33rd ANNUAL CONFERENCE OF THE GENETICS SOCIETY OF AUSTRALIA

The Flinders University of South Australia

27th – 30th August, 1986

Programme and Abstracts

GENERAL INFORMATION Supplement

TICKETS FOR THE SOCIAL EVENTS

Some tickets are still available both for the Annual Society Dinner and for the Winery Visit/Barbeque. Unless sold out previously, these will be available from the Registration Desk until the following times:

for the DINNER, not later than 9.00 am Thursday 28th.
for the WINERY VISIT/BBQ, not later than 4.00 pm Thursday 28th.

SUSTENANCE

ON CAMPUS In addition to being made honorary members of the University Club for the duration of the meeting, registrants at the GSA Meeting have also been made honorary members of the Students' Union. The following facilities will thus be available in the UNION BUILDING on Thursday and Friday:

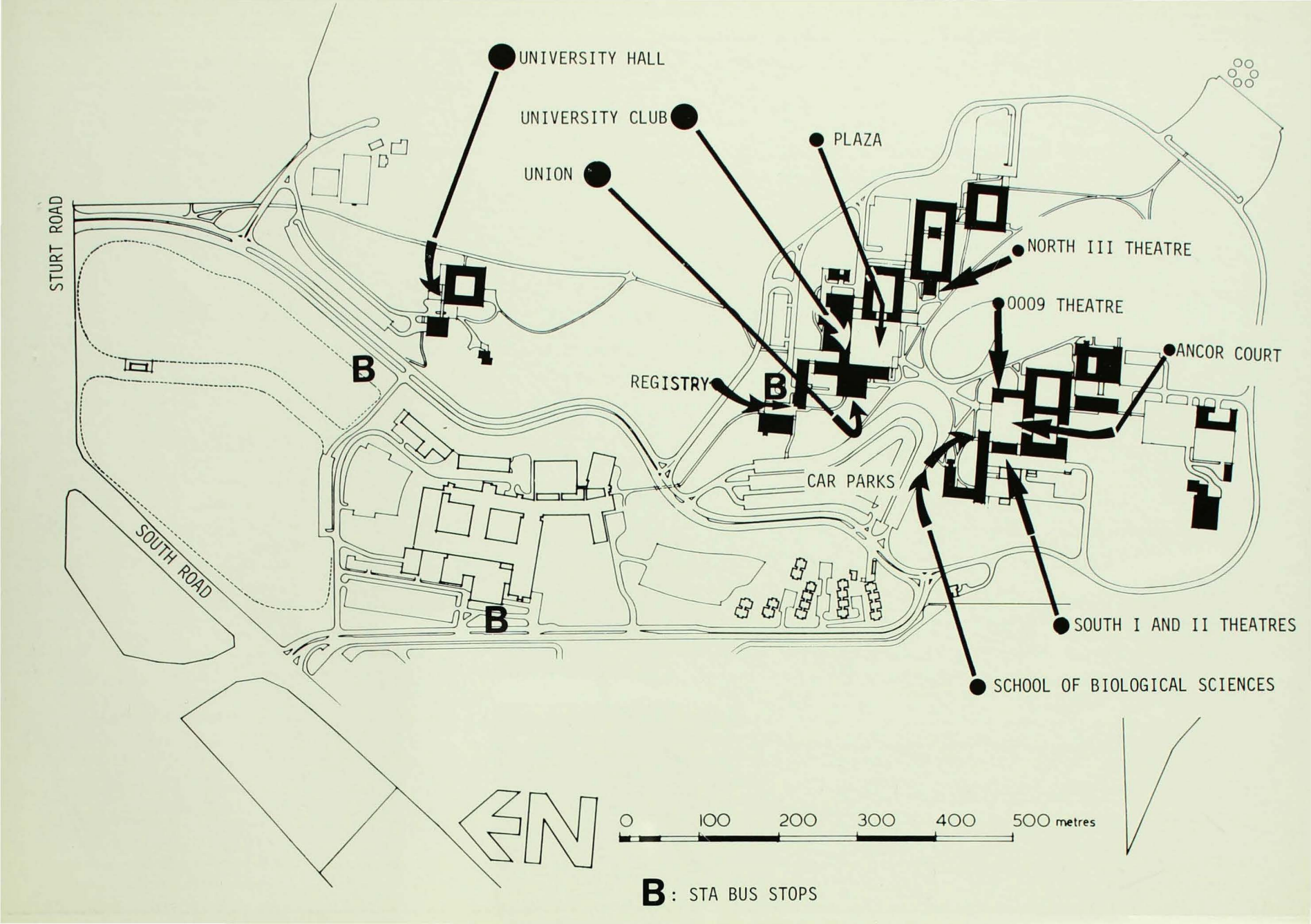
THE TAVERN (Undercroft level) 12.00 noon to 6.00 pm (or later according to trade). The Tavern will provide lunch from 12.00 to 2.00 (sandwiches and hot food).
THE UNIVERSITY CLUB (top floor) for lunch (restaurant style dining room and bar).
We are advised that the Refectory is not open this week.

University Hall have advised us that it is not economic for them to provide meals other than breakfast. However, Hall residents are eligible to use the Cafeteria in the Flinders Medical Centre for evening meals on Wednesday and Thursday. Vouchers to enable this can be obtained from the registration desk on Wednesday and on Thursday prior to 5.00 pm. and from University Hall in office hours.

OFF CAMPUS The closest restaurants are the Rickshaw and an Indian take-away on the corner of South Road and Rupert Street in Bedford Park. Other local restaurants include Lee's (ph2788859, 276 Shepherds Hill Road) and the Szechwan (ph2960396, 1518 South Road Darlington), both of which provide reasonably priced Chinese food, the Victoria Hotel at the top of Tapleys Hill, south along South Road, which has inexpensive counter meals, and the Contented Sole (ph2787099, 354 Shepherds Hill Road, Blackwood) that has been suggested to be the best fish restaurant in Adelaide but is in the upper price bracket.

Of those restaurants further afield, individual members of the local committee have found the following to their liking:

Bangles (Indian) ph513137, 10 Bank Street, City.
Bangkok (Thai) ph2235406, corner of Rundle Street and Frome Street, City.
Da Libero (Italian) ph310292, 69 Fullarton Road, Kent Town.
L'Epicurien (French) ph2714522, 296 Goodwood Road, Clarence Park.
Tandoori Oven (Indian) ph2720806, 259 Unley Road, Malvern.
The Adelaide Casino (bookings not accepted) North Terrace, City.


UNIVERSITY HALL
UNIVERSITY CLUB
UNION
PLAZA
NORTH III THEATRE
0009 THEATRE
ANCOR COURT
REGISTRY
CAR PARKS
SOUTH I AND II THEATRES
SCHOOL OF BIOLOGICAL SCIENCES
STURT ROAD
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0 100 200 300 400 500 metres
B: STA BUS STOPS

CONTENTS

GENERAL INFORMATION

REGISTRATION AND ENQUIRIES

Registration is $25.00 (Students $12).

The registration and enquiry desk will be located in the Seminar Room of University Hall from 7.00 pm to 10.00 pm on Wednesday 27 August, in the Foyer of North III Theatre from 8.30 am to 9.00 am on Thursday 28 August and from 9.00 am to 5.00 pm on both Thursday 28 and Friday 29 August in room 201 (The School Office) on level 2 in the School of Biological Sciences.

Members of the Organising committee would be pleased to assist with any difficulties. The committee comprises:
David Catcheside (Local Secretary)
Neil Brink
Rory Hope
Greg Kirby
Jeremy Timmis

SCIENTIFIC SESSIONS

The conference Opening and Plenary Sessions will be held in the North III Lecture Theatre. Concurrent Sessions will be held in Theatres opening off the Anchor Court. These are the South I and II Theatres adjacent to the School of Biological Sciences and Theatre 0009 in the School of Mathematical Sciences. Tea, Coffee, Posters and the Trade Display will be in Laboratories 1, 5, 6 and 7 in the School of Biological Sciences. Laboratories 5, 6 and 7 are located on the same level as the Anchor Court, adjacent to the foyer. Laboratory 1 is on level 1 at the foot of the foyer stairwell.

PAPERS

Proffered papers will be 15 minutes with an additional 5 minutes for questions. Chairmen are asked to keep strictly to time to permit switching between sessions. Invited papers of longer duration include 5 minutes for questions. Speakers should contact the chairman well before the starting time for the session. Facilities will be available for the projection of 35mm slides and overhead transparencies. Slides should be numbered consecutively in the top right hand corner of the mount (oriented for projection and viewed from behind the projector). Slides should be handed to the projectionist well before the starting time for the session.

POSTERS

Posters will be on view on both Thursday 28 and Friday 29 August. Presenting authors are asked to set up their posters by 2.00 pm on Thursday and to be in attendance at the poster sessions on both afternoons. Posters should be set up on Wednesday evening wherever possible. Please contact the registration desk for directions to set up a posters.

LUNCH

Registrants have been made honorary members of the University Club for the duration of the Conference. The Club has a bar and facilities for lunch, located on the top floor of the Union building. Hot food and sandwiches can also be obtained in the Refectory located on the middle level of Union building. By prior arrangement with the office in University Hall, lunch and evening meals can be obtained in the University Hall dining room.

TELEPHONES AND MESSAGES

Public pay phones are located in the Anchor Court adjacent to the South II Theatre, at University Hall and also outside the Registry building. During normal office hours, messages for persons attending the Conference can be received on (08) 2752280. Messages will be posted on the GSA Conference Notice Board which is located in the foyer on level 2 of the School of Biological Sciences.

SHOPPING AND BANKS

There is a Union Shop, Pharmacy, Bookshop, Post Office and a branch of the ANZ bank located in the vicinity of the Undercroft of the Union building. The State Bank has a branch on level 4 of the Flinders Medical Centre.

An extensive range of shopping and other facilities is located at the Marion Shopping Centre, about 3km west of the University along Sturt Road. Some buses to Marion stop at the Registry, others stop at the Flinders Medical Centre. Suburban shops are open until 9 pm on Thursday evening. The city shops are open until 9 pm on Friday evening.

Petrol can be obtained about 2km south of the University along South Road.

PARKING

Parking on campus requires a permit. Permits can be obtained from the registration desk.

BUSES AND TAXIS

Buses to the city can be obtained at the termini adjacent to the Registry building and also at the main entrance to the Flinders Medical Centre.

There is a taxi rank at the main entrance to the Flinders Medical Centre. Taxis can be contacted by phoning 223 3111, 211 8888 and 223 3333.

EMERGENCY

Emergency medical assistance can be obtained from the Health and Counselling Service located on the east side of the Plaza, internal telephone 2118, or at the Flinders Medical Centre, telephone 2759911.

The Flinders University Security Department can be contacted in an emergency or after hours by telephoning 2880 (internal) or 2752880 (external).

SOCIAL FUNCTIONS

MIXER

7.00 - 10.00pm Wednesday 27th August on the Mezzanine at University Hall.

ANNUAL SOCIETY DINNER

7.10 for 7.30pm Friday 29th August in the University Club. Tickets are $18.00. This includes pre-dinner drinks and a three-course meal. The bar will be open for purchase of beers, wines and spirits at members' prices.

WINERY VISIT AND BARBEQUE

A visit to Thomas Hardy & Sons at Reynella has been arranged on Saturday 30th August from 12.45 to 3.45pm. There will be opportunities to tour the Chateau and Homestead and to taste and purchase wines. Tickets will cost $19 inclusive of food (a spit roast) and drink (wines, beer and fruit juice). Bus transport returning to University Hall and direct to the Airport to connect with late afternoon flights will be available ($3).

PROGRAMME

Names of presenters are underlined.

WEDNESDAY 27 AUGUST

REGISTRATION
7.00 - 10.00pm University Hall, Seminar room

MIXER
7.00 - 10.00pm University Hall, Mezzanine

THURSDAY 28 AUGUST

REGISTRATION
8.30am Foyer of North III Theatre

WELCOMING ADDRESS
9.00 North III Theatre
Chairman: Dr. D.E.A. Catcheside
Emeritus Professor A.M. Clark, Pro Chancellor

PRINCIPAL GUEST SPEAKER
9.15 North III Theatre
Chairman: Professor A.M. Clark
Jack W. Szostak (Harvard Medical School)
Behaviour of artificial chromosomes and chromosome fragments in yeast.

MORNING TEA
10.15 - 10.45 School of Biological Sciences

SESSION 1A Symposium
GENE REGULATION IN EUKARYOTES
South I Theatre
Chairman: Professor J.A. Pateman

10.45 B.M. Tyler
Molecular approaches to gene regulation in Neurospora crassa.

11.15 J.R.E. Wells, P.D. Vize, A.Michalska, R. Ashman, A. Robins, and R.F Seamark
Selective gene transfer to single cell embryos: applications in research and agriculture.

11.45 M.J. Hynes
The 5' regulatory region of the amdS gene of Aspergillus nidulans.

12.15 T.G. Littlejohn and M.J. Hynes
Reverse genetic analysis of the amdS gene of Aspergillus nidulans.

12.35 A. Andrianopoulos and M.J. Hynes
Cloning of the regulatory gene amdR from Aspergillus nidulans.

SESSION 1B Symposium
THE GENETICS OF PLANT IMPROVEMENT
South II Theatre
Chairman: Dr. G.M.E. Mayo

10.45 B.S. Gill (Kansas state University)
Cytogenetic manipulations in resistance transfer in wheat.

11.15 K.W. Shepherd
Utilisation of rye genes in wheat improvement.

11.45 E.S. Dennis
Molecular contribution to plant breeding: probable and possible.

12.15 D.H.B. Sparrow
A plant breeder's perspective on molecular approaches to crop improvement.

LUNCH
12.55 - 2.00

SESSION 2A South I Theatre
Chairman: Professor J.S.F. Barker

2.00 J. Sved, A. Wilton, and M. Joseph
Can deleterious recessives explain heterosis in Drosophila melanogaster? I. Dichromosomal experiments.

2.20 A. Wilton, J. Sved, H. Kai and F. Ayala
Can deleterious recessives explain heterosis in Drosophila melanogaster? II. Half chromosome homozygotes.

2.40 A. Torkamanzehi, C. Moran, and F.W. Nicholas
Can P factors generate quantitative genetic variation in Drosophila melanogaster?

3.00 F.W. Nicholas
Replication of selection lines reduces response to selection.

SESSION 2B South II Theatre
Chairman: Dr. J.N. Timmis

2.00 R.I.S. Brettell, Z.Y. Xin, Z.M. Cheng, P.M. Waterhouse, P.J. Larkin and R. Appels
Identification of sources of resistance in wheat to barley yellow dwarf virus.

2.20 R. Appels
Distribution of rye DNA sequences among the grasses related to wheat.

2.40 C.L. McIntyre
Evolutionary change at the rDNA locus in the Triticeae.

3.00 C.E. May and R. Appels
Variability and genetics of rDNA spacer segments in Australian wheats.

AFTERNOON TEA, POSTERS AND TRADE DISPLAY
3.20 - 4.20 Laboratories 1, 5, 6 and 7, School of Biological Sciences

SESSION 3A South I Theatre
Chairman: Dr. J.A. McKenzie

4.20 J.G. Oakeshott and R.C. Richmond
Introduction to the molecular, biochemical and population genetics of Esterase 6 in Drosophila melanogaster.

4.40 C. Collet, K. Nielsen, R. Phillis, J.G. Oakeshott, R. Russell, S. Collet and R.C. Richmond
Esterase 6: A molecular analysis of the gene.

5.00 P.H. Cooke and J.G. Oakeshott
Allozyme and nucleotide variation at the Esterase 6 locus of a natural population of Drosophila melanogaster.

5.20 T. Petrovich and L.E. Kelly
Characterisation of a Suppressor of stoned mutant of Drosophila.

SESSION 3B South II Theatre
Chairman: Dr. P. Langridge

4.20 E. Vyse and P. Langridge
Restriction fragment length polymorphisms between wheat and barley.

4.40 C.B. Gillies
Action of ph gene on synaptonemal complex formation in wheat X Aegilops hybrids.

5.00 W.L. Gerlach, W.A. Miller, Z. Cheng and P.M. Waterhouse
Detection of plant luteoviruses.

5.20 D.J. Luckett
Colchicine mutagenesis in cotton.

SESSION 3C Theatre 0009, School of Mathematical Sciences.
Chairman: Dr. N.G. Brink

4.20 D.F. Catanzaro, B. West, P. Cattini, J.D. Baxter and T. Reudelhuder.
Protein-DNA interactions in the control of rat growth hormone gene expression.

4.40 M. King, N. Contreras and R. Honeycut
Nucleolar organizer activity and secondary constriction structure in Litoria (Amphibia).

5.00 M. Mahony
Nuclear DNA values and C-band heterochromatin of Australian myobatrachid frogs.

5.20 P.M. Gresshoff, A.C. Delves, A.J. Krotzky, B.J. Carroll and D.A. Day
Supernodulation mutants of soybean: host genetic analysis of symbiotic nitrogen fixation.

FRIDAY 29 AUGUST

SESSION 4A South I Theatre
Chairman: Dr. J.A. Sved

9.00 J. K. Davidson and P.A. Parsons
Extremes of climate and genetic heterogeneity in the dipteran species Drosophila melanogaster.

9.20 A.A. Hoffmann
Incompatibility among Drosophila populations: further examples and inheritance patterns.

9.40 J.S.F. Barker
Geographic variation in Drosophila buzzatii - drift, migration, or selection?

10.00 R.H. Thomas
Size and shape in natural and laboratory populations of Drosophila buzzatii.

10.20 J.A. Stoddart
Spatial patterns and populations.

SESSION 4B South II Theatre
Chairman: Dr V. Krishnapillai

9.00 R. Nordeen and B.W. Holloway
Chromosomal mapping of the phytopathogen Pseudomonas syringae pv syringae PS224.

9.20 A.D.G. Strom and A.F. Morgan.
Mechanism of plasmid integration in Pseudomonas putida PPN.

9.40 V. Korolik, V. Krishnapillai and P. Coloe
Cloning of Campylobacter jejuni antigen genes in Escherichia coli.

10.00 H.K. Mahanty and R. Kolter
Cloning and fine structure analysis of immunity and structural genes of Colicin V.

10.20 C. Cobbett
Regulation of the aroF tyrA operon of Escherichia coli.

SESSION 4C Theatre 0009, School of Mathematical Sciences.
Chairman: Dr. R. Appels

9.00 J.H. Ford
Independent control of chromosomal and spindle cycles: the relevance to errors of division.

9.20 C.G. Roberts and M.H.N. Tattersall
Cytogenetic analysis of clonal evolution in human ovarian carcinoma.

9.40 J. Brasch and D.R. Smyth
Amplification of specific rDNA sequences in a T-cell leukaemia line.

10.00 C. Paul and M. Frommer
In situ hybridisation of repeated sequences to human metaphase chromosomes using bromodeoxyuridine-labelled probes.

10.20 T. Shaw and R.H. Smillie
Human thymidine phosphorylase: significance and rapid assay by HPLC.

MORNING TEA
10.40 - 11.10 School of Biological Sciences

SESSION 5A Symposium
GENETICS OF INVERTEBRATE DEVELOPMENT
South I Theatre
Chairman: Professor J.A. Thomson

11.10 R.B. Saint and W. Kalionis
Genetic and molecular studies of Drosophila development.

11.40 L.E. Kelly
Genetic dissection of learning in Drosophila.

12.10 R.G. Tearle, D.B. Boyle, F.V. Morris and A.J. Howells
The structure, function and regulation of the White and Scarlet genes of Drosophila melanogaster.

SESSION 5B Symposium
MARSUPIAL GENOME EVOLUTION
South II Theatre
Chairman: Professor G.B. Sharman

11.10 D.L. Hayman
Chromosome evolution in marsupials.

11.40 J.A. Marshall Graves
Evolution of marsupial gene arrangements.

12.10 A. van Daal P.L. Molloy and D.W. Cooper
Evolution of phosphoglycerate kinase gene sequences in marsupials and eutherians.

LUNCH
12.40 - 1.40

SESSION 6A South I Theatre
Chairman: Dr. G.C. Kirby

1.40 B.P. Oldroyd
The effect of inbreeding in queen bees on honey production.

2.00 D. Rowell
Translocation heterozygosity and sociality in Delena cancerides.

2.20 W.B. Sherwin
Morphology and mating success in a frog hybrid zone.

2.40 T.A. McRae
A quantitative genetic analysis of life-history variation in two populations of Tribolium castaneum.

SESSION 6B South II Theatre
Chairman: Dr. R.M. Hope

1.40 A. van Daal, P.L. Molloy and D.W. Cooper
Methylation of PGK on the active and inactive X chromosome in kangaroos.

2.00 R.H.H. van Oorschot, J. Stratton and D.W. Cooper
Kangaroo red cell antigens recognised by monoclonal antibodies tend to be polymorphic

2.20 J. Wrigley and J.A. Marshall Graves
Gene mapping in monotremes: synteny between HPT and PGK in the platypus.

2.40 L.M. McKay and J.A. Marshall Graves
Marsupial-marsupial cell hybrids - at last

SESSION 6C Theatre 0009, School of Mathematical Sciences.
Chairman: Dr. D.R. Smyth

1.40 R. Frankham
A molecular hypothesis for position-effect variegation: transcription of anti-sense RNA.

2.00 J.W. Sentry, J.L. Joseph and D.R. Smyth
del, a dispersed DNA sequence in Lilium.

2.20 M. Walker
Molecular analysis of the new transposable system Mut in maize.

2.40 M.L. Arnold
Genomic organisation of highly repeated DNA from the grasshopper Caledia captiva.

AFTERNOON TEA, POSTERS AND TRADE DISPLAY
3.00 - 4.00 Laboratories 1, 5, 6 and 7, School of Biological Sciences

SESSION 7A South I Theatre
Chairman: Dr. J.A. Marshall Graves

4.00 E. Vyse, M. Cronin and D. Cameron (Montana State University)
Introgression of white-tailed deer mitochondrial DNA into mule deer in Montana.

4.20 A.D. Marchant, D.D. Shaw and G.D. Clark-Walker
Mitochondrial DNA variation in the Caledia captiva species-complex.

4.40 J.R. Ovenden, R.W.G. White and A. Sanger
Mitochondrial genome variation in two species of the Australian freshwater fish genus Gadopsis.

SESSION 7B South II Theatre
Chairman: Dr. M.J. Witten

4.00 A. Elizur, R.G. Tearle and A.J. Howells
Molecular analysis of the Topaz gene and its mutants from Lucilia cuprina.

4.20 G.M. Clarke and J.A. McKenzie
Fluctuating asymmetry and insecticide resistance in Lucilia cuprina.

4.40 J.A. McKenzie and A.Y. Game
Diazinon resistance in Lucilia cuprina: Mapping of a fitness modifier.

SESSION 7C Theatre 0009, School of Mathematical Sciences
Chairman: Dr. D.L. Hayman

4.00 C.R. Leach, and D.L. Hayman
The incompatibility loci as indicators of conserved linkage groups in the Poaceae.

4.20 J.S. Lonie and G.B. Peters
Genetic variability in the small and discrete populations of Eucalyptus pulverulenta (Sims).

4.40 N.D. Murray
Reproductive isolation in an introduced population of the beetle Chrysolina quadrigemina.

BUSINESS MEETING
5.00 South I Theatre

ANNUAL SOCIETY DINNER
7.10 for 7.30 Flinders University Club

SATURDAY 30 AUGUST

GUEST SPEAKERS North III Theatre
Chairman: Professor D.W. Cooper

9.00 David R. Wolstenholme (University of Utah)
Mitochondrial Genomes of Drosophila and other invertebrates.

9.45 Bryan C. Clarke (University of Nottingham)
Frequency dependent selection and metrical variation.

MORNING TEA
10.30 - 11.00 Foyer North III Theatre

PRESIDENTIAL ADDRESS
North III Theatre
Chairman: Dr. J. Langridge

11.00 D.W. Cooper
The effect of parental source on gene action in eukaryotes.

BARBEQUE
12.45 - 3.45 Hardy's Chateau Reynella
12.20 buses depart from University Hall
3.50 buses depart for University and Airport

POSTERS

For poster locations see the map inside the back cover.

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1. G.C. Webb and S.A. Fabb
Probing murine male meiosis using unique DNA flanking the immunoglobulin heavy chain genes.

2. C. Zhang and B.W. Holloway
A region selective transposon mutagenesis system for Pseudomonas aeruginosa.

3. J. Richter
A chromosomal study of Australian bats.

4. M. Delbridge and C. Cobbett
Bi-directional regulation at the aroF operator of Escherichia coli.

5. J.R. Andrews, G.M.F. Pasquini and P. Batterham
Further genetic analysis of the Lozenge locus in Drosophila melanogaster.

6. J.A. Saleeba, P.W. Atkinson, M.J. Hynes and C.S. Cobbett.
The use of a lac Z fusion to study the regulation of a gene of unknown function in Aspergillus nidulans.

7. R.A. Sandeman and M.J. Hynes
Cloning and regulation of acetate utilisation genes of Aspergillus nidulans.

8. A.E. Dollin, J.D. Murray and R.L. Close
Chromosome pairing abnormalities in interspecific hybrid cattle and rock wallabies.

9. W.R. Knibb, J.G. Oakeshott and J.S.F. Barker
Polymorphism for abnormal abdomen phenotypes in Drosophila buzzatii.

10. D.J. Schafer, M.M. Green, D.K. Fredline, W.R. Knibb and J.S.F. Barker
The formal genetics of Drosophila buzzatii.

11. F. Hannan and L.E. Kelly
Cloning and characterisation of the Stoned gene of Drosophila.

12. R. Ramsbotham and L.E. Kelly
The isolation of genes encoding nervous system phosphoproteins from Drosophila - a new methodology.

13. G.C. Webb, C.G. Keith and J.G. Rogers
Absence and hypoplasia of some ocular muscles in a child with the karyotype: 46,XY, der(2), inv? ins(2;7)(q21;q32q34)mat.

14. G.C. Webb, M.A. Leversha and L.E. Voullaire
Chromosome deletion at 11q23 in the child of a parent with fragility at the same site.

15. P. Mathew and S.W.McKechnie
Characterisation of a 37bp insertion in the first intron of the Adh gene of Drosophila melanogaster.

16. A. Game, and J. Oakeshott
Variation in the expression of Esterase 6 in Drosophila melanogaster.

17. M. Sandery and P. Langridge
Restriction fragment length polymorphisms between wheat and rye.

18. D. Rouch, J. Camakaris and B.T.O. Lee
Chromosomal gene involvement in plasmid-mediated copper resistance in Escherichia coli.

19. D. Bedo
Polytene chromosomes of the mediterranean fruit fly, Ceratitis capitata.

20. M. Westerman
Genome evolution in the genus Antechinus (Dasyuridae, Marsupiala).

21. L. McKay, J. Wrigley and J.A. Marshall Graves
Evolution of mamalian X-chromosome inactivation: sex chromatin in monotremes and marsupials.

22. J.L. Dempsey and A.A. Morley
Dose rate effects on mutation induction by ionizing radiation.

23. A.J. Pryor
Unstable genes for rust resistance in maize.

24. M. Fenech and A.A. Morley
Ageing in vivo does not influence micronucleus induction by X-irradiation in human lymphocytes.

25. D.R. Turner, A.A. Morley, M. Haliandos, R.F. Donnell and A.W. Skulimowski
Molecular basis for induced mutations at the HPRT locus.

26. P.A. Anderson and K.W. Shepherd
Cytogenetic analysis of recombination events between wheat and rye group 1 chromosomes.

27. C. Moritz and W. Brown
Large duplications in the mitochondrial DNA of lizards.

28. K.I. Matthaei and K.C. Reed
Sensitivity of in situ hybridisation with single-stranded RNA probes is increased dramatically by polyethylene glycol.

PARTICIPANTS

ADCOCK G.	MELBOURNE
ALLEN H.	MELBOURNE
ANDERSON P.A.	CSIRO PLANT IND.
ANDREWS J.	MELBOURNE
ANDRIANOPOULOS A.	MELBOURNE
APPELS R.	CSIRO PLANT IND.
ARNOLD M.	ANU
BARKER J.S.F.	NEW ENGLAND
BATTERHAM P.	MELBOURNE
BEDO D.	CSIRO ENTOMOLOGY
BRASCH J.	MONASH
BRETTELL R.	CSIRO PLANT IND.
BRINK N.G	FLINDERS
BULL A.	MELBOURNE
CATANZARO D.F.	SYDNEY
CATCHESIDE D.E.A.	FLINDERS
CATCHESIDE D.G.	WAITE INSTITUTE
CHESSON C.M.	ADELAIDE
CLARK A.M.	FLINDERS
CLARKE B.	NOTTINGHAM
CLARKE G.	CSIRO ENTOMOLOGY
CLAXTON J.H.	NEW ENGLAND
COBBETT C.	MELBOURNE
COLLET C.	ANU
CONSTABLE S.	MELBOURNE
COOKE P.	ANU
COOPER D.W.	MACQUARIE
COOPER S.	ADELAIDE
CROSS T.	MELBOURNE
DADAY H.	CSIRO PLANT IND.
DAVIDSON J.K.	LA TROBE
DAVIES S.	MURRUMBEENA, VIC
DAVIS M.	MELBOURNE
DEL BOSQUE A.	NEW ENGLAND
DELBRIDGE M.	ELTHAM, VIC
DEMPSEY J.	FLINDERS
DENNIS E.S.	CSIRO PLANT IND.
DOLLIN A.	CSIRO ANIMAL PR.
DONNELLON S.	SA MUSEUM
DOWZER C.	ADELAIDE
ELIZUR A.	ANU
ENGLAND P.	MARSFIELD, NSW
FABB S.	MELBOURNE
FARMER L.	MELBOURNE
FARRELL R.	MELBOURNE
FENECH M.	FLINDERS
FIDOCK D.	ADELAIDE
FORD J.	QE HOSPITAL SA
FRANKHAM R.	MACQUARIE
FRANKLIN I.	CSIRO ANIMAL PR.
FROMMER M.	CSIRO MOL. BIOL.
GABB B.W.	ADELAIDE
GAME A.	ANU
GERLACH W.	CSIRO PLANT IND.
GILL B.S.	KANSAS
GILLIES C.B.	SYDNEY
GLARE E.	FLINDERS
GRAVES J.M.	LA TROBE
GRESSHOFF P.M.	ANU
GRIGG G.W.	CSIRO MOL. BIOL.
HANNAN F.	MELBOURNE
HARDY S.M.	MELBOURNE
HATTHAEI K.I.	ANU
HAYMAN D.	ADELAIDE
HOFFMANN A.	LA TROBE
HOLLAND F.	FLINDERS
HOPE R.	ADELAIDE
HYLAND V.J.	CHILD. HOSP. SA
HYNES M.J.	MELBOURNE
JENKIN M.	CSIRO PLANT IND.
JONES D.	WAITE INSTITUTE
JOSEPH J.	MONASH
KALIONIS W.	CSIRO ENTOMOLOGY
KATZ M.	MELBOURNE
KELLY J.	ADELAIDE
KELLY L.E.	MELBOURNE
KING M.	DARWIN MUSEUM
KIRBY G.C.	FLINDERS
KNIBB W.R.	NEW ENGLAND
KOROLIK V.	MONASH
KRISHNAPILLAI V.	MONASH
KROTZKY A.J.	ANU
LANGRIDGE J.	CSIRO PLANT IND.
LANGRIDGE P.	WAITE INSTITUTE
LAWRINSON P.	FLINDERS
LATTER B.	SYDNEY
LEACH C.	ADELAIDE
LEE B.	MELBOURNE
LEETON P.	MALVERN, VIC
LEHESSON C.M.	ADELAIDE
LEONG W.	FAIRFIELD H.
LITTLEJOHN T.	MELBOURNE
LLEWELYN A.	QE HOSPITAL
LUCKETT D.	CSIRO COTTON R.
MAHANTY H.K.	CANTERBURY, NZ
MAHONY M.	PRINCE OF W. H.
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BEHAVIOUR OF ARTIFICIAL CHROMOSOMES AND CHROMOSOME FRAGMENTS IN YEAST

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What structural features of yeast chromosomes contribute to the remarkably high fidelity of chromosomal inheritance? Previous studies have shown that very short linear artificial chromosomes (5-15 kb) are inherited randomly at mitosis, but that longer artificial chromosomes (50-60 kb) segregate correctly in 99% of cell divisions. The random inheritance of the very short molecules is not due to failure to attach to the spindle, the use of Tetrahymena telomeres in their construction, or the short telomere centromere distance. We have shown that increasing the length of artificial chromosomes (to 100 kb) makes them more stable, and that decreasing the length of derivatives of chromosome III makes them less stable. Thus longer chromosomes are more faithfully inherited, at least through the size range of 10-300 kb. This result is consistent with a model in which sister chromatids are held together by catenation. Shorter chromosomes are more readily decatenated, and once sister chromatids have separated, they can only segregate randomly.

We have employed both artificial chromosomes and fragments of chromosome III in our studies of meiotic chromosome behaviour. Artificial chromosomes of 60 kb disjoin with high fidelity (90-95%) in meiosis I. Such fidelity is unexpected because these molecules are too short to engage in recombination at the frequency necesary for the generation of chiasmata in every meiosis. We have used multiply marked artificial chromosomes to show that correct disjunction can take place in the absence of recombination. In other crosses, we have shown that homology is not necesary for proper disjunction. Thus yeast has a distributive pairing mechanism, presumably a backup system that ensures the proper segregation of non-recombinant chromosomes.

MOLECULAR APPROACHES TO GENE REGULATION IN *NEUROSPORA CRASSA*

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The well-developed genetic system of *Neurospora crassa* has enabled several regulatory gene systems to be described in considerable detail, notably the quinic acid (*qa*), phosphorus and nitrogen utilisation systens. These systems have served as detailed models for more complex eukaryotic systems. More recently, the development of a DNA transformation system has enabled modified cloned genes to be reintroduced into *Neurospora* and has enabled the development of a sib-selection system for rapidly cloning genes corresponding to any known *Neurospora* mutation.

In order to combine the power of biochemical analysis with that of genetic analysis in *Neurospora*, I have developed crude extracts of *Neurospora* capable of faithfully transcibing cloned *Neurospora* genes *in vitro* . These include genes transcribed by RNA polymerase I (45S rRNA genes), RNA polymerase II (protein-coding genes), and RNA polymerase III (5S rRNA and tRNA genes) . I have used these transcription systems to analyse at the biochemical level, two *Neurospora* regulatory systems already well-characterised by classical and molecular genetic analysis. These are the *qa genes* and the ribosomal genes of *Neurospora.*

The *qa* genes, *qa*-2, *qa*-3 and *qa*-4, encode the first three enzymes of quinic acid (QA) catabolism(1).The transcription of these genes is induced by QA, and genetic analysis has shown that this induction is mediated by two reulatory genes *qa*-1S and *qa*-1F which are tightly linked to the three structural genes. The entire cluster of *qa* genes has been cloned into *E.coli* on the basis of the complementation of *E.coli aro* D mutations by the *Neurospora qa*-2 gene, and the individual *qa* genes have been identified in the cloned cluster by transformation of appropriate *Neurospora qa* mutants(1). The transciptional organisation of each of the *qa* genes has been determined in detail (1) and the complete nucleotide sequence of the 17.5 kb cluster has been determined. In particular, *qa* gene transcription has been examined in the presence of a wide variety of cis-acting and trans-acting regulatory mutations and the nucleotide sequences of many of those mutations have been determined(1). It has been shown that disruptive (rearrangement) mutations in *qa*-1F block *qa* gene transcription and that the effects of those mutations can be partially reversed in the case of *qa*-2 by promoter region mutations(1). Thus *qa*-1F is proposed to encode a protein directly required for *qa* gene transcription.In support of this hypothesis, cloned *qa* genes are not transcibed *in vitro* in the absence of *qa* -1F protein.Moreover, *qa* -1F protein, produced in an insect cell expression system or by *in vitro* translation of synthetic mRNA, has recently been shown to bind to a specific sequence upstream of each of the *qa* genes(J.Baum and N.Giles, unpublished data). In the case of *qa*-2, this target sequence lies in a region required for *qa*-2 induction.(1).This synthetic *qa* -1F protein will also be used to test whether *qa*-1F protein is required for *qa* gene transcription *in vitro.* Disruptive(nonsenese and frameshift) mutations in *qa*-1S cause constitutive transcription of the *qa* genes while some missense mutations are dominant and block induction(1). Thus *qa*-1S is inferred to encode a repressor which interacts with QA and which, in the absence of QA, blocks the positive action of *qa*-1F(1). The nature of this action is not yet determined but could involve the control of *qa*-1F mRNA translation, the binding of *qa*-1S protein to *qa* -1F protein, or the binding of *qa*-1S protein to a specific DNA sequence.

The expression of the ribosomal RNA genes of *Neurospora* is controlled co-ordinately by growth rate, by phosphorus deprivation and during conidial germination.To determine whether transcriptional regulation might contribute to this control, I have used the *Neurospora* RNA polymerase I and RNA polymerase III transcription systems to determine the promoter structures of the 45S and 5S rRNA genes in order to look for possible common control elements.Sets of point mutation clusters collectively spanning the promoter regions of each gene were constructed and assayed for their effects on the tramsciption of the respective genes *in vitro* . The results indicated that *Neurospora* 5S genes contain three internal promoter elements as well as an external "TATA-box" at -29 which fixes the start-point of transcription. In the case of the 45S rRNA gene, most of the sequences between -96 and +10 are required for transcription in the *in vitro* system. Within this region there are three sequences which show strong homology to three of the four 5S gene promoter elements, raising the possibility that in *Neurospora,* the 45S and 5S rRNA genes may be co-regulated via common promoter elements.

(1) Giles, NH *et al.* (1985) Microbiol. Rev. **49**, 338-358

SELECTIVE GENE TRANSFER TO SINGLE CELL EMBRYOS: APPLICATIONS IN RESEARCH AND AGRICULTURE

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Our long term aim is to produce livestock animals with superior growth rates and food utilization efficiencies. We have isolated a porcine growth hormone (PGH) cDNA clone and constructed an expression vector capable of producing PGH when reintroduced into the cells of a variety of different animals.

Both cDNA and genomic clones for PGH were isolated, the cDNA using a 27-base oligonucleotide probe and the cosmid using the cDNA. An expression vector, pHMPG.4, was then constructed using the human metallothionein IIA promoter, the PGH coding sequence from the cDNA and the 3' processing signals of the genomic clone.

The fidelity of the construct was tested by transfection into HeLa cells using calcium phosphate precipitation. ELISA's of extracts from Zn^{2+}-induced cells were found to contain PGH.

The expression vector was then further tested by microinjection into fertilized one-cell mouse embryos. To date 69 mice have developed from such eggs, 19 of which are transgenic and contain between one and thirty copies of the pHMPG.4 construct per diploid cell. Of these 19 mice, most are growing considerably larger than their normal littermates with the biggest being 1.9 times the size of non-transgenic mice.

Recently, the construct has also been used to create transgenic pigs by microinjection into one-cell pig embryos. Of the eight pigs which have developed from injected eggs born to date, three are transgenic and carry between three and ten copies of the construct per diploid cell. One animal has shown a strikingly increased growth rate.

THE 5' REGULATORY REGION OF THE amdS GENE OF Aspergillus nidulans

Michael J. Hynes

Department of Genetics, University of Melbourne

The acetamidase (amdS) gene of Aspergillus nidulans provides a suitable system for investigating the problems involved in gene regulation by multiple regulatory signals. Genetic analysis has allowed the definition of trans-acting regulatory genes as well as mutations in cis-acting sites adjacent to the structural gene. Molecular genetic analysis of this system aims at the identification of the sequences involved in regulation and the mechanisms of action of the regulatory gene products.

The amdS gene has been cloned and sequenced. It has a possible TATA box about 30 bp upstream from the start point for transcription and contains three introns, each with splicing signals consistent with those of other eukaryotes. A longer cDNA clone has been isolated and found to contain intron 3. Transformation studies show that both forms of cDNA are functional in specifying active acetamidase.

Proposed sites of action of the regulatory gene products have been determined by a combination of methods -

a) sequencing cis-acting mutations which specifically affect only one of the control mechanisms;
b) in vitro manipulation of the 5' end of the gene followed by transformation into Aspergillus;
c) the generation of multiple copies of 5' sequences by co-transformation of Aspergillus and the observation of titration effects on regulatory gene products, and
d) comparisons of 5' sequences of structural genes which share regulatory circuits with amdS.

REVERSE GENETIC ANALYSIS OF THE amdS GENE OF Aspergillus nidulans.

Tim G. Littlejohn and Michael J. Hynes.

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Analysis of gene regulation depends on identification of regulatory genes, their coffectors and their sites of action at the genes they regulate. Thorough analysis can only proceed, however, if a methodology for rapidly and accurately testing regulatory models in vivo can be facilitated by in vitro (molecular) techniques. This necessitates cloning the gene(s) under study, generating mutants of that gene's controlling region in vitro and assaying the regulatory consequences of such manipulations in vivo through transformation. Together these in vitro manipulations followed by in vivo analysis represent the "reverse genetic" approach to studying gene regulation.

The amdS (acetamidase) gene of Aspergillus nidulans has been subject to reverse genetic analysis. Only one aspect of this will be discussed here. Transformants carrying multiple copies of the amdS controlling region (amdI) can redily be generated, including those carrying many copies of mutant 5' regions. Through their property of titrating regulatory gene products, these transformants have allowed identification of the following:

a) a 100 b.p. fragment found 5' to amdS that titrates both the amdR and facB gene products

b) that DNA from the amdI93 mutation, which leads to loss of amdR control of amdS, also loses its ability to titrate the amdR gene product

c) that DNA from the amdI9 mutation, which leads to increased amdS expression via facB, titrates the facB gene product more readily than the wild type DNA

d) that DNA from the amdI66 mutation, which leads to increased expression of amdS via amdA7, titrates the amdA7 gene product more readily than the wild type DNA

e) that the areA102 gene product can be titrated by amdS DNA.

Titration analysis has identified DNA that binds most of the regulatory gene products known to control amdS expression. Further, in conjunction with amdS regulatory studies, these analyses identify sequences important for regulation and for regulatory gene product recognition and binding. Together these studies allow a precise method for dissection of the mechanism of gene regulation.

CLONING OF THE REGULATORY GENE amdR FROM Aspergillus nidulans

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Gene regulation generally involves the interaction of a regulatory gene product with a structural gene in a highly specific manner. The analysis of the 5' regulatory region of structural genes upon which regulatory gene products act, provides only half of the whole complex interaction involved in gene regulation. However, with the recent developments in cloning strategies and transformation systems, especially in lower eukaryotes, the ability to clone regulatory genes has afforded the possibilty of studying gene regulation from both structural and regulatory gene aspects.

In the ascomycete fungus, Aspergillus nidulans, the amdS gene encodes an acetamidase which permits utilization of acetamide as a carbon and/or nitrogen source. Extensive genetic analysis has shown that amdS regulation is mediated by a number of independently acting regulatory genes. One of these trans-acting regulatory genes is amdR. In addition to regulating amdS gene expression, amdR is known to coregulate the expression of three other genes involved in omega-amino acid utilization.

The amdR regulatory gene was cloned by transformation of a cosmid library into a trpC$^-$amdR$^-$ strain of A. nidulans by selecting for tryptophan prototrophy and then screening for growth on GABA as a nitrogen source. Genetic and molecular analysis of an amdR$^+$ transformant indicated that a single integrated cosmid resulted in this phenotypic change. The cosmid was subsequently rescued from genomic DNA of this transformant by transfomation into E. coli, and its identity confimed by retransformation into another amdR$^-$ strain of A. nidulans.

Cloning of the amdR regulatory gene has permitted the initial analysis of the molecular nature of amdR. A number of amdR$^-$ alleles have been analysed via Southerns and shown to possess detectable rearrangements, including one with a translocation. Characterization of amdR at the molecular level with respect to its regulation and its regulatory effect on the genes which it controls is currently being examined and will include DNA-protein binding studies.

CYTOGENETIC MANIPULATIONS IN RESISTANCE TRANSFER IN WHEAT

Bikram S. Gill

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Common wheat is a polyploid crop plant and is reproductively isolated from its putative progenitor species (homologous gene pool) and related taxa (homoeologous gene pool). The chromosomes of progenitor species readily pair with chromosomes of wheat and genetic transfer can be made by homologous recombination. Genetic manipulations are required in the production of F_1 hybrid plants and overcoming of chromosomal sterility caused by unbalanced gametes. The (alien) chromosomes of the taxa in the homoeologous gene pool do not pair with chromosomes of wheat mainly due to the action of homoeologous pairing suppressor gene Ph in chromosome 5B of wheat. Therefore, genetic manipulations are required to establish (i) the genetic relationship of an alien chromosome with a wheat chromosome(s), (ii) genetic effect of Ph gene is suppressed or eliminated to allow alien chromosome to pair with a wheat chromosome, and (iii) suitable cytogenetic assays are required for the identification of recombinant chromosomes. The recent developments in genetic analysis, chromosome identification, and molecular cytogenetics will be discussed in relation to above genetic manipulations for disease resistance transfer in wheat. Examples of homologous genetic transfer from Aegilops squarrosa and homoeologous transfer from Agropyron species will be discussed.

SESSION 1B

UTILISATION OF RYE GENES IN WHEAT IMPROVEMENT.

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MOLECULAR CONTRIBUTION TO PLANT BREEDING: PROBABLE AND POSSIBLE

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The goals of biotechnology in agriculture are generally the same as those of conventional plant breeding. In almost all programmes the principal goals are the improvement of yield, resistance to pests, diseases and environmental stress and quality of the product. Biotechnology offers the possibility of new kinds of solutions to problems.

Diagnostic tools will provide increased accuracy in selection of appropriate parents in selection schemes. Introduction of specific genes should add desirable characteristics to cultivars. Examples of such genes are disease resistance or herbicide tolerance. The addition of particular control signals enable different differently regulated expression of genes, either expression in different tissues, at a diffent stage of development, or at different levels.

Thus the benefits of genetic engineering lie in extending the range of available genes, (e.g. the induction of resistance to viral infection may depend on novel mechanisms, the herbicide tolerance genes may come from bacteria) and in decreasing the time and scale of operation in plant breeding programs.

SESSION 1B

A PLANT BREEDER'S PERSPECTIVE ON MOLECULAR APPROACHES TO CROP IMPROVEMENT.

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CAN DELETERIOUS RECESSIVES EXPLAIN HETEROSIS IN *DROSOPHILA MELANOGASTER*? I. DICHROMOSOMAL EXPERIMENTS

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In the balancer chromosome equilibration technique, population cages are set up containing a balancer chromosome and either a single or many wild type chromosomes. The technique has shown that chromosome homozygotes suffer very high fitness losses, up to 80-90%, compared to chromosome heterozygotes. At the level of individual loci, this chromosomal heterosis could indicate either heterozygote advantage (overdominance) or deleterious rcessives (dominance). We have carried out an experiment to test the second of these possibilities by setting up di-chromosomal cages containing just two chromosomes, sampling chromosomes from these cages at intervals of many generations, and measuring fitness using the balancer equilibration technique. During this period we would have expected a fall in the frequency of any deleterious recessive genes, accompanied by a rise in the fitness of the chromosome homozygote. In contrast to this, we found little or no rise in fitness of the chromosome homozygotes.

The major problem of interpretation in this experiment concerns the possibility of very large numbers of deleterious genes each with very small fitness effects, in which case the predictions become difficult to separate from the overdominance model. However based on estimates of gene numbers from band counting, we feel that it is possible to rule out such an explanation. An important consideration in the calculations is the assumed model for gene interaction, and it is argued that a threshhold model is the more likely one, which strengthens the conclusion.

CAN DELETERIOUS RECESSIVES EXPLAIN HETEROSIS IN DROSOPHILA MELANOGASTER? II. HALF CHROMOSOME HOMOZYGOTES.

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The reasons for studying the fitness of half chromosome homozygotes are to provide information on a) selective interactions about which there is little known making it difficult to construct realistic multiple locus models and on b) the selective values and distribution of individual loci or small chromosome regions. The fitness of whole chromosome homozygotes is drastically reduced to 20% of wild-type which makes it difficult to infer anything about the fitness of small regions since the consequences of assuming different selective interactions become more important as overall inbreeding depression becomes higher.

The approach taken was similar to that of Breese and Mather (1960). Using the multiply marked stack all (al dp b pr cn c px sp), lines homozygous for a wild-type left arm or right arm were constructed from 10 viable second chromosome lines. All combinations (10x10) of wild-type left arm with right arm were constructed and lines homozygous for only half the second chromosome were made by combining all of the lines with a particular chromosome arm in common. Very few half chromosome homozygous lines reached equilibrium with the balancer chromosome, SM5, when tested in population cages, but the SM5/+ heterozygotes were only 15% as fit as wild-type. The average fitness of the right arm homozygotes was 50% of wild-type and the left arm homozygotes were slightly less fit. The interpretation of these and other results will be discussed.

CAN P FACTORS GENERATE QUANTITATIVE GENETIC VARIATION IN *DROSOPHILIA MELANOGASTER*?

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When P strain males of *D. melanogaster* are crossed with M strain females, the resultant hybrids show a number of abnormal traits, known collectively as hybrid dysgenesis. This phenomenon is the result of the transposition of mobile segments of DNA called P factors. One of the results of the transposition of P factors is a substantially increased rate of mutation in the hybrids' germline. The effect of this increased mutation rate has been studied and reported for many single-locus traits. However, relatively little is known about the effect of transposition of P factors on loci that contribute to genetic variation in quantitative traits ("quantitative loci").

The P-M system in *D. melanogaster* provides an opportunity for such a study. If transposition of P factors in the dysgenic hybrids' genome creates new mutations at quantitative loci, then an increased response to selection is expected in the progeny of dysgenic crosses, when compared with non-dysgenic crosses.

Using the Harwich (P) and Canton S (M) inbred lines, 10 generations of divergent selection were conducted for abdominal bristle number in two replicate populations of the descendants of M♀♀ × P♂♂ (dysgenic) and P♀♀ × M♂♂ (non-dysgenic) crosses. *In situ* hybridisations confirmed that our samples of Harwich and Canton S were P and M stocks respectively. In addition, tests for ovarian dysgenesis and for the stability of the sn^{W} allele confirmed that transposition was occurring in the dysgenic cross but not in the non-dysgenic cross. However, the dysgenic replicates showed no higher response to selection than the non-dysgenic replicates. This contrasts with the results of a similar experiment by Mackay (1985), in which dysgenic lines showed on average twice the response of non-dysgenic lines. Possible reasons for the difference in results between these two experiments will be discussed.

REPLICATION OF SELECTION LINES REDUCES RESPONSE TO SELECTION

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A common criticism of artificial selection programmes that are conducted with just one selection line per treatment is that the selection line should have been replicated. However, when resources are limited, as they usually are, then in order to have r replicates of a selection line, the effective population size of each replicate will be only approximately $1/r$ times the effective size of the single selection line that could have been maintained instead of the r replicates. By reducing effective population size, replication reduces expected response to selection, because of the increased chance of losing favourable genes due to genetic drift.

A formula has been derived for predicting the decrease in selection response resulting from replication of selection lines, and the prediction agrees quite well with experimental results. In many practical situations, selection response can be reduced by more than 20% if selection lines are replicated.

IDENTIFICATION OF SOURCES OF RESISTANCE IN WHEAT TO BARLEY YELLOW DWARF VIRUS

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Wheat and wild grass lines were selected to screen for resistance to barley yellow dwarf virus (BYDV). A local isolate of the virus, serotype PAV, was chosen for these experiments. Reproducible infections were obtained when young seedlings were exposed to viruliferous aphids of the species Rhopalosiphum padi. Virus titres in infected plants were assessed using the ELISA technique. Barley plants carrying the Yd2 gene for resistance to barley yellow dwarf disease showed low titres of virus under the conditions used for the seedling infections, yet no significant resistance to virus accumulation was found in a range of wheat cultivars examined. Among a series of wild grasses tested, four species including two species of Agropyron (Thinopyrum) were identified as candidates for resistance. The low levels of virus measured in infected plants were confirmed in further experiments.

Screening for resistance has been extended to addition lines of alien grass species in wheat. One line obtained from China, Zhong 4, which shows field resistance to the GPV serotype of BYDV, consistently showed low levels of virus when seedlings were infected with the PAV serotype. Cytological analysis of this line indicated that it contains 14 Agropyron (Thinopyrum) chromosomes in addition to the 42 chromosomes of bread wheat. DNA was isolated from plants of Zhong 4 and, following endonuclease digestion, was probed with cloned ribosomal spacer sequences specific to the E and J genomes. The results of these experiments complement the cytological analysis and confirm that Zhong 4 contains alien chromatin from a species of Agropyron (Thinopyrum). Zhong 4 has now been successfully crossed to two Australian spring wheat cultivars with the eventual aim of introgressing the resistance character(s) into the bread wheat genome.

Distribution of rye DNA sequences among the grasses related to wheat

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Certain classes of rye (R genome) repetitive sequences show a very limited distribution among the grasses related to wheat. These sequences include the 350 family which is located in terminal heterochromatin and consists of extensive tandem arrays of a sequence 380 bp long, and the 5.3-H3 family which is dispersed throughout the rye genome. It was therefore surprising that both these sequences were prominent in Agroypron sp. which carry the P genome because this genome has not previously been identified as being closely related to the R genome. Studies with other repetitive sequences, including the rDNA spacer region and 5S DNA, in fact show that the R and P genomes are not closely related. To resolve this apparent conflict two possibilities are considered.

1) A relatively recent introgression of chromosomes from one genome into the other has occurred.
2) Independent amplification events of the 350 and 5.3-H3 families of DNA sequences have occurred in the R and P genomes in response to a selection pressure.

EVOLUTIONARY CHANGE AT THE rDNA LOCUS IN THE TRITICEAE.

C.L. McIntyre

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Ribosomal genes form an integral part of an organisms biology. In cereals, rDNA units consisting of 18S and 26S ribosomal genes seperated by a large spacer are tandemly repeated up to 2-3000 times. Previous work on the annual Triticum species had shown that the spacer region is diverging more rapidly than the surrounding gene sequences even though the spacer contains the regulatory sequences for 18S and 26S transcription. In this study, the spacer region has been cloned from a more diverse group of Triticeae species representing the R, S, P, E, N and J_1J_2 genomes. These grasses include both annual and perennial species.

Considerable polymorphism in spacer length exists both within and between the species. Much of the variation can be explained by variation in the number of 100-200 bp tandemly arranged subrepeats located in the spacer. A number of subrepeats have been isolated and sequenced for each of these grasses and for wheat. The sequences reveal overall similarity with some regions showing a much higher level of nucleotide sequence conservation than others. Despite this sequence homology, probes from this region hybridise preferentially to the genome of origin and can be used to detect alien chromatin carrying rDNA loci when introduced into wheat.

A 1 kb region spanning the spacer and 18S junction has also been cloned and sequenced for these grasses. The 18S gene region sequenced is perfectly conserved in the 7 grasses examined. 5' to the gene, a 9 bp region is perfectly conserved before an abrupt transition to less highly conserved spacer sequences. Within this section of the spacer, other regions of up to 40 bp show almost perfect homology, while the surrounding sequences exhibit less homology.

The start of transcription site was identified by comparison with published maize sequences and is perfectly conserved between wheat, rye and maize. This sequence (GGTATAGTAGGG) is also recognisable in sea urchin and provides a useful reference point.

The data also allows conclusions to be made about the relationships between the genomes as we expect greater homology between more closely related genomes.

SESSION 2B

VARIABILITY AND GENETICS OF rDNA SPACER SEGMENTS IN AUSTRALIAN WHEATS

C.E. May[1] and R. Appels[2]

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Using restriction enzyme digests of genomic DNA extracted from the leaves of twenty-five hexaploid wheat (*Triticum aestivum* L. em Thell) cultivars and their hybrids, fragment-length polymorphisms of the spacer DNA that separates the highly repeated ribosomal-RNA genes of wheat have been examined. The bulk of these genes are borne on chromosomes 1B and 6B of hexaploid wheat. We show that there are three distinct alleles of the 1B locus, designated *Nor1a, Nor1b* and *Nor1c,* and at least five allelic variants of the 6B locus, designated *Nor2a, Nor2b, Nor2c, Nor2d* and *Nor2e*. Chromosome 5D has only one spacer variant (*Nor3*). Whereas the 1B alleles differ only by the loss of one or two of the 130 bp sub-repeat units within the spacer DNA, the 6B allelic variants show major differences in their composition and lengths. The evolutionary and practical implications of these differences are discussed.

Introduction to the Molecular, Biochemical and Population Genetics of Esterase 6 in *Drosophila melanogaster*

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Esterase 6 (EST6) is the major β carboxyl esterase of *D. melanogaster* and most of its sibling species. In *D. melanogaster* and *D. simulans* it is synthesised in the sperm ejaculatory duct of the adult male and transferred to the female during mating. Once in the female it stimulates sperm utilisation and egg laying and delays receptivity to remating. However, in *D. yakuba* EST6 is not localised to the reproductive tract, in *D. teissieri* the enzyme is not specific to males and *D. seychellia* lacks any EST6 activity at all.

D. melanogaster and *D. simulans* are both polymorphic for the same two major EST6 allozymes. Frequencies of these allozymes vary clinally with latitude and the directions of the clines are consistent across the two species and the three continents sampled. The scale and repeatability of the clines can only reasonably be explained by the action of natural selection.

Clones of the EST6 gene have been isolated as the first step in a molecular analysis of the structure, regulation and evolution of the gene. The cloning strategy involved: purification of EST6 protein, sequencing five tryptic peptides from the protein, synthesis of corresponding oligonucleotides, and screening of genomic and cDNA libraries with the oligonucleotides. Two clones hybridising to the probe were isolated which mapped *in situ* to the chromosomal location of EST6. Four other clones were isolated during the screen, two each of which mapped to the locations of the other major esterases of *D. melanogaster*, Esterase C (ESTC) and Acetylcholinesterase (ACE). Papers below describe experiments confirming the identity of the putative EST6 clones and using them for molecular analyses of the structural and regulatory EST6 polymorphisms. The putative ESTC and ACE clones have yet to be characterised further.

Esterase 6: A Molecular Analysis of the Gene

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The evidence for the cloning of the Esterase 6 gene and a molecular analysis of the RNA transcripts and the gene structure will be presented.

The paper by Oakeshott and Richmond above outlines the procedure by which cDNA clones were isolated which hybridised to synthetic oligonucleotides complementary to peptides from purified EST6 protein. We believe that these clones contain *Est6* sequences for the following reasons: 1. The two cDNA clones were isolated on the basis of homology to the protein sequence. 2. The clones hybridise in-situ to the correct chromosomal location. 3. The developmental profile of the RNA transcripts hybridizing to the clones is identical to that of the Esterase 6 protein. 4. Esterase 6 null flies lack the RNA transcripts and have a B104 transposable element inserted in the gene. 5. Tryptic peptides of EST6 not used in the oligonucleotide construction have been identified in the translated cDNA sequence.

Northern blotting reveals the presence of two transcripts of sizes 1.83kb and 1.68kb and both follow identical developmental profiles. The region of homology to the cDNA clones has been localised to a 2.3kb EcoR - Hind fragment of genomic DNA. The direction of transcription was determined using riboprobes in the Northern analysis and from the cDNA sequence.

Sequencing of the two cDNA clones suggests that the difference between the two transcripts is the result of differential RNA splicing of internal sequences within the 3' half of the gene.

Partial sequencing of the genomic DNA has revealed the presence of an intron of 52bp approximately 400bp from the 3' end of the gene. Another gene begins almost immediately 3' of Esterase 6 and shows considerable DNA and amino acid homology to Esterase 6.

ALLOZYME AND NUCLEOTIDE VARIATION AT THE ESTERASE-6 LOCUS OF A NATURAL POPULATION OF DROSOPHILA MELANOGASTER

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One hundred and fifty-seven third chromosome lines isoallelic for Est-6 were extracted from a natural population of Drosophila melanogaster from Coffs Harbour, N.S.W. Est-6 electrophoretic class was determined by using high resolution cellulose acetate electrophoretic plates. Ten allozyme classes were resolved from the sample where only four had been evident from traditional electrophoretic procedures. Genetic analyses confirmed that the additional electrophoretic variation mapped at or near the Est-6 locus. Two of the new classes were associated with significantly higher third chromosome viabilities than other classes, while another class was significantly associated with the linked polymorphic inversion In(3L)P.

The cellulose acetate plates also were used to score 14 D. melanogaster lines previously shown to represent six different Est-6 alleles on the basis of differences in the in vitro thermostability of the EST-6 protein. Allelic classes determined by the two criteria, i.e. electrophoresis and thermostability, were largely uncorrelated. This implies an even larger degree of variability at the Est-6 locus than that detected by either criterion alone.

Est-6 clones have been isolated from genomic libraries made from four of the electrophoretic classes. Clones from two of these classes have now been partially sequenced. The nucleotide polymorphism revealed is discussed in relation to the electrophoretic and thermostability variation at the protein level.

Characterization of a Suppressor of stoned mutant of Drosophila.

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A supressor of the stoned allele, stn^{ts2}, has been isolated in Drosophila melanogaster. Genetic characterization of this suppressor, su(stn) , has been achieved. The study revealed a number of interesting phenomena. It has been shown that the supressor allele, su(stn), is co-dominant with the $su(stn)^{+}$, wild-type allele, and the supressor gene maps to the X chromosome between the markers singed and ocelliless (approx. map position 22.0). The suppressor appears to suppress both the behavioral component of the stn phenotype and the reduced viability of the stoned alleles. Studies of the su(stn) - stn interactions suggest that that the suppressor acts as a metabolic rather than a genetic suppressor. The suppression mechanism is proposed as involving the accomodation of the reduced or missing function in the stn mutants.

Restriction fragment length polymorphisms between wheat and barley

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Restriction fragment length polymorphisms (RFLPs) have been proposed as the new generation of genetic markers for plant breeding. Wheat, *Triticum aestivum*, and Rye, *Secale cereale*, cDNAs from seeds and leaves have been employed as probes to identify RFLP between wheat and barley, *Hordeum vulgare*. The six barley-wheat disomic addition lines have been used to identify the chromosomal location of the barley markers.

SESSION 3B

ACTION OF *Ph* GENE ON SYNAPTONEMAL COMPLEX FORMATION IN WHEAT X *AEGILOPS* HYBRIDS

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Bivalent pairing at metaphase I in allohexaploid wheat (*Triticum aestivum*, 2n=6x=42) is under the control of a gene designated *Ph*, located on chromosome 5B. In the presence of *Ph*, 21 bivalents are found at metaphase I, as a result of pairing and crossing over between strictly homologous chromosomes - 7 bivalents from each of the A, B and D genomes. A mutant allele *ph* has been described which, when homozygous, results in the occurrence at metaphase I of homoeologously paired multivalents.

When wheat carrying either the *Ph* or *ph* allele is crossed with *Aegilops peregrina* (=*T. kotschyi* = *A. variabilis*, 2n=28), the presence of the *Ph* allele in the hybrid results in almost exclusively univalent formation at metaphase I. In hybrids carrying the *ph* allele there are variable amounts of metaphase I pairing - as few as 6 univalents, plus bivalents, trivalents and occasional higher multivalents. Investigation of pachytene pairing in these hybrids has been carried out by electron microscopic examination of surface spread nuclei. Extensive synaptonemal complex formation has been found in both genotypes, probably involving both intra- and interchromosomal pairing. Limited qualitative and quantitative differences in pachytene pairing are suggested between the two genotypes. The relationship of the pachytene and metaphase I behaviours will be discussed, together with its bearing on various hypotheses about the mode of action of the *Ph* gene.

DETECTION OF PLANT LUTEOVIRUSES

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Luteoviruses are a group of phloem limited virus pathogens of plants. Barley yellow dwarf virus is the type member of the group. Infected plants show stunting and yellowing, but symptoms alone are not sufficient for accurate diagnosis. ELISA assays are presently the most reliable, routine system for diagnosis. Nucleic acid probes from cloned segments of luteovirus genomes have the potential to provide unlimited supplies of standardized reagents for diagnostic purposes.

Genome fragments from two serotypes of BYDV have been cloned and tested as hybridization probes. Dot blot assays using plasmid probes detect BYDV in crude extracts of infected plants at a level at least as sensitive as ELISA. Some probe sequences are specific for distinct serotypes. Others hybridize more generally with RNA of both serotypes and other luteoviruses. These could be used for the general diagnosis of infection by members of the luteovirus group, and have been used to verify GPV, a new serotype of barley yellow dwarf virus.

COLCHICINE MUTAGENESIS IN COTTON

D.J. Luckett

CSIRO Cotton Research Unit, Narrabri

Heritable mutations produced by the application of colchicine to the apical meristems of young seedlings have been reported in four crop species: sorghum, flax, barley and sunflower.

Since the production of somaclonal variants in cotton (*Gossypium hirsutum*) is difficult and the facilities are not presently available in Australia, an attempt was made to produce novel genetic variants for incorporation in our plant breeding programmes using the colchicine technique.

The response of cotton to the treatment (1% colchicine in lanolin applied to the shoot apex of 4-day old seedlings) was very similar to that in the other reported species. Of 293 treated individuals of cv. Sicot 1, 64 (22%) survived to produce flowering shoots which yielded viable C1 seeds. 84 C1 single-boll families were raised in the field, 18 of which were selected for extreme character expression and grown again as spaced C2 plants the following season. Although no gross morphological mutants were produced, considerable heritable variation was created in quantitative characters of economic importance; yield and fibre quality. The range of the mutant types was large enough to make this technique useful as a plant breeding tool in cotton.

PROTEIN-DNA INTERACTIONS IN THE CONTROL OF RAT GROWTH HORMONE GENE EXPRESSION.

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Growth hormone (GH) gene expression is confined almost exclusively to the anteriory pituitary gland and, in the rat (r), is induced by glucocorticoid and thyroid (T_3) hormones. Amongst cultured cells, this regulated expression of the rGH gene is retained only in the rat pituitary tumour cell-lines, GH and GC. Our studies have shown that elements responsible for tissue-specific expression and also hormonal regulation are contained within DNA sequences flanking the 5' end of the rGH gene. We prepared nuclear extracts from GC and other cell-lines and, using a DNaseI protection assay ('footprinting'), we examined the binding of factors in these nuclear extracts to 5'-flanking DNA of GH genes. The functional role of factor binding sites was assessed through either the deletion or specific mutation of these sequences. These mutants, as well as wild-type promoter sequences, were ligated to a marker gene (chloramphenicol acetyl transferase, CAT) and transfected into GC cells by calcium phosphate co-precipitation which were then assayed for transient expression of CAT acctivity.

Using nuclear extracts from GC cells, two sites in the rGH 5'-flanking DNA were found to be protected from DNaseI digestion. One protected region occurred between nucleotides -97 and -62 (numbered backwards from the cap site), while the other was found between nucleotides -139 and -109. The putative factors binding to these sites were named GC-1 and GC-2, respectively. These factors were thought to be unique to GC cells since nuclear extracts from HeLa (human cervical carcinoma) and Rat-2 (rat fibroblasts) afforded no protection from DNaseI cleavage. Using a nuclear extract from rat liver (a rich source of T_3 receptor) an additional site, not protected by nuclear extracts from GC cells, was detected further upstream between nucleotides -327 and -304. The corresponding factor was named RL-1.

Analysis of transient expression from deletion mutants of the rGH promoter showed that the GC-2, but not the GC-1 binding site was required to maintain basal levels of transcription. Site-directed mutagenesis of the GC-1 binding site showed that it was required for the full induction afforded by T_3, but not glucocorticoids.

Deletion of the RL-1 binding site led to an almost two-fold increase in transient expression, suggesting that this sequence, or sequences in its vicinity may mediate a repressor function. Site-directed mutagenesis is being performed on the RL-1 binding site in order to investigate its role in the regulation of rGH expression and its possible interaction with the T_3 receptor.

SESSION 3C

NUCLEOLAR ORGANIZER ACTIVITY AND SECONDARY CONSTRICTION STRUCTURE IN LITORIA (AMPHIBIA).

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Previous C-banding studies have shown that five distinct secondary constriction classes are recognizeable in the Amphibian genus Litoria. This paper presents the results of further studies on some of these species using both G-banding and the in situ hybridization of 18S+26S ribosomal cistrons derived from Xenopus. This analysis shows us that four of the secondary constriction types are nucleolus organizers. These have most unusual characteristics with some being highly concervative in their form, and others varying between species, and also varying between cells within individuals. The remaining constriction type appears to be induced by the presence of heterochromatin and its direct affect on the chromosomal substructure of adjacent regions.

NUCLEAR DNA VALUES AND C-BAND HETEROCHROMATIN OF AUSTRALIAN MYOBATRACHID FROGS

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Nuclear DNA values showed a large range in Australian Myobatrachid frogs, from a low value of 1.9 picograms to a high of 38 pg. The median value for the family was between 7 and 8 pg. This median value is similar to that reported for several other anuran families, but the number of species with high values is unusual. Excluding increases due to polyploidy, the range observed in the myobatrachids is greater than for any other anuran family, but is well below the range reported in urodeles.

Although nuclear DNA values differed considerably, the diploid number and chromosome relative lengths of the various species were conservative. An overall uniformity of C-band pattern was observed; centromeric bands occurred on all chromosomes; interstitial bands were common; telomeric bands occurred, but not on all chromosomes; large blocks or whole C-banded chromosome arms were uncommon. While the location of C-bands was very similar in many species the size of the bands differed. Those species with large bands showed high nuclear DNA values. It is proposed that increases in nuclear DNA value were partially due to increases by addition or duplication of the C-band regions, without changing chromosome morphology. However, increases in nuclear DNA could not be wholly explained by these means. The possible functional significance of variation in nuclear DNA value during development will be considered.

SUPERNODULATION MUTANTS OF SOYBEAN: HOST GENETIC ANALYSIS OF SYMBIOTIC NITROGEN FIXATION

Peter M. Gresshoff, Angela C. Delves, Arno J. Krotzky, Bernard J. Carroll and David A. Day

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We have used induced mutagenesis (EMS) and M2 family selection in soybean (Glycine max cult. Bragg) to isolate a range of symbiotically altered mutants (1, 2, 4). Three non-nodulation mutants (nod49, nod139 and nod772) were isolated (4). Inheritance was by a single recessive gene. The phenotype is associated with a non-diffusable root factor. Mutants nod49 and nod139 lack curled roothairs and subcortical cell divisions. Mutant nod772 has few curled root hairs. Genetic complementation analysis has been completed. The second class of mutant shows a nitrate tolerant symbiosis (nts) (1, 2). A total of 15 nts mutants were isolated; 12 were studied in more detail. The nitrate tolerance of nodulation (up to 1000 nodules per plant in the presence of nitrate) is caused by a mutational alteration of the autoregulation mechanism. They thus are termed to be supernodulation mutants. In all tested cases the genotype of the shoot controls the phenotype of the root (3, 6). Genetic complementation analysis suggests at least three complementation groups. We have been able to suppress supernodulation phenotypically (through the shoot application of phytohormones) and genetically (through a recessive suppressor gene). Growth analysis suggests that high supernodulation restricts plant growth (5). Growth of mutant nts382 in the absence of inoculum on nitrate shows similar growth parameters as wildtype, suggesting that the mutation has only insignificant effects on plant fitness in the absence of nodulation. Moderate supernodulation mutants (like nts1116) give good seed yields. Preliminary tests suggest that some nts mutants have acid soil tolerance. Future work will focus on the gene products of the described mutant genes. Combined biochemical, genetical and molecular analysis is hoped to extend our insight into this important symbiotic interaction.

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EXTREMES OF CLIMATE AND GENETIC HETEROGENEITY IN THE DIPTERAN SPECIES *DROSOPHILA MELANOGASTER*

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Cold and desiccation tolerance varied among populations of *Drosophila melanogaster* collected from the climatically different habitats of Melbourne in the temperate zone, Townsville in the humid subtropics and, Darwin in the wet/dry tropics. The following relative rankings of the populations were predictable from climatic origins and were found.

Cold tolerance: Melbourne > Townsville > Darwin.
Desiccation tolerance: Melbourne, Darwin > Townsville.

A random sample of 15 isofemale strains per population were tested. For both characters, population differentiation was repeatable across trials, and there was significant heterogeneity within populations. For the Melbourne sample, in 6 between-trial comparisons, a significant positive correlation between stresses was not found, indicating that tolerance to cold and desiccation are distinct ecological characters of importance in the determination of distribution patterns.

INCOMPATIBILITY AMONG *DROSOPHILA* POPULATIONS: FURTHER EXAMPLES AND INHERITANCE PATTERNS

Ary A. Hoffmann

La Trobe University

Previous work has indicated the presence of unidirectional incompatibility among two Californian populations of *Drosophila simulans*. Crosses between males from a Riverside (R) population and females from a Watsonville (W) population yield only a few progeny. This incompatibility was maternally inherited over one generation and "curable" by tetracycline treatment.

Additional sampling of *D.simulans* populations from America and Australia indicate that most populations are of the W type. Repeated backcrossing suggests that the ability of males to cause incompatibility in W females is maternally inherited over several generations, but that female susceptibility to R males is not always maternally inherited. The implications of these results and those from other experiments for the maintenance of the two compatibility types will be discussed.

Preliminary results from crosses among *Drosophila melanogaster* populations will be presented. These suggest that unidirectional incompatibility which is curable by tetracycline also occurs in this species.

GEOGRAPHIC VARIATION IN DROSOPHILA BUZZATII - DRIFT, MIGRATION, OR SELECTION?

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Department of Animal Science
University of New England

Patterns of geographic variation in allele frequencies within a species may provide valuable information on past and current evolutionary forces affecting the genetic structure of the species. Using spatial autocorrelation methods and an analytical technique new to population biology (directional spatial autocorrelation analyses - Oden and Sokal 1986), the geographic variation of allozyme frequencies in Drosophila buzzatii in eastern Australia has been analyzed and the results used to infer the population biological processes that have given rise to the observed patterns.

The data were 12 allozyme frequencies (6 loci - one allele omitted for each locus) from 57 localities. In spatial autocorrelation analyses, 6 alleles show significant spatial structure, but there was essentially no correlation between allele frequency surfaces. However, this analysis cannot show the directionality of any spatial variation pattern - hence the introduction and use of directional spatial autocorrelation. There was substantial correspondence between the results of the two analyses, with 5 of the same 6 alleles showing significant two-dimensional spatial patterns. Thus of the 12 alleles, 4 lack spatial patterns altogether, 3 show patterns that are difficult to interpret, and 5 show significant spatial variation, but with patterns that vary in directionality.

The clear continent-wide patterns rule out genetic drift as a tenable interpretation of the significant genetic heterogeneity among localities. The absence of correlations among loci across localities is evidence against two or more loci tracking the same environmental gradient or participating in the same recent migration wave across the continent.

Differential selection must be affecting at least some of the allozyme frequencies, and this selection is interpreted as operating on different spatial scales for different alleles - from continental to strictly local.

(Work done in collaboration with R.R. Sokal, State University of New York, Stony Brook, and N.L. Oden, Brookhaven National Laboratory.)

SIZE AND SHAPE IN NATURAL AND LABORATORY POPULATIONS OF *DROSOPHILA BUZZATII*

R.H. Thomas

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Several populations of *Drosophila buzzatii* were examined for patterns of within and between population variation in body size and shape measures, following work initiated by F.W. Robertson. Two objectives in this work are, firstly, to determine the nature and extent of genetic variation in quantitative traits and, secondly, to use patterns of phenotypic variation to provide information on environmental variation on a scale perceived by the flies.

Two populations (Brisbane and Hunter Valley) were examined in the laboratory at 18° and 25°C. Patterns of heritabilities and genetic correlations were similar between the two populations. However, significant genotype-environment interactions were detected only in the Hunter Valley population. Possible explanations for this result are discussed.

Patterns of phenotypic variation in wild and laboratory populations were examined to give estimates of temperature during larval development and degree of nutritional stress experienced by larvae. These methods allow measurement of fine-scale temporal and spatial environmental variation. This is the scale at which processes are apparently operating to maintain allozyme variation.

SPATIAL PATTERNS AND POPULATIONS

J. A. Stoddart

AUSTRALIAN INSTITUTE OF MARINE SCIENCE

As a result of their defining populations more by sampling than by theory, population genetics studies are sometimes unable to address significant ecological problems. The use of models to suggest appropriate spatial and temporal scales for sampling is discussed in conjunction with a study in progress which examines geographic patterns in genetic variation in the reef coral Acropora digitifera. In this species, small scale recruitment processes produce misleading results in estimates of gene flow made at larger scales.

CHROMOSOMAL MAPPING OF THE PHYTOPATHOGEN PSEUDOMONAS SYRINGAE pv SYRINGAE PS224

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The IncP-10 plasmid R91-5 and its derivative pMO22 (R91-5::Tn501) mobilise the chromosome of Pseudomonas syringae pv. syringae PS224. Chromosomal markers are mobilised by these donors at frequencies up to 10^{-2} per donor. An interrupted mating system has been established and time of entry studies have revealed nine different donor origins. Twenty-seven auxotrophic markers and three catabolic markers have been mapped by time of entry. Twenty-one PS224 markers were found to be functionally equivalent to characterized P. aeruginosa PAO or P. putida PPN markers by IncP-1 R prime complementation

A Tn5 mutagenesis system utilizing pMO75 (R91-5::Tn5) has allowed the isolation of auxotrophic and catabolic Tn5 mutants. From 13,450 kanamycin resistant exconjugants, a total of 83 stable auxotrophic mutants were isolated (0.6%). The following Tn5 auxotrophic phenotypes have been obtained: Arg, Cys, His, Ilv, Leu, Met, Phe, Pro, Pur, Pyr and Trp. In addition eleven catabolic mutants were obtained from 1,250 Km^r exconjugants (0.9%). Southern hybridizations of EcoRI digested DNA from four Tn5 auxotrophs, including two Cys auxotrophs, and one Tn5 catabolic mutant, revealed unique fragments when probed with pBR322::Tn5. The size of the fragments ranged from 9-21 kb. This data indicates that Tn5 insertion is random and most likely involves a single Tn5 element.

MECHANISM OF PLASMID INTEGRATION IN PSEUDOMONAS PUTIDA PPN

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The IncP-10 plasmid R91-5 is unable to maintain itself in Pseudomonas putida PPN, although it has apparently normal transfer functions. Introduction of pM075 (R91-5::Tn5) into PPN chromosome::Tn5 derivatives resulted in two classes of Hfr donor strains. Each class transferred chromosomal DNA from the site of the chm::Tn5 insertion, but in opposite directions. A model will be presented in which both Hfr classes are explained by homologous recombination between the plasmid and chromosomal Tn5 elements.

Southern blot analysis of pairs of Hfrs derived from different chromosomal Tn5 inserts showed that, as predicted by the model, pM075 had integrated at the site of chromosomal Tn5 insertion, resulting in R91-5 DNA flanked by Tn5 elements. Furthermore, the two Hfr classes differed in the orientation of integrated R91-5 DNA and in the orientation of the flanking Tn5 elements. The genetic consequences of this work will be discussed.

CLONING OF CAMPYLOBACTER JEJUNI ANTIGEN GENES IN ESCHERICHIA COLI

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The bacterium Campylobacter jejuni causes severe diarrhoeal disease in man. SDS-PAGE profiles of membrane proteins of the organism showed that it had a unique array of membrane proteins. Therefore it was decided to clone genes for those unique proteins with a view to developing specific DNA probes for rapid and reliable identification of bacterium.

To this end a genomic library of C. jejuni was constructed in Escherichia coli using plasmid pBR322 as the vector. The clones were then screened by Western blotting using antisera raised to whole cells of C. jejuni and to outer membrane proteins. Eight recombinant plasmids were selected. Physical maps of three were constructed. Using Southern hybridization these recombinant plasmids were tested for their specificity as probes to a number of C. jejuni isolates and to other Campylobacter and non-Campylobacter species likely to occur in the same clinical environment. Data pertinent to the use of these probes will be presented.

CLONING AND FINE STRUCTURE ANALYSIS OF IMMUNITY AND STRUCTURAL GENES OF COLICIN V.

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The immunity and structural genes of colicin V from a plasmid pCol V-K30 were cloned into pBR 322 and pACYC 184 and renamed as pHK 11 and pHK 22 respectively. Insertion of transposon Tn_5 followed by selection and *in vivo* complementation confirmed three complementation groups which were further analysed by restriction maps. Two loci were involved in the production of colicin and one in conferring immunity. The DNA sequence was also analysed.

REGULATION OF THE aroFtyrA OPERON OF ESCHERICHIA COLI

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The *aroFtyrA* 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. Two such sequences, known as TYR R Boxes, have been identified in the *aroF* promoter region and a third possible TYR R Box has been postulated.

This paper describes the isolation and characterization of *aroF* regulatory mutants which are no longer repressed by *tyrR+*. The *aroF* promoter region has been fused to the structural gene for chloramphenicol acetyl transferase (*cat*) which confers resistance to chloramphenicol on the cell. The level of resistance is repressed in the presence of *tyrR+*. This fusion has been used to select for regulatory mutants with increased resistance to chloramphenicol in the presence of *tyrR+*. Nucleotide sequencing of the these mutants has confirmed that three TYR R Boxes are required for repression of *aroF* by *tyrR+*.

INDEPENDENT CONTROL OF CHROMOSOMAL AND SPINDLE CYCLES: THE RELEVANCE TO ERRORS OF DIVISION

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In the cell cycle, cytoplasmic and nuclear events appear to occur synchronously. Such synchrony is particularly evident in the cycles of chromosome replication/condensation and spindle polymerization/organization. In a series of experiments, attempts were made to disturb the synchrony of the chromosomal and spindle cycles in 3 ways:

1. Cultures were treated with the DNA antagonist methotrexate (10^{-7} M for 18 hours followed by washing and a further 5 hours culture in normal media) in order to retard chromosome replication. Under such conditions, cytoplasmic events do not seem to be retarded.

2. Spindles were treated with microtubule inhibitors, either colcemid (0.03ug/ml) or nocodazole (10^{-7} M) to effect reversible metaphase arrest. Under such conditions, chromosomal replication is unaffected.

3. Cells were induced to enter anaphase precociously. This was achieved by treating actively growing cultures with warm hypotonic solution. In each of 3 different hypotonic solutions, dilute media (10%), 0.075 M KCl and 0.08 % NaCl, the number of anaphase cells was doubled after 1 minute.

The first of the 3 treatments resulted in large spindles which were very frequently multipolar. Further culture of these cells resulted in rapid changes in chromosome number. The second treatment led to endomitosis and subsequent polyploidization and tetraploidy. Aneuploidy was frequently found in the cells which had not been arrested in metaphase by the treatment. Cells exposed to the hypotonic solutions were examined at anaphase or telophase for lagging chromosomes or other aberrations. No difference in the number of aberrant divisions was noted between the control and the experimental cultures.

These data show that the spindle and chromosomal cycles, although usually synchronous, are under independent regulation. Sustained disturbances in the synchrony result in dramatic changes in chromosome number. The results give some insight into the different roles of chromosomes and spindles in maintaining regular chromosome segregation. They also suggest means of loss of control in pathological conditions such as invasive cancer.

SESSION 4C

CYTOGENETIC ANALYSIS OF CLONAL EVOLUTION IN HUMAN OVARIAN CARCINOMA

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Tumour progression over time involves the sequential selection of mutant subpopulations. Although some characteristics of tumours are relatively constant, the selective pressures of varied local conditions result in marked heterogeneity for others.

Cytogenetic studies of solid tumour tissue have revealed a high level of heterogeneity. Analysis of chromosomal rearrangement/breakage as well as aneuploidy may provide information about the degree of genetic instability in a tumour. Sequential studies over time would provide data concerning tumour progression and the role which karyotypic alterations play in tumour evolution.

An alternative method of analysis is to order the chromosomal rearrangements seen at any one time, according to their frequency. Thus, a rearrangement seen in all cells must have occurred before one which is present in only 50% of cells. Using these data, a pattern of clonal evolution may be inferred which allows comparison of the development, not only of tumours of similar type but in different patients but also of different metastatic sites of the same tumour. In patients with multifocal coelomic tumours, cytogenetic evolution may give a measure of the degree of heterogeneity at each site and thereby identify older tumour sites.

Amplification of specific rDNA sequences in a T-cell leukaemia line

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Human ribosomal RNA genes are polymorphic for at least five length variants. The variation is localized to one region of the non-transcribed spacer. Tandem copies of ribosomal RNA genes are found at five sites in the human genome - on the NORs of acrocentric chromosomes 13, 14, 15, 21 and 22. Individuals always carry more than one repeat length.

Family studies have indicated that specific repeats are not necessarily localized to specific chromosomes. However analysis is difficult because of complex polymorphic patterns. In an alternative approach single human NOR chromosomes have been investigated in somatic cell hybrids. In these, usually only one length variant can be detected per NOR. However, specific length variants are apparently not chromosome specific.

We have investigated an amplified NOR which was detected in a cell line derived from CCRF-CEM, a human T-cell leukaemia line. In this sub-line the NOR on chromosome 14 is present in four tandem copies each carrying rDNA genes as shown by in situ hybridization. Looking at rDNA length variants, the third shortest variant is the most abundant in the ancestral line. However in the 14p+ line the shortest repeat is now by far the most frequent.

This result supports studies from somatic cell hybrids. It seems that one chromosome 14 in CCRF-CEM carries predominantly the shortest length variant and this has been preferentially amplified in the derived line. Also, it is of interest that it is not the same variant as that located on another chromosome 14 studied in somatic cell hybrids.

IN SITU HYBRIDISATION OF REPEATED SEQUENCES TO HUMAN METAPHASE CHROMOSOMES USING BROMODEOXYURIDINE-LABELLED PROBES

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Human highly-repeated satellite sequences cloned into M13 were used as templates to prepare single-stranded DNA probes containing bromodeoxyuridine in place of thymine. The probes were hybridised to human metaphase chromosome spreads under standard hybridisation conditions and excess probe was washed off at various levels of stringency. Bound probe was visualised using an indirect immunological detection procedure. First, the hybridised preparations were reacted with a monoclonal antibody against bromodeoxyuridine. The slides were then incubated with a peroxidase-conjugated polyclonal antibody and with peroxidase-antiperoxidase complex. Peroxidase activity was located by reacting with DAB to yield a strong, brown precipitate at the site of activity. The slides were finally mounted in a permanent medium.

The procedure is reliable and fast (the washing and detection steps can be carried out in an eight hour working day). The sensitivity is similar to that of other in situ hybridisation techniques, but the resolution is very high, allowing accurate localisation of satellite sequences within heterochromatic regions. Slides have been kept at room temperature for several months with little or no fading of the DAB precipitate. We believe that this procedure should be very useful for detecting RNA transcripts in cells, and could be modified for detecting single-copy DNA.

HUMAN THYMIDINE PHOSPHORYLASE: SIGNIFICANCE AND RAPID ASSAY BY HPLC

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Nucleobase residues for cellular nucleic acid synthesis may be acquired by salvage or synthesised de novo. Several assays for genotoxicity (and, therefore, presumed carcinogenic and mutagenic potential) rely, with some justification, on the assumption that salvage is the principal source of nucleosides in mature mammalian tissues. In such assays the possible role of nucleoside catabolism in regulating the available nucleoside pool is usually overlooked.

Nucleoside concentrations in most mammalian tissues are regulated by reversible phosphorylytic reactions:

$$\text{nucleoside} + P_i \rightleftharpoons \text{nucleobase} + \text{pentose phosphate}$$

Using reverse-phase HPLC, we have developed a rapid, sensitive and inexpensive method to measure nucleoside phosphorylase activity in in human blood. The assay can be readily applied to ex vivo tissues other than blood and also to cells cultured in vitro.

Only deoxyribonucleoside-specific phosphorylase activity is detectable in peripheral blood obtained from normal human adults but preliminary results suggest that both enzyme activity and the extracellular nucleotide pool are altered in most leukaemias.

GENETIC AND MOLECULAR STUDIES OF DROSOPHILA DEVELOPMENT

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The systematic genetic analysis of development and the subsequent analysis of developmental genes isolated by recombinant DNA techniques, have resulted in major advances in our understanding of the regulation of animal development.

The most significant advances have been made in the study of genes which act as primary developmental switches in the processes of segment formation and differentiation in Drosophila melanogaster. Mutations at these loci result in disruption of segment formation or, in the case of the homeotic mutations, in the formation of normal structures in abnormal positions. Analysis of the cloned products of these genes shows they are expressed immediately before, and/or at the formation of the cellular blastoderm, in a metameric pattern (in the case of the segment genes), or in a series of anterior-posterior specific positions (in the case of the homeotic genes). Several of the homeotic and segment genes contain a highly conserved sequence termed the"homeobox". This sequence, which is also highly conserved in many complex metazoans including man, has characteristics of a DNA binding domain. The logical inference is that the homeobox genes and segment genes that contain this sequence are a group of DNA binding proteins which act as transcription factors regulating the expression of sets of other genes and thus act to trigger specific developmental pathways.

We have sought to extend the study of this gene family in Drosophila melanogaster by isolating DNA fragments homologous to a synthetic oligonucleotide encoding part of the conserved homeobox sequence. We have isolated four novel clones with varying degrees of homeobox homology. Characteristics of these clones in relation to other homeobox genes will be discussed.

Genetic Dissection of Learning in Drosophila.

L.E. Kelly.

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The development of a simple paradigm by Quinn and his colleagues, enabled the isolation of a number of X-linked learning mutants in Drosophila. Two of these mutants dunce and rutabaga, are of particular interest in that the molecular defects underlying their abnormal behavior have been characterized. Dunce appears to be the structural gene for an isozyme of cyclic nucleotide phosphodiesterase (PDE), whereas rutabaga alters the activity of the cAMP generating enzyme adenylate cyclase. These data along with the observation that mutants in the dopa decarboxlase (ddc) gene also cause learning defects, lend support to the model proposed by Kandel which suggests that the cellular processes of sensitization and habituation are the bases of learning and short term memory. Also implicit in these findings is that protein phosphorylation is involved in the learning /memory processes.

In our laboratory we have been taking two approaches to the understanding of learning and memory in Drosophila. The first of these involves the study of protein phosphorylation in mutants at the stoned locus, and the characterization of the stoned gene and its product. Mutations at the stoned locus show unusual hypersensitivity. The second approach involves the characterization of genes encoding the proteins that are integral parts of the Kandel model.

THE STRUCTURE, FUNCTION AND REGULATION OF THE WHITE AND SCARLET GENES OF DROSOPHILA MELANOGASTER

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Dept. Biochemistry, Faculty of Science, Aust. Natl. Univ.

Lesions in only four ommochrome pathway genes of D. melanogaster completely block the formation of pigment - vermilion (v), cinnabar (cn), white (w) and scarlet (st). The first two code for pathway enzymes and w and st affect the uptake of pigment precursors. The current major aim of this laboratory is to analyse and compare the structure, expression and regulation of these four genes. This talk addresses w and st.

The biochemical consequences of lesions at w and st and the temporal requirement for their gene products are virtually identical, suggesting a close relationship at either the regulatory or functional level. We have analysed clones carrying the st region and compared the sequences with those published for the w gene. The two genes show significant nucleotide and amino acid homology, suggesting a) derivation from a common ancestral gene and b) that their similar mutant phenotypes reflect shared functions. Hydropathy analysis of the amino acid sequences delineates membrane spanning regions in both proteins, and both also have a cytoplasmic anchor sequence at the carboxy-terminus. This membrane association is consistent with a role in precursor transport across the plasma membrane and possibly across intracellular membranes as well.

Quantitative and qualitative changes in the mRNAs have been detected for both genes. A lesion in one gene does not affect the transcription of the other, ruling out a regulatory relationship between w and st. Similarly lesions in several other eye colour genes have no major effect on the transcription of w and st.

Given that w and st are expressed in largely the same tissues we have also compared sequences of regions that may have regulatory function. Five small regions of homology 5' to the two genes have been detected and some of these are located at the sites of lesions in putative w and st regulatory mutations.

CHROMOSOME EVOLUTION IN MARSUPIALS.

D.L. Hayman

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An overview is given of the principal pathways of chromosome evolution in Marsupials. Evidence is presented for a conserved chromosome complement in many of the major marsupial taxonomic groups and for the derivation from this complement of the different chromosome complements present in other groups.

EVOLUTION OF MARSUPIAL GENE ARRANGEMENTS

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Comparisons of gene arrangements in different eutherian species have been used to construct independent phylogenies of a number of different groups and to reveal the course of genome evolution within and between groups. A combination of comparative gene mapping and cytology has revealed remarkable conservation of autosomal gene arrangement, making it possible to deduce gene arrangements in a common ancestor. This conservation is most striking for the X, which seems to have been entirely conserved in eutherian mammals, this has been proposed to be a consequence of its isolation by the X chromosome inactivation mechanism. Marsupials have evolved independently from eutherians for more than 130 million years; thus comparative gene mapping of marsupial can provide data particularly valuable for building up a "big picture" of mammalian genome evolution, since homology will reveal extremely ancient gene arrangements.

A major effort has been put into marsupial gene mapping using somatic cell genetic techniques, recently supplemented by molecular techniques; these studies complement the few data from classic genetic studies. In my laboratory, we have concentrated on the X chromosome in macropodid and dasyurid species, using hybrids between marsupial cells and HPRT-deficient rodent cells to establish synteny and to make chromosome assignments. This has been accomplished by correlating the expression of a gene, or its presence revealed by Southern blot analysis, with the expression of other genes or the retention of a particular chromosome. We have shown that the genes *Pgk*, *Gpd* and *Ags*, which are borne on the X in all eutherians, are also on the X in macropodid and dasyurid species.

However, two gene, *Sts* and *Otc*, which are X linked in eutherians, are excluded from the X in a number of marsupial species; *Otc* has recently been localised to an autosome by *in situ* hybridization. These two genes lie on the short arm of the human X. *Sts* is within the "pseudoautosomal" X-Y pairing region and is not subject to inactivation, while *Otc* is outside this region, and is inactivated. We suggest that a region within a larger pseudoautosomal segment was translocated from the X to an autosome early in the marsupial lineage.

This suggestion is consistent with our recently proposed model for a progressive differentiation of X and Y chromosomes, accompanied by a gradual spreading of inactivation.

EVOLUTION OF PHOSPHOGLYCERATE KINASE GENE SEQUENCES IN MARSUPIALS AND EUTHERIANS

Angela van Daal, P.L. Molloy, and D.W. Cooper

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There are two forms of the glycolytic enzyme phosphoglycerate kinase (E.C. 2.7.2.3, PGK). The first is a housekeeping enzyme found in all cells except sperm. It is controlled by an X-linked locus. The second is sperm-specific in eutherians and is the only form found in marsupial sperm. In marsupials it is also present at lower levels of activity in all other cells. It is autosomally inherited and in mouse has been found linked fairly closely to the MHC and t regions on chromosome 17. DNA sequences are available for five PGK genes: (1) the human X-linked functional gene, (2) the mouse X-linked functional gene, (3) a human X-linked pseudogene, (4) a human autosomal pseudogene, (5) and a kangaroo pseudogene derived from a Macropus robustus genomic library (see A. van Daal et al. this conference). We have used Felsenstein's maximum likelihood method for reconstructing phylogenies (DNAML) to examine the evolutionary relationships between these genes. The data suggest the hypothesis that the kangaroo gene is equidistant from the other four. Further data are needed to test this hypothesis. It will be interesting to attempt to relate the time of gene duplication - into the autosomal and X-linked forms - to the time of separation of the eutherian and marsupial lineages.

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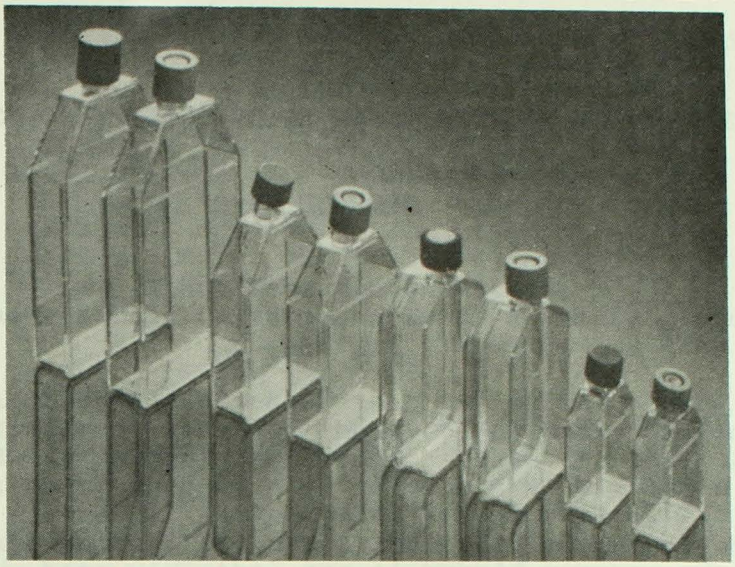

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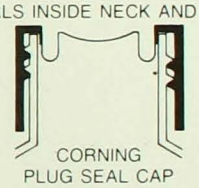

The black phenolic style caps are best for open culture systems requiring gas exchange with the environment. These use a traditional nontoxic liner for sealing the cap against the rim of the flask neck ensuring leak free use. With the black cap slightly loosened, gas exchange is permitted without the danger of a plug accidentally engaging and sealing off the culture from the environment. Many researchers also find they are easier to put on and take off than the plug seal caps.

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THE EFFECT OF INBREEDING IN QUEEN BEES ON HONEY PRODUCTION

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It has been demonstrated that in certain rare crosses between different lines of honey bees, honey production is increased by worker heterosis. However, the commercial exploitation of this kind of heterosis is difficult as it requires control of mating, which is expensive.

Another possibility for the exploitation of heterosis in honey bees is the use of hybrid queens. This is an attractive concept as matings need only be controlled in the production of breeding stock.

Fitness characters such as rate of oviposition tend to display heterosis. In bees, brood area (a character of queens; not workers) has been shown to have a high phenotypic correlation with honey production. Thus the use of hybrid queens might substantially increase yields.

In the present experiment, the performance of colonies headed by inbred queens was compared with that of colonies headed by queens resulting from the cross between those inbreds. The characters measured were brood area and weight gain. Significant increases in brood area were observed in the hybrid queen colonies. Colony weight gain (an index of honey production) was phenotypically correlated with brood area. Thus there is potential for increases in production by the use of hybrid queens.

TRANSLOCATION HETEROZYGOSITY AND SOCIALITY IN DELENA CANCERIDES

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The social huntsman spider *Delena cancerides* is composed of a number of distinct chromosomal races. The ancestral telocentric karyotype is found in Queensland and south-eastern Victoria. In other regions, however, wholesale centric fusion has resulted in a largely metacentric karyotype. In western Victoria, both males and females are homozygous for ten fusions, resulting in normal bivalent formation at meiosis. In contrast to this, populations in Victoria and NSW carry fusions in an obligate heterozygous state in the males, and consequently chromosome chains occur at meiosis. Two distinct races carrying sex-linked fusion heterozygosity have been identified, one with a complex of five chromosomes, and the other with nine.

Recent evidence indicates that the heterozygous races have resulted from chance hybridization between parental races homozygous for fusions. Since complex heterozygosity of this type also occurs in the social Isoptera, however, it is believed that this has been fixed and maintained in *Delena* for selective reasons relating to its social lifestyle.

MORPHOLOGY AND MATING SUCCESS IN A FROG HYBRID ZONE.

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The distributions of Litoria ewingi and L. paraewingi meet in a narrow hybrid zone north of the Great Dividing Range in central Victoria. The species are distinguished by one-way developmental incompatibility in experimental interspecific crosses. There is no other reliable method for identifying individuals at any stage of the life cycle. It has been postulated that, in spite of the developmental incompatibility, hybrids may be selectively superior in at least one part of the zone.

A discriminant analysis of morphometric data was used to classify adults from each locality as phenotypically most similar to individuals from the hybrid zone, or from allopatry of one or other species. These phenotypes were assumed to have some genetic basis; their frequencies showed seasonal variation which would result in some allochronic assortative mating without deliberate mate choice. Except for males at one locality in the hybrid zone, a high level of success at achieving amplexus was seen among individuals which were classified with samples from the hybrid zone. This pattern may be the result of active mate choice, and offers a partial explanation for the paradox that the northwestern end of the zone is a "hybrid swarm", despite the incompatibility of parental genomes.

A QUANTITATIVE GENETIC ANALYSIS OF LIFE-HISTORY VARIATION IN TWO POPULATIONS OF TRIBOLIUM CASTANEUM

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University of New England

A major contention of life-history theory is that the traits that compose an organism's life-history are coadapted and moulded by natural selection to solve a particular ecological problem. The biometrical approach of quantitative genetics is particularly appropriate for the study of life-history characters because these characters typically show continuous variation due to underlying polygenic determination.

A quantitative genetic analysis was performed on two populations of Tribolium castaneum derived from separate geographic locations. These populations having evolved under presumptively different selective regimes may have evolved different organizations of life-history traits.

A nested or hierarchal experiment using F_3 female descendents of wild caught beetles was done under two constant laboratory temperatures (33°C and 37°C) to examine the potential existence of genotype-environment interactions.

METHYLATION OF PGK ON THE ACTIVE AND INACTIVE X CHROMOSOME IN KANGAROOS

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In the female marsupial the X chromosome derived from the father becomes inactivated. This contrasts with eutherians where either the paternally or maternally derived X chromosome may be inactivated. In both cases, however, the inactivation is stable and is inherited through somatic cell generations. Methylation has been proposed as a means of maintaining the inactive X chromosome in that state, since the pattern of DNA methylation is copied during DNA replication. We are interested in the involvement of methylation in marsupial X chromosome inactivation.

A Macropus robustus EMBL4 EcoRI genomic library was screened with the human full length PGK cDNA clone pHPGK7e (gift of Dr. A. Michelson). Two cross-hybridising clones were isolated with 11.5 kb and 16 kb inserts. Both clones hybridised to almost the entire 1.3 kb coding region of the human cDNA clone. The region of hybridisation on both of the kangaroo genomic clones was shown to be approximately 1.2 kb which suggested that the clones contained PGK pseudogenes, or that the kangaroo PGK gene contained no introns. One of the clones was sequenced and demonstrated to be a pseudogene. It has a 25 amino acid region out of register caused by deletion of an A, followed 25 amino acids on by insertion of an A. At the amino acid level there is an 83% homology and at the nucleotide level an 82% homology between the kangaroo PGK pseudogene and the human PGK gene. This PGK pseudogene has been used to probe male and female kangaroo DNA digested with methylation sensitive enzymes for both eastern grey kangaroos (Macropus giganteus) and tammar wallabies (Macropus eugenii). In the 3' region of the X-linked PGK gene of both these species there is a CCGG site which is methylated on the active X, but which is not methylated on the inactive X chromosome. The methylation status at the 5' end of the gene is being investigated.

KANGAROO RED CELL ANTIGENS RECOGNISED BY MONOCLONAL ANTIBODIES TEND TO BE POLYMORPHIC

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Twenty-eight monoclonal antibody reagents were generated against red blood cells of four different animals from three different species of the family Macropodidae. These were used to type 381 animals from 24 species of the superfamily Macropodoidea. Three of the reagents (B, 1 and 18) were found in most species of the superfamily. The antigens recognised by these reagents were usually monomorphic within a species. By contrast the remaining reagents detected antigens found in a small number of species, within which they were frequently polymorphic. The mouse immune system from which the monoclonal agents are derived seems to react preferentially against polymorphic antigens.

Most species had their own characteristic antigenic repertoire. The comparison of typings between species, genera and families revealed phylogenetic relationships broadly equivalent to those currently accepted.

In the species Macropus robustus at least 6 different loci were found to be involved in separate blood group systems.

Apart from being useful in studying phylogenetic relationships the reagents have also proved to be useful for identification of individuals and studying hybrid zones and sympatric species.

GENE MAPPING IN MONOTREMES:

SYNTENY BETWEEN HPT AND PGK IN THE PLATYPUS

Jacki Wrigley and Jennifer A. Marshall Graves

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Comparative mapping studies of eutherian and metatherian chromosomes have revealed a surprising degree of synteny conservation between diverse mammalian groups. In particular the X chromsome appears to have benn conserved *in toto*, perhaps due to the mechanism of X-chromosome inactivation.

It is thus of great interest to extend mapping studies to the third group of mammals, the monotremes, which diverged from the therian line 150-200 million years ago. To this end HPRT- rodent cells and playtpus cells were fused and hybrids retaining platypus HPRT were selected. These hybrids also expressed platypus PGK, but not G6PD or GLA. Subclone analsysis indicates that Hpt and Pgk are syntenic in the platypus making this the most ancient mammalian syntenic group known.

Karyological analysis of the hybrids and their revertants do not reveal the chromosomal locations of the synteny group. However it permits the exclusion of chromosome 1 as a possible location.

MARSUPIAL-MARSUPIAL CELL HYBRIDS - AT LAST

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Over the years, a major effort has been made to map marsupial genes using rodent-marsupial and human-marsupial somatic cell hybrids. These hybrids have proved very difficult and tedious to analyse because they display extreme growth inhibition, and rapid and extreme chromosome fragmentation and segregation; problems that seem to characterize hybrids between cells from widely divergent species.

We have therefore sought to construct hybrids between cells of two marsupial species, in order to:

1) Provide hybrids with stable partial chromosome sets suitable for gene mapping.
2) Study, in an independent system, the empirical rules and the mechanism of chromosome segregation.

In order to construct marsupial-marsupial cell hybrids, it was necessary to derive at least one marsupial parent line carrying a selected marker. We have used an established swamp wallaby (*Macropus bicolor*) fibroblast line with a passable cloning efficiency, and selected and tested thioguamine-resistant, phenotypically HPRT-dificient variants, choosing one which is stable and has a low reversion rate in HAT medium. We have fused this with wild type lymphocytes and fibroblasts from brushtail possum (*Trichosurus vulpecula*) and a near diploid *Dasykaluta rosamondae* fibroblast line, and selected hybrids which are detected cytologically. Swamp wallaby-possum *M.bicolor* X *T.vulpecula* cell hybrids initially retained virtually intact chromosome sets from both parents, but have undergone progressive loss of possum chromosomes; thus they seem to have characteristics suitable for gene mapping.

A MOLECULAR HYPOTHESIS FOR POSITION-EFFECT VARIEGATION: TRANSCRIPTION OF ANTI-SENSE RNA

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Position-effect variegation is the mosaic expression of a gene lying near a breakpoint of a chromosomal rearrangement. There is currently no comprehensive hypothesis to account for this phenomenon.

I propose that position-effect variegation is due to the transcription of anti-sense RNA that hybridises to and thereby inactivates sense mRNA transcripts. The anti-sense transcription is induced as a consequence of a chromosomal rearrangement that places the anti-sense strand next to a promoter.

There is extensive experimental evidence for three of the assumptions inherent in this hypothesis, namely that anti-sense RNA can inactivate sense mRNA, that position-effect variegation results from changes in the position of genes, and that transcription is affected in position-effect variegation "mutations".

This hypothesis accounts for the observed features of position-effect variegation, including the variegated phenotype and the occurrence of both dominant and recessive variegating "mutations". The hypothesis leads to the novel predictions (1) that position-effect variegating "mutations" will contain sense mRNA transcripts, anti-sense RNA for affected loci, and hybrid sense mRNA/antisense RNA molecules, (2) that the extent of the "spreading effect" will depend on the length of the anti-sense RNA, and (3) that the breakpoints in position-effect variegation "mutations" will be in strictly defined positions.

del, a dispersed DNA sequence in Lilium

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We are studying one catgegory of abundantly repeated sequence from the very large genome of Lilium henryi. Initially a rapidly reannealing probe (C_0t < 1 Ms) was used to identify abundant fragments released from genomic DNA digested with BamHI. A sample of fragments were cloned, and their dispersion throughout the genome demonstrated by in situ hybridization. They were shown to arise from subregions of a larger sequence which we have named del (after dispersed element of lilies).

del units have been identified and characterized from a partial genomic library of L. henryi. Three clear arrangements have been observed. The most frequent is a full 8.5 kbp unit which has long terminal direct repeats of about 1,500 bp. Less frequently, 1,500 bp termini have been found in isolation. Also a clone containing only del in tandem arrays has been observed.

Sequencing of the terminal repeats of a full 8.5 kbp del unit is underway. The middle third of each terminus has been examined to date. Both are AT rich (70%) and they share 95% sequence homology.

In L. henryi we calculate that 1.25% of the genome is homologous to del (equivalent to about 50,000 copies of the full repeat). In a screening of 13 other Lilium species, del sequences were universally present but at greatly differing levels. Between species, homology to del ranged from about 100 fold less to 5-10 fold more than that in L. henryi.

Long direct terminal repeats are characteristic of some classes of transposable elements such as copia in Drosophila, Ty elements of yeast and retroviruses. In plants however, most mobile elements identified to date have short inverted terminal repeats, so del may represent an additional category.

MOLECULAR ANALYSIS OF THE NEW TRANSPOSABLE SYSTEM *MU7* IN MAIZE.

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The characterization of maize transposable element systems at the genetic level has led to construction of a model of a two element system. Each system appears to be composed of two types of elements, one the autonomous active element which encodes its own tranposition as well as that of the other responding element. This element moves only in the presence of the active controlling element.

The *Mut* system is so far composed of a non-transposing controlling element (*Mut*) on chromosome 2 which acts on a responding element in the *Bronze 1* gene (chromosome 9) to convert the mutant allele *bz1-mut* into an active gene. By analogy with the *Ac/Ds* system which has been examined at the molecular level the mutant *bz1-mut* may contain an insertion sequence that prevents proper gene function. The insertion would be absent in the progenitor allele and have been excized from the *Bronze 1* gene in germinal revertants.

Comparison between the *Bronze 1-McClintock* allele and *bz1-mut* has indicated that there is a small insertion in the mutant *bz1-mut* in the 5' region of the gene. However this insertion sequence may also be present in the two germinal revertants that have been studied, suggesting that *Mut/bz1-mut* is not a standard type of transposable element system.

GENOMIC ORGANISATION OF HIGHLY REPEATED DNA FROM THE GRASSHOPPER *CALEDIA CAPTIVA*

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A detailed molecular analysis was carried out on a family of highly repeated sequences from the grasshopper *Caledia captiva*. This "144bp" family was detected in two reproductively isolated taxa (Papuan Torresian and Daintree). Restriction endonuclease experiments revealed minor variation between the two taxa, in the overall genomic organisation of this family. DNA sequencing of individual repeats from both the Papuan Torresian and Daintree genomes revealed high levels of intra- and interspecific variation. Repeats from both somatic and meiotic material were sequenced in order to assay the levels of variation. *In situ* hybridisation of a cloned 144bp sequence to Daintree chromosomes revealed hybridisation to the procentric regions of most of the chromosomes. The molecular data along with results from cytogenetic, allozymic and hybridisation studies suggest that these two taxa may have undergone hybridisation since their divergence.

Introgression of white-tailed deer mitochondrial DNA into mule deer in Montana

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White-tailed deer, *Odocoileus virginianus*, and mule deer, *O. hemionus*, are closely related species which occur in sympatry in parts of Western North America. Each species has a distinctive morphology, behaviour and habitat preference. Interspecific hybridization has been reported in Alberta and fertile hybrids have been produced in captivity. PAGE electrophoresis of serum albumin indicate that 1-2% of deer in Montana are hybrids. Restriction endonuclease digests of mitochondrial DNA suggest that both sexes contribute to hybrid production. Samples from a wide geographic area in the United States suggest that introgression of white-tail deer mitochondrial DNA into mule deer has occurred in the past.

SESSION 7A

Mitochondrial DNA variation in the Caledia captiva species-complex

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Within the Caledia captiva grasshopper species-complex, the "Torresian" and "Moreton" taxa have extensive karyotypic and genic differences. They are parapatric, and form a narrow hybrid zone which has been shown to be stable and to be maintained by hybrid breakdown largely attributable to the chromosomal differences. The "Moreton" and "South-East Australian" (SEA) taxa, however, have similar genic characteristics, show no reproductive isolation from each other, and are connected by gradual clines in the frequencies of chromosome morphs.

A study of mitochondrial DNA (mtDNA) variation in these three taxa, using differences in the digestion patterns of six restriction enzymes as markers, has shown that the Moreton and SEA insects form one polythetic taxon, and insects from certain sites in the Torresian range fall into a separate taxon. There is an area, however, within the range of the chromosomally defined Torresian taxon, in which the mtDNA is found to be exclusively of the Moreton/SEA type. This area of apparent introgression of mtDNA extends much further into the range of the Torresian taxon than any reported introgression of chromosomal or allozymic markers from the Moreton taxon. It will be suggested that the hybrid zone (as described by nuclear characters) has migrated southwards, somehow leaving Moreton-type mtDNA behind.

MITOCHONDRIAL GENOME VARIATION IN TWO SPECIES OF THE AUSTRALIAN FRESHWATER FISH GENUS GADOPSIS.

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The River Blackfish consists of two species, Gadopsis marmoratus Richardson and G. bispinosus Sanger. G. marmoratus is endemic to Victorian and northern Tasmanian rivers and streams and has been introduced to southern Tasmanian rivers. G. bispinosus has been found in Victorian rivers and streams north of the Great Dividing range, often in sympatry with G. marmoratus. A recent isozyme study reported five fixed differences between G. marmoratus and G. bispinosus which is equivalent to Nei's genetic distance of between 0.51 and 0.69. Within each species, geographically isolated populations were found which differed by one (G. bispinosus, Nei's D - 0.05) or two (G. marmoratus, Nei's D = 0.09) fixed differences. To test for the presence of cryptic species, and to construct robust phylogenies between populations, the sequence variation of 39 G. marmoratus and 12 G. bispinosus mitochondrial genomes has been analysed with five informative restriction endonucleases. We have found that geographically isolated populations from each species represent mitochrondrial genome clones which correspond to and extend those identified by the isozyme study. The magnitude of mitochondrial DNA sequence differentiation between these clones is similar to that reported between salmonid and sunfish species. This degree of differentiation may have been enhanced by the lack of migration between populations in geographically isolated drainage patterns, but it may also indicate specific or subspecific status for some of the geographically isolated populations.

MOLECULAR ANALYSIS OF THE TOPAZ GENE AND ITS MUTANTS FROM LUCILIA CUPRINA

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Lucilia cuprina (the sheep blowfly) and Drosophila melanogaster are two dipteran species that diverged about 100 million years ago. In spite of this evolutionary distance and considerable difference in genome size the two species have similar genetic linkage groups and much of their biochemistry appears similar. Of the genes which encode one biochemical pathway, the ommochrome biosynthetic pathway which leads to the deposition of brown eye pigment, the homologous scarlet gene of D. melanogaster and topaz gene of L. cuprina have been analysed extensively in our laboratory.

The topaz gene was isolated by virtue of its homology to already cloned scarlet sequences. The regions containing the two genes have been sequenced extensively and show considerable homology at the nucleotide level. This homology appears confined to exons. Although the sizes of exons and the amino acid sequence of the protein they encode have been conserved the sizes of introns have not. Two striking differences between the homologous genes are the much lower GC content and presence of repeated sequences within the topaz region

We have also analysed two spontaneous topaz mutants at the nucleotide level. Although there are sequence differences between the wild-type and mutant strains the high level of nucleotide polymorphism in this species cautions against drawing meaningful conclusions about the relationship between molecular changes and genetic lesions.

SESSION 7B

FLUCTUATING ASYMMETRY AND INSECTICIDE RESISTANCE IN LUCILIA CUPRINA

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Fluctuating asymmetry is used to assess potential background modification of developmental processes in resistance genotypes of Lucilia cuprina. Results provide evidence for modification of diazinon resistance genotypes with the major gene(s) responsible or such modification localised to chromosome III. No modification was evident for dieldrin resistance genotypes.

Results are discussed with reference to the fitness modification of diazinon genotypes previously described (McKenzie et al., 1982; McKenzie and Purvis, 1984), and the implications these results have for models of the evolution of insecticide resistance and strategies for insecticide usage.

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DIAZINON RESISTANCE IN LUCILIA CUPRINA : MAPPING OF A FITNESS MODIFIER

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In the absence of insecticide the fitness set of susceptible and resistant genotypes is generally considered to be SS>RS>RR. Population cage studies showed such a fitness set at the diazinon resistance locus of L. cuprina at the time resistance first evolved following the introduction of diazinon for blowfly control.

After a further decade of diazinon usage the fitness set evolved to SS=RS=RR in the absence of the chemical. However, when the field genome was disrupted by repeated backcrossing to a laboratory strain the initial fitness set was re-established.

Chromosome substitution line analysis localised the gene(s) responsible for this modification to chromosome III. The gene, or gene complex, has subsequently been mapped to the region of the w locus.

The influence of genotypes at the modifier "locus" on single generation fitness components of diazinon resistance genotypes will be discussed.

THE INCOMPATIBILITY LOCI AS INDICATORS OF CONSERVED LINKAGE GROUPS IN THE POACEAE.

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Gametophytic self-incompatibility controlled by two loci has been found in all genetically analysed self-incompatible species belonging to the Poaceae. The incompatibility system leads to differential transmission of different pollen genotypes and thus genes which are linked to the self-incompatibility genes may have a disturbed rate of transmission.

Diploid grass species with a basic number of n=7 and a two-locus gametophytic self-incompatibility system coming from five tribes well separated on the evolutionary time scale were chosen and examined for disturbed segregation ratios of various isozyme loci in crosses where the compatibility relationship between the plants was known.

The five species so far analysed (one from each tribe) have shown linkage of GPI (glucose phosphoisomerase) to one of the incompatibility loci though there are differences in the estimates of the recombination frequency between the species. All species have also shown undisturbed segregation ratios for at least one other isozyme in the cross from which the recombination estimate is derived and also undisturbed segregation ratios for GPI in other crosses indicating that the disturbances may be attributed to linkage and not viability effects.

Very few plants are well mapped genetically and this process is more complicated in plants than in animals. The behaviour of the incompatibility system means that the linkage group in which these genes are located can be readily identified. These results indicate that it might be feasible to extrapolate from linkage relationships in one grass species to possible linkage relationships in another.

GENETIC VARIABILITY IN THE SMALL AND DISCRETE POPULATIONS OF EUCALYPTUS PULVERULENTA (Sims).

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Within the eucalypts, some species exist in large and continuous populations, while others may be found in very small stands separated by distances up to hundreds of kilometres (1). Eucalypts have mixed mating systems, which favour outcrossing but tolerate selfing, to the extent that 20-30% of seed result from self-fertilisation (2). If such a mating system is consistent in all eucalypts, the varying population sizes and structures should, in theory, produce measurable effects on the level of genetic variability. Drift and inbreeding should decrease species-wide variability, but increase between population differences. Moran and Hopper (3) measured population gene frequencies in the rare West Australian species E.caesia and found them to be consistent with such effects. Here we report on the levels of genetic variability in another rare eucalypt, E.pulverulenta, from the central and southern highlands of NSW. Electrophoretic variants were scored within half-sib arrays of seeds or seedlings, from a total of 46 parent trees in 3 populations. 16 loci were studied.

This species shows very low levels of heterozygosity within populations (He=0.03-0.11). It possesses a level of selfing typical of known eucalypts (=28% overall), although this value varies greatly between the 3 populations studied. E.pulverulenta ($\bar{H}$=.134) has a genetic diversity much lower than those of most tree species. Indeed, this value, like that for E.caesia, is comparable with those for herbaceous inbreeders (4). Furthermore, pop. differentiation (Dst/$\bar{H}$) is high (=43%). Together, these results suggest that drift, dependent on small pop. sizes, has played a major part in distribution of genetic variation throughout the species.

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REPRODUCTIVE ISOLATION IN AN INTRODUCED POPULATION OF THE BEETLE *CHRYSOLINA QUADRIGEMINA*

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Studies of speciation in Hawaiian *Drosophila* have led to models of the way that rapid changes in mating behaviour, and hence speciation, might follow founder events. Deliberate introductions of insects used in the biological control of weeds can provide suitable circumstances to test these models. The possible existence of reproductive isolation between ancestral and derived populations has therefore been investigated in one such case, the chrysomelid beetle *Chrysolina quadrigemina*.

C.quadrigemina, originally from France, has been used to control the pasture weed *Hypericum perforatum* (St. John's Wort), first in Australia, and later in the U.S.A., Canada, and elsewhere. Canadian populations are derived from Australian animals *via* an apparent bottleneck. Results of mating tests between Australian and Canadian beetles reveal strong mating isolation, especially between Australian males and Canadian females.

These results are reported, and their significance for current ideas on the evolution of reproductive isolation (and for biological control programs) are outlined.

MITOCHONDRIAL GENOMES OF *DROSOPHILA* AND OTHER INVERTEBRATES

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We have sequenced and analyzed the entire mitochondrial (mt) DNA molecules of *Drosophila yakuba* and the nematodes *Ascaris suum* and *Caenorhabditis elegans*, and portions of the mtDNA molecules of *D. melanogaster*, *D. virilis* and the trematode, *Fasciola hepatica*. The mtDNA molecule of *D. yakuba* contains the same genes (for 13 proteins, two rRNAs and 22 tRNAs) that are found in vertebrate mtDNAs. The relative arrangements of the genes in *D. yakuba* and vertebrate mtDNAs are well conserved. In contrast, nematode mtDNA molecules lack one of the genes (ATPase8) found in *D. yakuba* and vertebrate mtDNAs, and the relative arrangements of genes between nematode mtDNAs and *D. yakuba* or vertebrate mtDNAs differ greatly. All of the tRNA genes (except $tRNA^{ser}_{AGY(A)}$) of *D. yakuba* and vertebrate mtDNAs can be folded into the characteristic secondary structures of bacterial and eukaryotic nuclear-coded tRNAs, although the dihydrouridine (D) and TψC loops are poorly conserved. However, in 21 of the 22 tRNA genes of nematode mtDNAs, the TψC arm and variable loop are replaced with a loop of four to 12 nucleotides. The tRNA genes of *F. hepatica* resemble those of *D. yakuba* and vertebrates with regard to secondary structure. The $tRNA^{ser}_{AGY(R)}$ genes of all invertebrates and vertebrates have a TψC arm and variable loop, but lack a D-loop. In *D. yakuba* mt-protein genes ATG, ATA and ATT, and in one case ATAA are used as translation initiation codons. In nematode mtDNAs, TTG (but not ATG) together with ATA and ATT are used for this purpose. In the nematode mitochondrial genetic code AGA and AGG specify serine rather than arginine (standard genetic code) or termination (vertebrate mt-genetic code). In *D. yakuba* mtDNA AGA, but not AGG is used to specify serine, and in *F. hepatica* mtDNA at least AGG specifies serine.

This work was supported by National Institutes of Health Grants GM 18375 and RR 07092.

FREQUENCY DEPENDENT SELECTION AND METRICAL VARIATION

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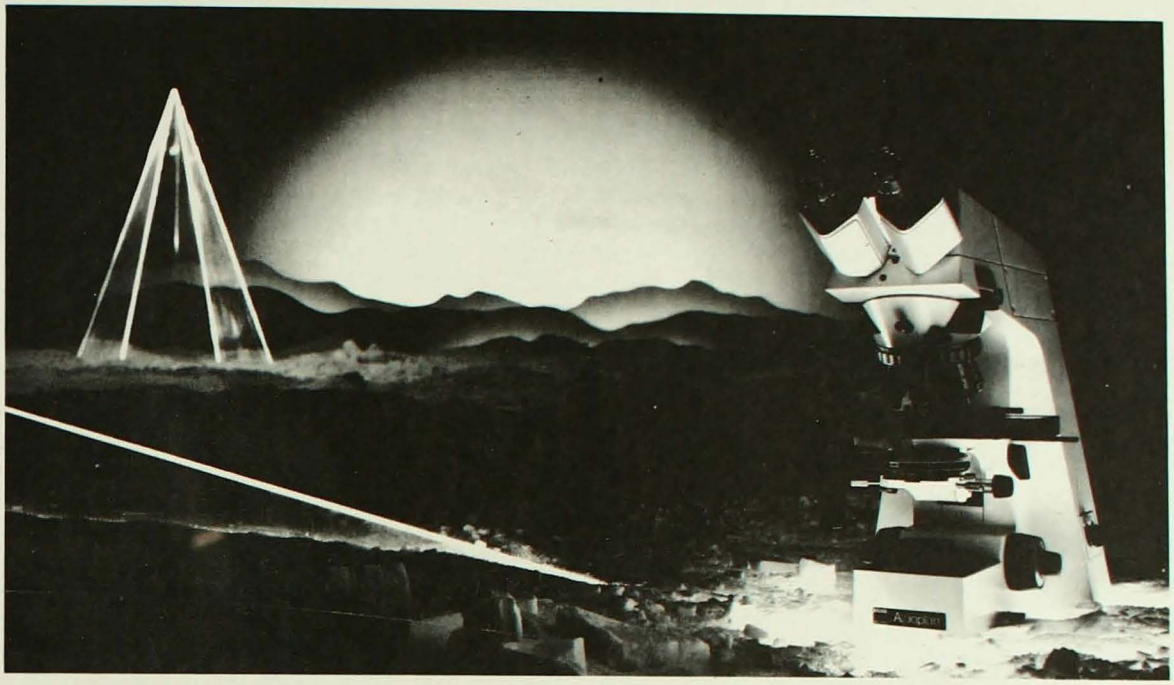
Studies on predation of artificial prey by wild birds indicate that the birds tend to overeat common colour varieties of prey and to undereat rare ones, thus exercising frequency-dependent selection. Recent experiments have shown that this behaviour extends to prey that vary continuously in shape. The predators overeat the common forms that are near the mean, and undereat the rarer forms that are near the extremes. Thus predators can act to preserve or increase the variance of metrical characters. There is reason to believe that parasites and competition act in the same way. In these cases the characters may be biochemical as well as morphological.

Computer simulations of frequency-dependent and stabilising selection acting on a metrical character show that a combination of the two forces can maintain polymorphisms among the genes that contribute to the character. It is argued that selection on metrical characters may be responsible for many polymorphisms in proteins and DNA.

THE DAWN OF A NEW ZEISS AGE



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PROBING MURINE MALE MEIOSIS USING UNIQUE DNA FLANKING THE IMMUNOGLOBULIN HEAVY CHAIN GENES.

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Murine male meitotic preparations were probed by *in situ* hybridization to localize 10.5 kb of unique DNA which flanks the C 1 heavy chain immunoglobulin gene on the 5' side. The probe, inserted into the plasmid vector pBR 322, was labelled with ^{125}I dCTP using nick translation. After exposing autoradiographs, made by standard techniques of *in situ* hybridization, for 40 days, a strong signal was obtained on one bivalent of size appropriate to pair 12. The signal was in the distal position on chromosome 12 and it could be traced from spermatogonial divisions through to mature sperm. The signal was strongest in some well-spread stages of prophase of first spermatocytes, weakest in early spermatids, then surprisingly strong in sperm heads. This variation is probably due to differences in the packaging of the target sequence at the various stages of meiosis.

POSTER 2

A REGION SELECTIVE TRANSPOSON MUTAGENESIS SYSTEM FOR PSEUDOMONAS AERUGINOSA

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We have sought to develop a method for isolating a large number of Tn5 inserts in a pre-selected short region of the bacterial chromosome. Prime plasmids derived from the kanamycin sensitive ECM plasmid pMO1632 were isolated by selecting for the catA marker situated at 64' on the P. aeruginosa PAO chromosome. One such prime, pMO1811, was transferred to an E. coli strain with Tn5 inserted into the chromosome. Inserts of Tn5 into this prime plasmid were selected by transferring pMO1811 to another E. coli strain selecting for kanamycin resistance. Transconjugants carrying the Tn5 loaded prime plasmid were then mated to a P. aeruginosa catA mutant selecting for catA+ and carbenicillin resistance. Suitable exconjugants were then grown for about 25 generations without any selective pressure to enable any chromosomal fragment carrying Tn5 from the prime plasmid to reinsert into the P. aeruginosa chromosome by homologous recombination and for the remaining plasmid to be lost. 557 such inserts were obtained. So far 10 ben (benzoate non utilising) and 18 ant (anthranilic acid non utilising) mutants have been isolated, these being genes which are known to be linked to catA. Two new mutant sites have been identified - gcu (glycine non utilising) and oap a gene thought to affect the permeability of a variety of organic acids and alcohols. The prime plasmid pMO1811 complements all the mutants isolated. The prime plasmid has been used to construct a cosmid bank of this chromosomal region. A preliminary linkage map of the region covered by the prime plasmid isolated has been constructed.

A CHROMOSOMAL STUDY OF AUSTRALIAN BATS.

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Karyotypes from a wide range of species of Chiroptera (bats) are characterized on the basis of general morphology, C- and G-banding.

The karyotypes are compared to determine the degree of difference which exists at the various taxonomic levels, i.e. species, genus, family.

They are also compared with the primitive karyotype derived by Bickham (1979) to determine the degree to which the karyotypes have evolved since the American and Australian groups separated from the original population in Eurasia.

The results generally support the view that the karyotype remains conservative at the family level although one notable exception was found.

POSTER 4

BI-DIRECTIONAL REGULATION AT THE aroF OPERATOR OF ESCHERICHIA COLI

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The *tyrR* gene product plays an important role in the regulation of the metabolism of the aromatic amino acids in *E. coli*. The *aroFtyrA* operon is is one of a number of transcription units regulated by the *tyrR* gene product. Three sequences in the *aroF* promoter region known as TYR R Boxes are involved in this regulatory mechanism. Nucleotide sequencing has identified the presence of an open reading frame (ORF) of unknown function translated divergently from the *aroF* regulatory region. The promoter of this gene was expected to overlap one or more of the TYR R Boxes involved in the regulation of *aroF* suggesting it too may be regulated by *tyrR*.

This poster demonstrates that transcription of this ORF is also regulated by the *tyrR* gene product. The promoter region of the ORF has been fused to the structural gene for chloramphenicol acetyl transferase (cat). Expression of this fusion can be regulated over a 100-fold range by *tyrR*. In addition, mutations which abolish regulation of *aroF* have also been shown to cause derepression of the ORF promoter. The function of the ORF gene has yet to be determined.

FURTHER GENETIC ANALYSIS OF THE *LOZENGE* LOCUS IN *DROSOPHILA MELANOGASTER*

J.R. Andrews, G.M.F. Pasquini and P. Batterham

Department of Genetics, University of Melbourne

The *lozenge* (*lz*) gene complex was subjected to extensive genetic analysis in the pioneering work of M.M. and K.C. Green. Fine structure mapping placed 19 mutations into 4 sub-loci which span a 0.140 map unit region around map position 27.7 on the X-chromosome (polytene bands 8D4-8E2). Complementation analysis revealed an overall pattern of non-complementation. However, in some instances complementation was observed to occur - even for pairs of alleles mapping to the same sub-locus.

Genetic complexity at this locus is paralleled by the pleiotropy observed in the phenotype of *lz* mutants. Typically *lz* mutants have:-

a) Narrower eyes than wild type with patches of irregular or fused facets.
b) Reduced tarsal claws.
c) Sterility in females due to abnormal differentiation of the genital disc leading to the absence of parovaria and spermathecae.
d) Extreme *lz* mutants totally lack the activity of two phenol oxidase enzymes, one of which (PHOX) is encoded by a structural locus (*Phox*) which is unlinked to *lz* (chromosome II:80.6).
e) Five mutations at the *lz* locus suppress the expression of the gene *Bc* which is either allelic or closely linked to the *Phox* gene.

Our research aims to determine the molecular structure and function of the *lz* gene complex. In the analysis of structure our strategy is to isolate overlapping clones spanning the entire locus, thus generating a physical map of this region. Genetically mapped chromosomal rearrangement mutations will then be localized at the molecular level allowing the correlation of genetic and physical maps. At this stage we report the results of fine structure mapping and complementation analysis with recently generated spontaneous, P-M and MR induced *lz* mutations. A physical map will be presented for 30kb of cloned DNA derived from the *lz* region.

POSTER 6

THE USE OF A lacZ FUSION TO STUDY THE REGULATION OF A GENE OF UNKNOWN FUNCTION IN Aspergillus nidulans.

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The amdS gene in Aspergillus nidulans has a number of independent regulatory mechanisms governing expression of the gene. aciA is a gene regulated in a similar manner to amdS.

aciA has an unknown function,thus a fusion of aciA with E.coli lacZ was constructed to yield regulatory data for aciA. The fusion produces an enzymatically active beta-galactosidase protein under the control of aciA. Assays were performed in an array of mutant backgrounds to give regulatory infomation for the fusion. This regulatory data combined with 5'sequence infomation of aciA and amdS has allowed putative regulator binding sites to be identified in the aciA sequence. The fusion has also allowed the aciA locus to be mapped.

CLONING AND REGULATION OF ACETATE UTILISATION GENES OF *Aspergillus nidulans*.

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The *amdS* gene of *Aspergillus nidulans*, acetamidase, is under the positive control of a number of independently acting regulatory genes. One of these genes, *facB*, also regulates three genes involved in acetate utilisation. The *facA* gene, encoding acetyl CoA synthase, converts acetate to acetyl CoA. The *acuD* gene (isocitrate lyase) and the *acuE* gene (malate synthase) constitute the glyoxylate bypass which allows acetate to be used as a carbon source.

Two of these acetate utilisation genes, *facA* and *acuE*, have been cloned. The *facA* gene produced a message of 2.6kb and the *acuE* gene a message of 1.85kb. The direction of transcription of each gene has been determined using single stranded subclones as probes against RNA and both genes are currently being sequenced. cDNA clones have also been obtained for *facA* and will be sequenced to determine the number and position of introns and the 3' endpoint.

The regulation of *facA* and *acuE* is being studied in two ways, by Northern analysis and titration of *facB* gene product. Titration of *facB* gene product is investigated by transforming small plasmid subclones from the 5' untranslated region of each gene into *Aspergillus* and screening for decreased acetate or acetamide utilisation. This should localise the *facB* product binding site and, together with sequence data from *facA*, *acuE* and *amdS*, will provide more information on the regulation of these genes by *facB*.

POSTER 8

CHROMOSOME PAIRING ABNORMALITIES IN INTERSPECIFIC HYBRID CATTLE AND ROCK WALLABIES

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The synaptonemal complex is a protein matrix which forms between the pairs of chromosomes as they synapse during pachytene stage of meiosis. Examined by electron microscopy, the regularity of the synaptonemal complex indicates the extent of homology between the two pairing chromosomes. In first cross hybrids, one set of chromosomes is contributed from each parent, therefore an analysis of the synaptonemal complexes of an interspecific hybrid provides useful information about the karyotypic differences between the species involved.

In this study the synaptonemal complexes of Bos indicus x Bos taurus hybrid cattle, and of assimilis x Mt. Claro, assimilis x Mareeba and Mt. Claro x Mareeba hybrid rock wallabies were analysed. A wide range of pairing abnormalities has been observed and will be illustrated here including:

trivalent, quadrivalent and pentavalent pairing, inversion pairing, partial failure to pair, triple pairing and non-homologous pairing between autosomes and sex chromosomes.

POLYMORPHISM FOR ABNORMAL ABDOMEN PHENOTYPES IN DROSOPHILA BUZZATII

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The abnormal abdomen syndrome in the cactophilic repleta group Drosophila mercatorum is one of the rare examples of a DNA polymorphism that manifestly is maintained in wild populations by natural selection (Templeton and Johnson 1982; DeSalle et al. 1986). It is argued that deletions of rDNA on the Y, and inactivating rDNA insertions on the X, limit juvenile hormone esterase translation. The resulting high juvenile hormone titres in pupae lead not only to a juvenilized adult cuticle (at certain temperatures), but also to a profound alteration of life history traits, encompassing precocious sexual maturity, elevated fecundity, but decreased longevity. Such genotypes are selected preferentially in marginal desiccating environments, where the adverse conditions reduce longevity of all flies irrespective of genotype.

In another cactophilic repleta group species, D. buzzatii, abnormal abdomen-like phenotypes are ubiquitous, but at low frequencies in wild and laboratory stocks. Genetic, biochemical and molecular studies on five isolated abnormal abdomen-like D. buzzatii stocks reveal a variety of genetic changes (including rDNA deletions) that are unrelated at the level of the DNA, but which give rise to superficially similar phenotypes. This may be because all the different genetic changes have a common effect of disruption of normal translation and the adult abdominal epidermis is differentiated late in pupation.

Templeton, A.R. and J.S. Johnson, 1982. Life history evolution under pleiotropy and K-selection in a natural population of Drosophila mercatorum. In Ecological Genetics and Evolution (J.S.F. Barker and W.T. Starmer, eds), pp. 225-239. Academic Press, Australia.

DeSalle, R., J. Slightom and E. Zimmer, 1986. The molecular through ecological genetics of abnormal abdomen. II. Ribosomal DNA polymorphism is associated with the abnormal abdomen syndrome in Drosophila mercatorum.

POSTER 10

THE FORMAL GENETICS OF *DROSOPHILA BUZZATII*

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University of New England

Thirty-one visible recessive and seven dominant mutants were produced by inbreeding and X-ray irradiation. These mutants and the polymorphic allozyme loci are currently being mapped.

Assignment of markers to linkage groups was facilitated by monitoring segregation with or from two translocations both marked by a dominant mutant *Delta wings*.

Delta-one is a (3;5;2) translocation and *Delta-two* is a (2;3) translocation.

The linkage map to date will be illustrated and homologies with other *Drosophila* species will be discussed.

Cloning and characterization of the stoned gene of Drosophila.

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Stoned (stn) mutations are recessive neurological mutations that map to region 20 at the base of the X-chromosome of Drosophila melanogaster. Stn^{ts} mutants have an unusual jumping reaction in response to a light-off stimulus and are unable to coordinate their movements at the restrictive temperature. In addition to the ts alleles, one stress-sensitive allele and a number of lethal alleles have been identified.

A library of 50 unique λ clones, microdissected from the base of the X-chromosome, has been used to probe Southern blots of DNA from two strains of flies each of which carries a P-element induced lethal mutation at the stn locus. Clones λDm20-3 and λDm20-5 hybridize to altered bands in the DNA from strains 30A2 and PH1 respectively. The 30A2 mutation is a large deletion spanning the stn gene and penetrating into the two adjacent loci. The PH1 mutation, however, is confined to the stn locus. The clone λDm20-5 hybridizes to at least two species of fly RNA, and thus probabiy contains some part of the stn gene.

POSTER 12

The isolation of genes encoding nervous system phosphoproteins from *Drosophila* - a new methodology.

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A general method has been devised for the isolation of genes that encode substrates for the mammalian cAMP-dependent protein kinase. A *Drosophila* head cDNA library in phage λ-gt11 has been used in these studies, although similar results have been obtained with a rat liver cDNA library in the same vector. The technique involves screening plaques for the presence of phosphorylatable proteins using the catalytic subunit of cAMP-dependent protein kinase and δ-ATP^{32}. This technique allows for the isolation of the genes encoding proteins that act as substrates for cAMP-dependent protein kinases, but may also be useful as a method for the isolation of genes encoding substrates for other protein kinases, including the Ca^{++}/calmodulin-dependent protein kinases, C-kinase and the tyrosine-kinases associated with hormone receptors.

ABSENCE AND HYPOPLASIA OF SOME OCULAR MUSCLES IN A CHILD WITH THE KARYOTYPE 46,XY,der(2),inv? ins(2;7)(q21;q32q34)mat.

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Absence of one of the ocular muscles is a very rare event and an unusual cause of squint. We describe a child who had minor dysmorphic features and congenital esotropia which, at operation, was found to be caused by absence of the right and hypoplasia of the left lateral rectus and muscles. Chromosomal analysis revealed an insertion of a small segment of chromosome 7 (7q32-7q34) into chromosome 2 at band 2q21 causing trisomy of that segment of chromosome 7.

A balanced insertional rearrangment was found in the mother, aunt and sister of the propositus, who were all phenotypically normal. It is uncertain whether the shifted piece of 7 is inserted direct or inverted into 2.

POSTER 14

CHROMOSOME DELETION AT 11q23 IN THE CHILD OF A PARENT WITH FRAGILITY AT THE SAME SITE.

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The folate-sensitive fragile site at 11q23.3 in man has been so rarely seen it is regarded as provisional only. In contrast, deletion of the terminal segment 11q23.3 to 11qter has been frequently observed and a distinct syndrome has been associated with this deletion.

We present the case of a male baby who died as a neonate who had multiple dysmorphic features. His fibroblasts showed the karyotype 46,XY,del(11)(q23.3). His lymphocytes were mosaic 46, XY/46,XY,del(11)(q23.3) with three normal cells in 463 counted, showing that deletion of chromosome 11 was a post-zygotic event.

When the lymphocytes of the mother were grown in medium 199, an average of 37% of cells showed fragility at 11q23.3, while a further 8% showed deletion beyond the fragile site; addition of folic acid depressed expression to 9%. Addition of 5 FdU to lymphocytes growing in RPMI 1640 raised the frequency of expression from 4% to 46%.

The mother subsequently had a second, normal son who carried the fra(11)(q23.3) site at a level of 14% in medium 199; expression was depressed to 6% by folic acid; and in RPMI 1640, 5 FdU raised expression from zero to 38%.

This case is of interest because it strongly suggests that fragility can occur *in vivo* for a site which to date has only be observed to be fragile *in vitro*.

CHARACTERISATION OF A 37 bp INSERTION IN THE FIRST INTRON OF THE *Adh* GENE OF *DROSOPHILA MELANOGASTER*

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A 37 bp point insertion, described by Kreitman (1983), occurs in populations of Drosophila in various countries. Of 11 alleles sampled it was found in only 3 of the 5 *Adh*-F alleles, indicating possible linkage disequilibrium. Due to the complementary nature of the sequences involved the insert could potentially form a secondary structure. The insert occurs 125 bp 5' to the larval promotor within the first intron of the primary adult transcript. The detection of this insert in isogenic lines from an Australian population required the probing of Southern blots of genomic DNA with a specific probe. The probe was a subcloned 158 bp *Hind*III fragment of the *Adh* gene which was known to flank the reported position of the insert.

After detecting the insert expression tests were carried out on the lines to detect differences between those with and those without the insert. These tests included enzyme inducibility, tissue specific control and quantification of mRNA levels.

The results from these tests, when combined with recent discoveries of trans-acting transcription initiation proteins which bind specifically to the DNA surrounding the site of insertion (Heberlein *et al.*,1985), indicate a possible role for the insert in transcription control of *Adh* in larval tissue.

POSTER 16

Variation in the Expression of Esterase 6 in *Drosophila melanogaster*

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Forty-two third chromosome isoallelic lines of *Drosophila melanogaster* were isolated from the Coffs Harbour population and 4 day old virgins of each sex assayed for esterase 6 activity. The assay was repeated on extracts prepared from several different generations.

Both male and female activity showed large differences among lines which were repeatable over generations. For females a 2.5 fold range of activity was found across lines and for males a 3.2 fold range. Males were found to have 3 to 9 times the activity of females of the same line, and most of the variation between lines was uncorrelated between males and females. This suggests possible differences in the regulation of EST6 activity between the sexes.

Little association was found between EST6 electromorphs and activity for either sex.

Radial immunodiffusion assays with polyclonal EST6 antibody were used to determine the levels of EST6 cross reacting material (CRM) in males from each of the lines. The specific activity was then obtained as the ratio of activity to CRM. It was found that the activity in males was highly correlated with CRM but not correlated with specific activity.

The results suggest that inherited variation for EST6 activity is largely due to regulatory rather than structural polymorphism, that is, to differences in the number of EST6 protein molecules produced rather than the specific activities of those molecules.

POSTER 17

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS BETWEEN WHEAT AND RYE

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Wheat and rye cDNA and genomic clones have been used as probes in hybridization experiments to detect restriction fragment length polymorphisms (RFLPs) between Chinese Spring wheat (*Triticum aestivum*), 2n = 42, and Imperial rye (*Secale cereale*), 2n = 14.

Polymorphisms can be localized to a rye chromosome by probing digests of genomic DNA isolated from a series of wheat/rye addition lines. Genomic and c DNA clones have been compared in their efficiency in detecting RFLPs.

This application of RFLPs should quickly generate a large number of useful genetic markers in wheat and rye.

POSTER 18

CHROMOSOMAL GENE INVOLVEMENT IN PLASMID-MEDIATED COPPER RESISTANCE IN *ESCHERICHIA COLI*.

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Plasmid pRJ1004 encodes inducible resistance to Cu in *E. coli*. Two plasmid determinants are involved in the resistance, *pco*, which encodes Cu transport and sequestration functions, and *cdr* which specifies repair of Cu damage to DNA. In addition, a number of chromosomal genes contribute to the resistance. A number of chromosomally encoded Cu transport functions are essential components of the *pco* mediated Cu efflux system. This is shown by the failure of the *pco* determinant to confer Cu resistance in the Cu uptake mutant GME111 and the Cu efflux mutant GME138. Also, the enhanced Cu efflux associated with the resistance is not expressed in an *unc*A mutant, which indicates the enhanced Cu efflux is energised by cellular ATP.

POLYTENE CHROMOSOMES OF THE MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA*

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Several larval and pupal tissues of *Ceratitis capitata* were examined for the presence of polytene chromosomes suitable for detailed cytological analysis. Polytene chromosomes were found in salivary glands, malpighian tubules, mid gut, hind gut and fat body but cannot be easily spread for analysis because of high levels of ectopic pairing and chromosome fragmentation. Trichogen cells from pupal bristles provided the only polytene material in which chromosomes could be adequately separated and spread. Generally these chromosomes are thin and difficult to analyse. However the enlarged superior orbital bristle trichogen cells of male pupae have well banded highly polytene chromosomes suitable for full cytological analysis. A standard polytene chromosome map for *C. capitata* is now being compiled.

In polytene cells five banded chromosomes and a heterochromatic network are found. Comparison of the mitotic and polytene complements using C and Q banding shows that the heterochromatic network represents the sex chromosomes which are differentially underreplicated in polytene tissues. Only the autosomes are represented by the usual banded polytene chromosomes.

POSTER 20

GENOME EVOLUTION IN THE GENUS ANTECHINUS (DASYURIDAE, MARSUPIALA)

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Evolution in the Australasian marsupial group Antechinus has been studied at the cytological and DNA level. All species investigated agree in having the standard Dasyurid karyotype, 2n=14 (XX , XX) which probably represents the ancestral marsupial complement (Hayman and Rofe, 1985). With the exception of Parantechinus apicalis, which shows massive addition of heterochromatin to the X chromosome and fairly large procentric C-bands on the autosomes, arm ratio measurements and a battery of chromosome banding techniques show few obvious karyotypic changes associated with speciation in this group.

DNA determinations showed that the virtually identical G-band patterns mask some alterations in the DNA amount per haploid genome. These DNA changes have occurred in both the unique and repeated sequence classes of the genome. There is no evidence that in the antechinuses C-banded material 'is greatest in species with the largest nuclear DNA content' (Hayman and Rofe, loc. at.)

Filter hybridization studies using probes made to unique DNA sequences suggest the presence of three major species groupings of antechinuses, a result in agreement with those derived using other taxonomic tools (e.g. penis morphology, isozyme studies etc.). The New Guinean species A. melanutus and A. wilhelmina are probably not true antechinuses.

Hayman, D. and Rofe, R. (1985) Cytogenet. Cell Genet. 39: 40-50.

EVOLUTION OF MAMMALIAN X-CHROMOSOME INACTIVATION: SEX CHROMATIN IN MONOTREMES AND MARSUPIALS

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The heterochromatic sex chromatin body, formed by the inactive X chromosomes, is a characteristic feature of interphase cells in females in many animal species.

It has been proposed that X-inactivation in eutherian mammals is a multistep process which has evolved from a more primitive system, features of which are found in monotremes and marsupials. In eutherians heterochromatinization and DNA methylation act together to maintain inactivation. Therefore, it is important to know if these mechanisms are present in the marsupial and monotreme systems.

Earlier researchers on marsupials have claimed to observe sex chromatin in both sexes; however, visualisation of the sex chromatin may have been confused by the presence of condensed constitutive heterochromatin. For this reason we have chosen to study the brushtail possum as it has little constitutive heterochromatin, and the platypus as a representative of the monotreme group.

We have studied nuclear morphology in a range of tissues. A female-specific chromatin body was observed in nuclei of possum corneal epithelia, but not in other tissues. No sex differences were observed in any monotreme tissue. This suggests, therefore, that inactivation of the X chromosome by heterochromatinization is tissue specific in marsupials and absent in monotremes.

POSTER 22

DOSE-RATE EFFECTS ON MUTATION INDUCTION BY IONIZING RADIATION

by

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A clonogenic assay to quantify thioguanine-resistant spleen lymphocytes in the mouse has been developed,(Jones *et al*,1985;Dempsey and Morley,1986). This has been used to study the *in-vivo* mutation frequency at the hypoxanthine phosphoribosyltransferase (hprt) locus after whole-body irradiation of mice with high(15Gy/hr) and low-dose-rate(0.025Gy/hr) gamma rays. A dose response curve for induced mutants was obtained for each dose rate over the range 0-3Gy.The induced mutant fraction differed between the two dose rates and the shapes of the dose response curves may be different.These differences may be explicable in terms of induction of DNA repair mechanisms.

These results suggest that dose-rate effects may be especially significant when projecting the mutagenic or carcinogenic consequences of low-dose-rate irradiation from various sources.

Jones, I.M., K.Burkhart-Schultz, A.V.Carrano (1985) A method to quantify spontaneous and *in vivo* induced thioguanine-resistant mouse lymphocytes, Mutation Research, 147, 97-105.

Dempsey, J.L., A.A.Morley (1986) Measurement of *in vivo* mutant frequency in lymphocytes in the mouse, Environmental Mutagenesis, 8, 385-391.

UNSTABLE GENES FOR RUST RESISTANCE IN MAIZE

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The Rp1 gene conferring resistance against rust shows inherent instability giving rise at high frequency to susceptible mutations independant of any known insertional event. The frequency is allele specific varying from 8×10^{-3} for Rp1g to 1.5×10^{-4} for Rp1d. The Rp1d allele has been used as a target in an attempt to recover an insertional mutation using the Ac-Dc controlling element system. Most of the mutants recovered are fully susceptible but 2 other classes have been recognised. One class has an intermediate resistance phenotype and is highly unstable mutating to a fully susceptible phenotype. It is not yet known if this is due to Ac-Dc excision events. The other class shows not only an altered resistance phenotype but also an additional specificity and now recognizes a new rust race. The stability of this class has not been tested. The origin of these events is unknown, but since resistance is known to be a complex locus in a chromosomal region of at least 3-5 map units involved in resistance against rust pathogens the possibility of some sort of genetic reassortment giving rise to new specificities must be likely. Any molecular analysis of rust resistance in maize will rely heavily on genetic studies of events at the loci.

POSTER 24

AGEING IN VIVO DOES NOT INFLUENCE MICRONUCLEUS INDUCTION BY X-IRRADIATION IN HUMAN LYMPHOCYTES

by
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Abstract

The biological basis for the age-related decline in the stability of the genome is not determined, however it is possible it could be the consequence of an increasing deficiency in DNA repair during the life-span of an organism. To test this hypothesis in humans we compared the extent of chromosomal damage, after X-irradiation, in lymphocytes from healthy young and old individuals.

Chromosome damage was estimated by the cytokinesis-block micronucleus method which is more precise and sensitive than the conventional micronucleus methods. In the cytokinesis-block method micronuclei are scored only in those cells that have completed one nuclear division and these cells are recognised by their binucleate appearance following inhibition of cytokinesis by cytochalasin-B. The level of X-ray induced micronuclei, after exposure to 75cGy and 150cGy, was measured by subtracting the base-line micronucleus frequency in the control unirradiated cultures from the observed frequency in the irradiated cultures.

Comparison of the results for lymphocytes from the young and old subjects showed that there was no difference between the two groups thus indicating that cells from aged subjects do not exhibit increased chromosomal instability following X-irradiation. These results suggest that the repair of those DNA lesions that lead to chromosome breakage does not decline with age.

MOLECULAR BASIS FOR INDUCED MUTATIONS AT THE HPRT LOCUS

by
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Abstract

Human peripheral blood lymphocytes were exposed *in vitro* to mutagenizing agents and were maintained in culture to allow for expression of resultant mutations. 8 days post insult, lymphocytes were cloned in microwells in a conditioned medium containing T-cell growth factors and the selective agent 6-thioguanine (TG). Lymphocytes were cloned at an appropriate dilution such that microwells showing growth after 10 days had a high probability (>90%) of containing monoclonal populations. Such monoclonal populations are functionally HPRT negative. Clonal lymphocyte populations were expanded to about 10^8 cells per clone and DNA was extracted. Southern analysis of clonal DNA was carried out using a human full length c-DNA for HPRT. Results will be presented for mutations induced by X-rays, melthyl-nitrosourea (MNU) and mitomycin C (MMC). X-rays caused frequent deletion of the entire HPRT gene or of segments of the gene, many of which would have been submicroscopic. Inversions were also observed. MNU induced changes not resolvable by Southern analysis using restriction enzymes EcoRI, HindIII, BamHI or PstI and these changes are believed to involve point mutations within the gene or in controlling regions. MMC caused some total deletions of the HPRT gene. This observation is discussed with reference to models of DNA crosslink repair.

POSTER 26

CYTOGENETIC ANALYSIS OF RECOMBINATION EVENTS BETWEEN WHEAT AND RYE GROUP 1 CHROMOSOMES

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Koebner and Shepherd (1985) used the ph1b mutant in wheat, to induce the first genetically substanciated example of homoeologous recombination between wheat and rye chromosomes. The recombination events were determined by a break in linkage between the rye glutelin locus, tightly linked to the centromere, and a heterochromatic telomere, both present on the long arm of rye chromosome 1R (1RL). The glutelin phenotype was determined by SDS-PAGE and the heterochromatic telomere was cytologically observable by Giemsa C-Banding. The recombinant lines that were anlysed, lacked the rye glutelin locus but contained the rye telomere.

The cytogenetic analysis involved crossing the recombinant lines to wheat group 1 monosomic, ditelosomic and double ditelosomic stocks. By observing the pairing behaviour of the recombinant chromosome in the progeny of these crosses, the wheat chromosome involved in the recombination event could be determined. Of the three recombinant lines analysed, it appeared that each of the group 1 wheat chromosomes (1A, 1B and 1D) had recombined with 1RL.

Reference: Koebner R.M.D. and Shepherd K. W. (1985). Induction of recombination between rye chromosome 1RL and wheat chromosomes. Theor. Appl. Genet. 71:208-215.

LARGE DUPLICATIONS IN THE MITOCHONDRIAL DNA OF LIZARDS.

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Whereas the great majority of animal mtDNAs are between 16 to 18 kilobases (kb) in length, the mtDNAs of some lizards from the genera Cnemidophorus (Teiidae) and Heteronotia (Gekkonidae) vary from 17 to 26 kb. Detailed analyses of several long mtDNAs from Cnemidophorus have shown that, in each case, the additional DNA stems from a large tandem duplication. The duplications vary in genetic content, although all but one include the control region. The variation in the location of the duplications suggest that they have arisen independantly. In one of the duplication genomes, a secondary modification (deletion) has probably created a pseudogene. Preliminary data for Heteronotia mtDNA suggest that the additional mtDNA is endogenous and probably derived by duplication. The size of the duplications is more variable in Heteronotia and the long mtDNAs appear to be restricted to the triploid parthenogenetic form. The presence of duplicate regulatory sequences and structural genes in these genomes provides a unique system to investigate (i) the control of mtDNA transcription and replication, and (ii) the dynamics of sequence divergence in the absence of functional constraints.

POSTER 28

SENSITIVITY OF *IN SITU* HYBRIDIZATION WITH SINGLE-STRANDED RNA PROBES IS INCREASED DRAMATICALLY BY POLYETHYLENE GLYCOL.

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In situ hybridization is a uniquely potent tool for DNA sequence analysis, providing a direct link between classical genetics, cytogenetics, and molecular genetics. Applications vary from routine qualitative analyses of interphase cells (to discriminate between individual cells containing different levels of a particular sequence) to sub-chromosomal mapping of single-copy genes on differentially-stained metaphase spreads. The resolution required varies accordingly. In contrast, sensitivity remains a prime consideration in all applications since it is clearly desirable to obtain results as quickly as possible while minimising expense.

The resolution attainable can vary between the extremes afforded by [^{125}I]-labeled probes (low resolution, high sensitivity) and affinity-labeled probes (high resolution but relatively poor sensitivity). A satisfactory compromise in most cases is to use [^{3}H]-labeled probes, an option that is made even more attractive by their stability and ease of preparation. The high sensitivity required for hybridization to single-copy sequences has previously been achieved by using self-complementary DNA probes in the presence of dextran sulphate, the probe being prepared with multiple [^{3}H]-labeled nucleotides by nick translation.

Nevertheless, RNA probes are preferable to DNA, due in part to the higher rate of formation and stability of hybrids and partly to the ability to minimise background by post-hybridization treatment with ribonuclease. Preparation of such probes has become quite trivial with the advent of cloning vectors containing strong, highly specific promoters for bacteriophage RNA polymerases (SP6, T7, T3).

A recent report (Amasino, 1986) has shown that the inclusion of polyethylene glycol (PEG) in Southern hybridizations with single-stranded probes provides sensitivity comparable to that of self-complementary DNA in dextran sulphate. In extending these studies to *in situ* hybridizations, we find similarly that PEG dramatically increases the sensitivity afforded by single-stranded RNA probes. We have yet to test multiple-labeled probes under these conditions, but it seems safe to assume that their use would greatly facilitate the mapping of single-copy genes.

Amasino, R.M. (1986) Anal. Biochem. **152**, 304-307.

LOCATIONS OF TRADE DISPLAYS AND POSTERS