the Dictyostelium discoideum actin 15 promoter in an E. coli - D. discoideum shuttle vector. When this construct was introduced into a D. discoideum thymidylate synthase mutant strain HPS400, stable transformants were obtained at high frequency. These transformants grew in standard axenic medium without requiring exogenous thymidine supplement. This construct provides a second selectable marker for use in transformation of D. discoideum.

POSTER 34

Genetic Analysis of Post-Translational Modifications of Cell-Surface and ECM Glycoproteins in *Dictyostelium discoideum*

Andrew A. Gooley, Alan Champion and Keith L. Williams
School of Biological Sciences, Macquarie University, N.S.W. 2109

Post-translational modifications in eukaryotes are assumed to be important although specific functions for particular types of modification are yet to be fully understood.

We have focused our studies on a relatively simple eukaryote, the multicellular slime mould *Dictyostelium discoideum*. *D. discoideum* is an excellent system for the study of development and also a promising eukaryote host in the field of biotechnology.

We are particularly interested in the function glycoconjugates play on developmentally, cell-type specific (including ECM) glycoproteins. The approach we have taken is to use monoclonal antibodies (McAbs) to identify and study such molecules. We have generated mutants which lack specific oligosaccharides recognised by a panel of McAbs and in association with the parasexual genetic system of *D. discoideum* we are beginning to characterise the genetics of glycosylation in this eukaryote.

Three major types of glycosylation will be discussed, the N-linked and O-linked oligosaccharides and the unique glycophosphatidylinositol lipid tail which anchors the glycoprotein to the cell surface.

AAABG

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MEMBERSHIP

The current membership of AAABG is 400, of which approximately 10% are from New Zealand. It is obvious that the committee would like to see a larger representation of New Zealanders. For 1989, the membership subscription of AAABG is NZ$30 (incl. GST), plus a joining fee of NZ$15 for new members. For new members wishing to join retrospectively for 1988 and so obtain a copy of the conference proceedings from Armidale (the theme of which was "The Economics of Livestock Improvement"), the joining fee is NZ$30. Cheques (NZ$45 or NZ$60) should be made payable to AAABG and be sent to:

Dr B.W. Wickham
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1990 CONFERENCE OF AAABG

To emphasise that New Zealand is an integral part of the AAABG, the 1990 conference is to be held in Hamilton and Palmerston North. As stated in the objectives, the committee will be encouraging participation from all parties interested in animal genetics - breeders, extension workers, consultants and scientists. A large contingent of Australians is expected to participate in the conference. The following gives a brief resume of the conference programme.

Theme: Technology Transfer

Dates: Sunday 4 February to Friday 9 February 1990

Programme:
4 February - register at Waikato University
5 February - papers on Available Technologies
6 February (Waitangi Day) - international visitors hosted with farming families
7 February - fielday at Palmerston North Showgrounds
8 February - Species Specific papers at Massey University
9 February - papers with a View to the Future and recognition of the retirement of Professor A.L.Rae by AAABG and Massey University

BODEN RESEARCH CONFERENCES

With the generous support of Dr A. Boden, FAA, company director and science writer, the Academy conducts a series of small specialist research conferences in the biological sciences — called the Boden Research Conferences — to enable active research workers in rapidly advancing fields to discuss current advances and problems. To the extent of available resources they are intended to fill the niche in Australia filled by the Gordon Research Conferences in the United States. The initiative and financial responsibility for each meeting is in the hands of the relevant national scientific society, which thus acts as a joint sponsor with the Academy.

Two conferences are organized each year, with a membership of 20-30, and of about 2 days' duration; the two conferences are held at Thredbo in the Snowy Mountains of New South Wales in the first week of February one following the other. Each year the Academy issues invitations to relevant scientific societies to forward proposals for Boden Conferences, from which two are selected. Societies are invited to consult with their members and to put forward one or more proposals. Proposals submitted in previous years will be reconsidered if resubmitted.

Scientific societies should submit proposals on application forms which are available from the Academy secretariat on request.

The organizers of approved conferences are responsible for the detailed arrangements, and the society acting as joint sponsor receives and is accountable for the sum allocated for the meeting, up to $5,000 for each conference. The supplementation of this sum from other sources is encouraged.

The Academy now cordially invites societies to prepare proposals for February 1991. They should reach the Executive Secretary by the **31st July, 1989.** Secretaries of societies intending to submit proposals should give preliminary notice to the Academy immediately.

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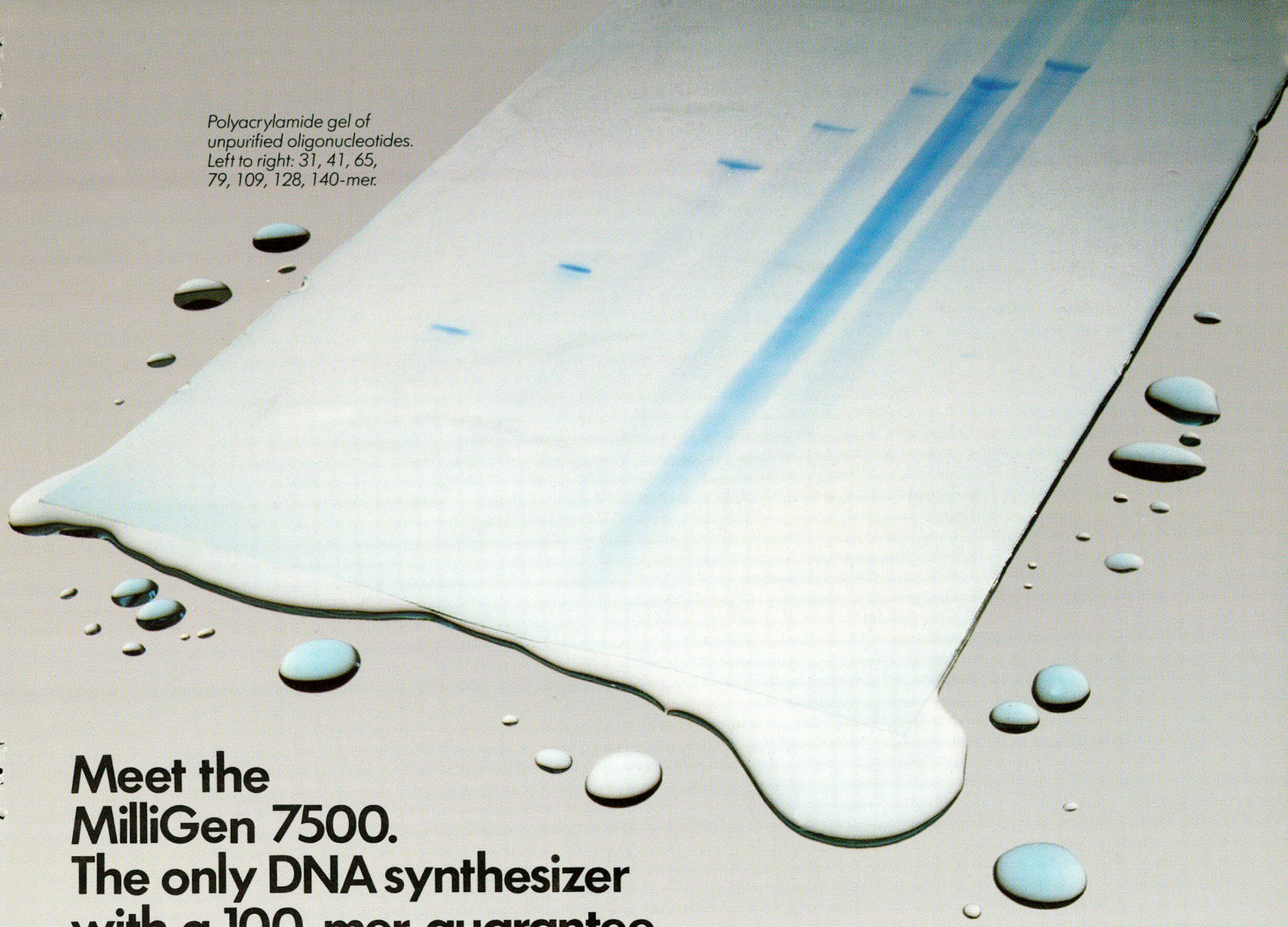

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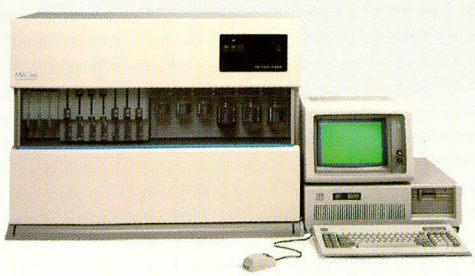

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- Waste bottle: 6 liter capacity, with level detector
- Synthesis scale: 0.1 to 30 micromoles
- Cycle time per coupling: <4 minutes, 3 column parallel synthesis; <7 minutes, single column
- Coupling efficiency: 99 + %
- Reagent consumption: 5-10mg of amidite per cycle; <8ml of other reagents per cycle

Control and software

- Voice synthesizer: proofreads entered sequences
- Light pen bar-code reader: Automatic notebook entry of bar-coded reagent labels
- Protocols: Standard beta-cyanoethyl phosphoramidite protocol supplied, 21 cycles per protocol; unlimited user modification, creation of protocols

Self-diagnostics

- Continuous real-time monitoring during synthesis
- Trityl monitoring system: 505nm photometer with 3 separate flow cells and feedback shutdown; range of 0.01-0.3 micromoles
- Waste level detector
- Amidite depletion detector
- Liquid overpressure transducer
- Gas pressure transducer for high and low input gas pressure

Power and operating environment

- Power requirements: 110-120/220-240 VAC ± 10%, 50-60 Hz, 200 VA
- Temperature: 10-40°C operating; 0-50°C storage
- Humidity: 0-90% non-condensing
- Gas requirements: argon or nitrogen, 80 psig
- Ventilation: Directed internal reagent ventilation system with fan and 4" exhaust duct
- Dimensions: 96.5 cm (38") long by 76.2 cm (30") high by 43.8 cm (17.25") deep
- Weight: Approximately 73 kilograms (160 lbs.)

Options

- 3-column temperature controller: programmable range – ambient to 60°C
- Battery back-up power supply

For more information about the MilliGen 7500, or any of our other products, call toll-free at (800) 872-0071; in Massachusetts, (617) 275-5208. Or write Millipore Corporation, MilliGen Division, 75 Wiggins Avenue, Bedford, MA 01730.

Ordering Information

Catalogue Number	Description
103001	MilliGen 7500 DNA Synthesizer with 3-column parallel synthesis capability (includes MilliGen Express-DNA software with standard protocols, and IBM PC-AT computer)
103010	Same as 103001, without IBM PC-AT computer
103006	IBM PC-AT computer with hard disk, PC-DOS, monochrome graphics card and monochrome monitor
103007	Epson FX86 printer
103008	Power supply battery backup
103009	3-column block heater
103004	Auxiliary parts and tools
103005	Complete oligonucleotide synthesis kit (includes sufficient amidites, resins and chemicals for 200 cycles)

Warranty

One year limited warranty on parts and labor, ninety days on travel within U.S.A.

New BioSyntech™ Reagents for DNA Synthesis

BioSyntech Reagents for DNA synthesis include the following kits of amidites, supports, and chemicals:

900030	Cyanoethyl Phosphoramidite Mixed Kit (0.5 g each of A, C, G, T)
900031	Cyanoethyl Phosphoramidite Mixed Kit (1.0 g each of A, C, G, T)
901005	dNucleoside-Porous Glass Mixed Kit (0.1 g each of A, C, G, T)
901006	dNucleoside-Porous Glass Mixed Kit (1.0 g each of A, C, G, T)
900002	Complete chemicals kit (Consists of Acetic Anhydride, Acetonitrile, Dichloroacetic Acid, Dimethylaminopyridine, Iodine, Tetrazole, and Triethylamine solutions in sufficient quantities for 200 couplings)

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