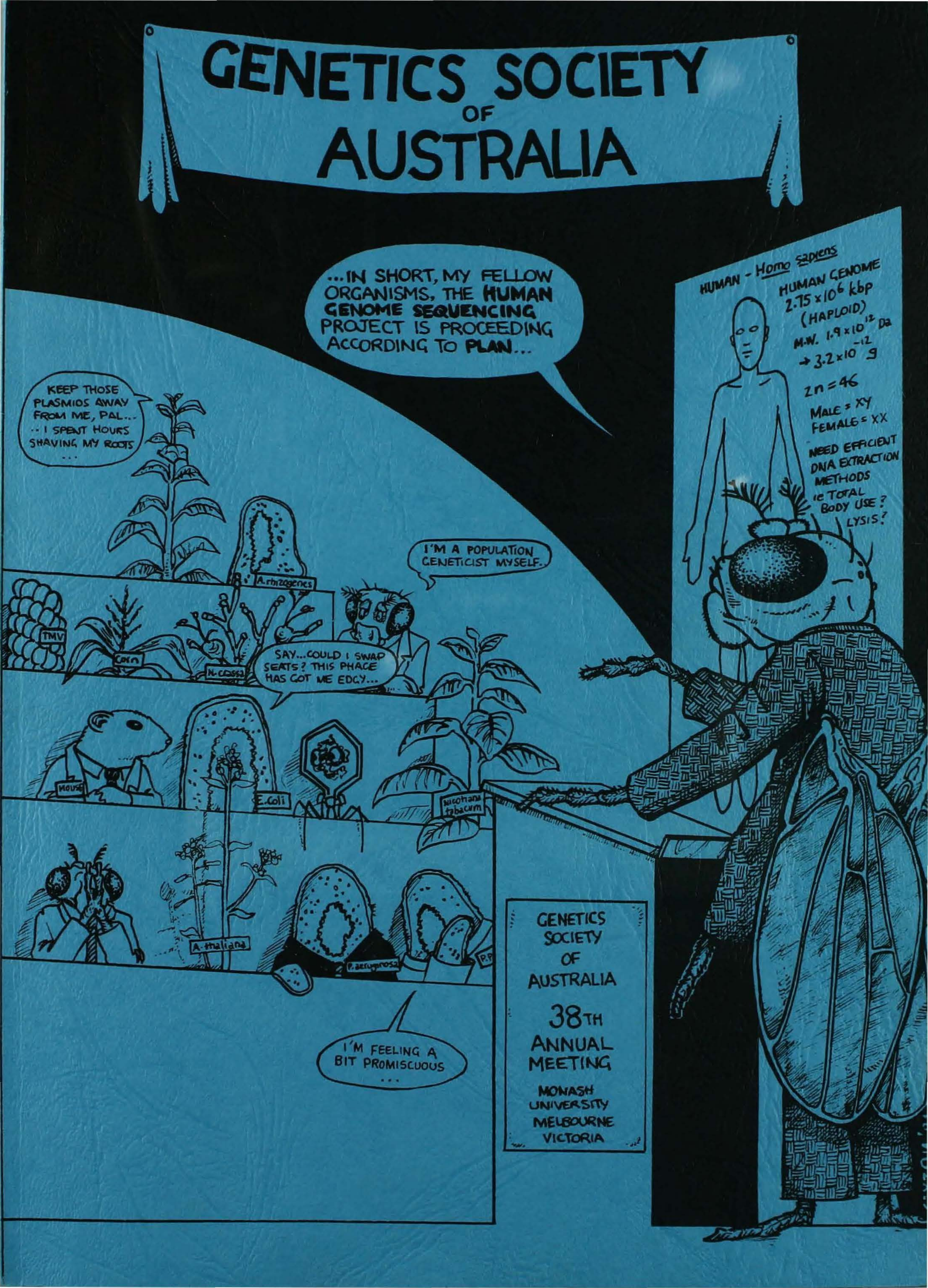
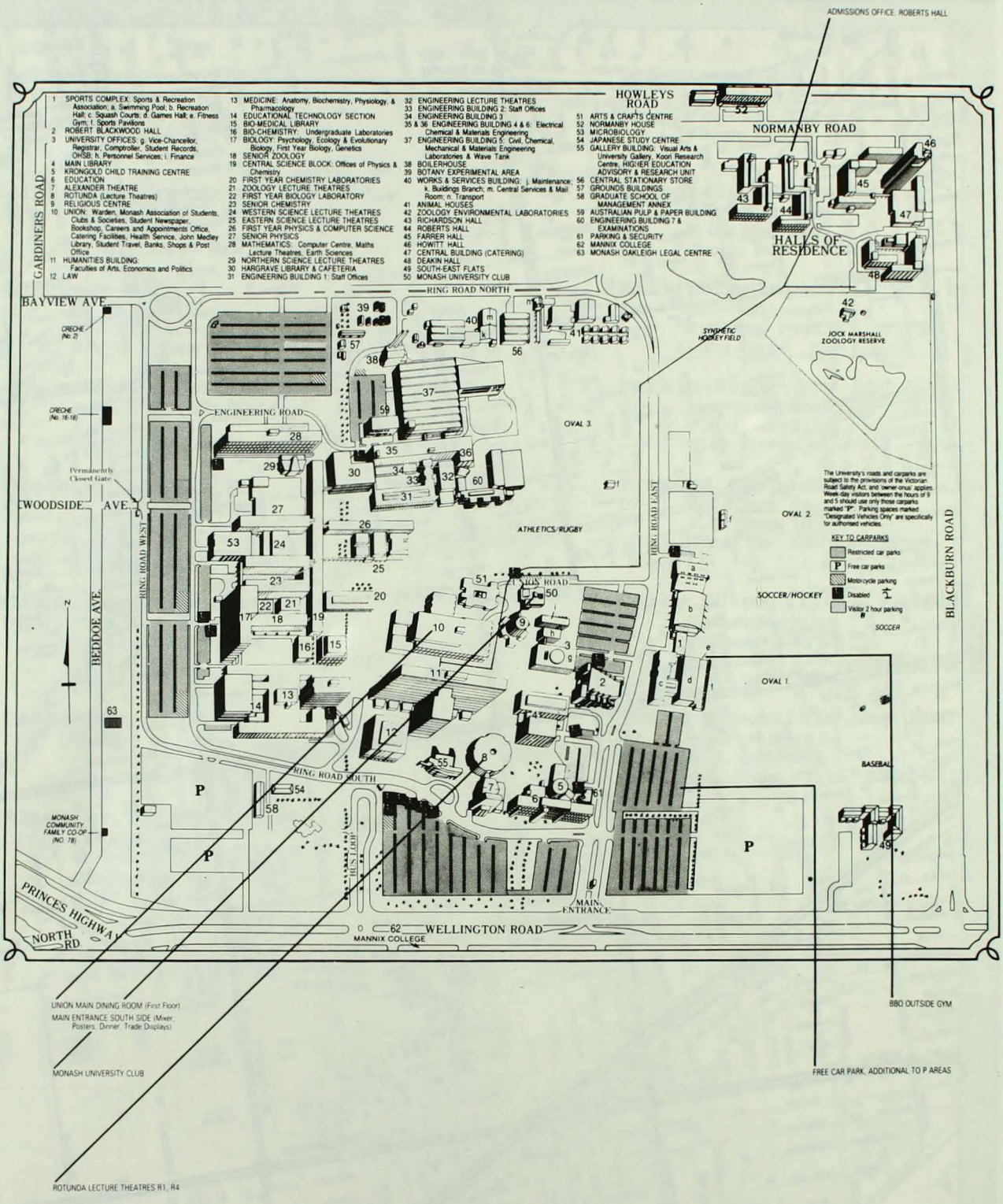

GENETICS SOCIETY OF AUSTRALIA
...IN SHORT, MY FELLOW ORGANISMS, THE HUMAN GENOME SEQUENCING PROJECT IS PROCEEDING ACCORDING TO PLAN...
KEEP THOSE PLASMIDS AWAY FROM ME, PAL... ..I SPENT HOURS SHAVING MY ROOTS ...
HUMAN - Homo sapiens
HUMAN GENOME
2.75 x 10^6 kbp
(HAPLOID)
M.W. 1.9 x 10^12 Da
→ 3.2 x 10^-12 g
2n = 46
MALE = XY
FEMALE = XX
NEED EFFICIENT DNA EXTRACTION METHODS
ie TOTAL BODY USE? LYSIS?
I'M A POPULATION GENETICIST MYSELF.
A.rhizogenes
TMV
corn
N.crassa
SAY...COULD I SWAP SEATS? THIS PHAGE HAS GOT ME EDGY...
MOUSE
E.coli
Nicotiana tabacum
A.thaliana
P.aeruginosa
P.P
I'M FEELING A BIT PROMISCUOUS ...
GENETICS SOCIETY OF AUSTRALIA
38TH ANNUAL MEETING
MONASH UNIVERSITY
MELBOURNE
VICTORIA

DRSmyth


ADMISSIONS OFFICE: ROBERTS HALL
HOWLEYS ROAD
NORMANBY ROAD
HALLS OF RESIDENCE
GARDINERS ROAD
BAYVIEW AVE
CRECHE (No. 2)
CRECHE (No. 16-18)
Permanently Closed Gate
EWOODSIDE AVE
BEDDOE AVE
RING ROAD WEST
RING ROAD NORTH
RING ROAD EAST
RING ROAD SOUTH
ENGINEERING ROAD
OVAL 3
OVAL 2
OVAL 1
ATHLETICS/RUGBY
SOCCER/HOCKEY
SOCCER
BASEBALL
SYNTHETIC HOCKEY FIELD
JOCK MARSHALL ZOOLOGY RESERVE
BLACKBURN ROAD
BUS LOOP
MAIN ENTRANCE
WELLINGTON ROAD
MANNIX COLLEGE
PRINCES HIGHWAY
NORTH RD
MONASH COMMUNITY FAMILY CO-OP (NO. 78)
1 SPORTS COMPLEX: Sports & Recreation Association; a. Swimming Pool; b. Recreation Hall; c. Squash Courts; d. Games Hall; e. Fitness Gym; f. Sports Pavilions
2 ROBERT BLACKWOOD HALL
3 UNIVERSITY OFFICES: g. Vice-Chancellor, Registrar, Comptroller, Student Records, OHSB; h. Personnel Services; i. Finance
4 MAIN LIBRARY
5 KRONGOLD CHILD TRAINING CENTRE
6 EDUCATION
7 ALEXANDER THEATRE
8 ROTUNDA (Lecture Theatres)
9 RELIGIOUS CENTRE
10 UNION: Warden, Monash Association of Students, Clubs & Societies, Student Newspaper, Bookshop, Careers and Appointments Office, Catering Facilities, Health Service, John Medley Library, Student Travel, Banks, Shops & Post Office
11 HUMANITIES BUILDING: Faculties of Arts, Economics and Politics
12 LAW
13 MEDICINE: Anatomy, Biochemistry, Physiology, & Pharmacology
14 EDUCATIONAL TECHNOLOGY SECTION
15 BIO-MEDICAL LIBRARY
16 BIO-CHEMISTRY: Undergraduate Laboratories
17 BIOLOGY: Psychology, Ecology & Evolutionary Biology, First Year Biology, Genetics
18 SENIOR ZOOLOGY
19 CENTRAL SCIENCE BLOCK: Offices of Physics & Chemistry
20 FIRST YEAR CHEMISTRY LABORATORIES
21 ZOOLOGY LECTURE THEATRES
22 FIRST YEAR BIOLOGY LABORATORY
23 SENIOR CHEMISTRY
24 WESTERN SCIENCE LECTURE THEATRES
25 EASTERN SCIENCE LECTURE THEATRES
26 FIRST YEAR PHYSICS & COMPUTER SCIENCE
27 SENIOR PHYSICS
28 MATHEMATICS: Computer Centre, Maths Lecture Theatres, Earth Sciences
29 NORTHERN SCIENCE LECTURE THEATRES
30 HARGRAVE LIBRARY & CAFETERIA
31 ENGINEERING BUILDING 1: Staff Offices
32 ENGINEERING LECTURE THEATRES
33 ENGINEERING BUILDING 2: Staff Offices
34 ENGINEERING BUILDING 3
35 & 36 ENGINEERING BUILDING 4 & 6: Electrical Chemical & Materials Engineering
37 ENGINEERING BUILDING 5: Civil, Chemical, Mechanical & Materials Engineering Laboratories & Wave Tank
38 BOILERHOUSE
39 BOTANY EXPERIMENTAL AREA
40 WORKS & SERVICES BUILDING: j. Maintenance k. Buildings Branch; m. Central Services & Mail Room; n. Transport
41 ANIMAL HOUSES
42 ZOOLOGY ENVIRONMENTAL LABORATORIES
43 RICHARDSON HALL
44 ROBERTS HALL
45 FARRER HALL
46 HOWITT HALL
47 CENTRAL BUILDING (CATERING)
48 DEAKIN HALL
49 SOUTH-EAST FLATS
50 MONASH UNIVERSITY CLUB
51 ARTS & CRAFTS CENTRE
52 NORMANBY HOUSE
53 MICROBIOLOGY
54 JAPANESE STUDY CENTRE
55 GALLERY BUILDING: Visual Arts & University Gallery, Koori Research Centre, HIGHER EDUCATION ADVISORY & RESEARCH UNIT
56 CENTRAL STATIONARY STORE
57 GROUNDS BUILDINGS
58 GRADUATE SCHOOL OF MANAGEMENT ANNEX
59 AUSTRALIAN PULP & PAPER BUILDING
60 ENGINEERING BUILDING 7 & EXAMINATIONS
61 PARKING & SECURITY
62 MANNIX COLLEGE
63 MONASH OAKLEIGH LEGAL CENTRE
The University's roads and carparks are subject to the provisions of the Victorian Road Safety Act, and owner-onus applies. Week-day visitors between the hours of 9 and 5 should use only those carparks marked "P". Parking spaces marked "Designated Vehicles Only" are specifically for authorised vehicles.
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P Free car parks
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UNION MAIN DINING ROOM (First Floor)
MAIN ENTRANCE SOUTH SIDE (Mixer, Posters, Dinner, Trade Displays)
MONASH UNIVERSITY CLUB
ROTUNDA LECTURE THEATRES R1, R4
BBQ OUTSIDE GYM
FREE CAR PARK, ADDITIONAL TO P AREAS

Map 70

See Page 11 for Map Symbols.

1 kilometre (1000m) Australian Map Grid (AMG) shown in Red

Joins 61
Joins 69
Joins 79

Mt. Waverley
Jordenville
Syndal
Huntingdale
Oakleigh
Oakleigh East
Oakleigh South
Mt. Waverley
Glen Waverley
Clayton
Clayton North
Clayton South
Notting Hill
Melbourne
Ferntree Gully
Waverley
Stephensons
Forster
Blackburn
Princes
South Eastern
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Dandenong
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GENETICS SOCIETY OF AUSTRALIA
38TH ANNUAL MEETING
7TH – 10TH JULY 1991
MONASH UNIVERSITY, MELBOURNE

PROGRAM AND ABSTRACTS

General Organization

The Department of Genetics and Developmental Biology welcomes you to Monash University for our 38th Annual General Meeting.

All oral presentations will be held in the Rotunda lecture theatres R1 and R4. Chairpersons must keep strictly to time to permit movement between concurrent sessions. Slides should be given to the projectionist, located in the office at the first floor, before the start of sessions for loading onto carousels. Speakers are requested to contact their chairperson prior to the start of their session. Please include 5 minutes for discussion within the 20 or 30 minutes allocated for your talks.

Tea/coffee will be served in the foyer of R1/R4 or in the Main Dining Room of the Union. Initial Registration, Posters and Trade Displays will be in the Main Dining Room of the Union (first floor).

Posters

Posters will be viewed on Monday 8th July from 3.40 p.m. onwards in the Main Dining Room of the Union. Posters should be assembled on the boards by Monday lunchtime. Tea/coffee will be served. Posters will again be viewed during the lunch breaks on Monday 8th and Tuesday 9th July. People presenting posters are urged to be at their posters during these times. Posters are to be removed by 1.00 p.m. Wednesday, 10th July.

Registration

A desk will be set up for this purpose at the Mixer to be held at 7.00 p.m. on Sunday 7th July in the Main Dining Room of the Union. A similar desk will also be set up in the foyer of R1 during the meeting (8th–10th July). The cost of registration is $45 (full member), $25 (student) and free for students presenting paper/poster.

Meals

Arrangements will be made for boxed lunches (roast chicken, bread-roll, fruit cake, fruit and fruit juice) to be available from the Main Dining Room of the Union. Food/drinks are also available at the Union but service delays may be experienced due to limited facilities. The Monash University Club is available for meals and alcohol till 7.30 p.m. but temporary membership is essential for entry. Membership is acquired by the filling out of a card (free of charge) which will be available at the Registration Desk. A list of local restaurants will also be provided.

Conference dinner

This will be at 7.30 p.m. on Tuesday 9 July in the Banquet Room of the Union (first floor). The dinner will be of two courses with beer, wines and orange juice supplied. Music will be provided for dancing after the dinner. The cost is $35 per person (non-students) and $25 per person (students).

Conference barbecue

This will be from 1.00 p.m. on Wednesday 10 July outside the Fitness Gym of the Sports Complex. The cost is $10 per person.

Accommodation

This has been booked at the Halls of Residence. Those having bookings should go to the Admissions Office, Roberts Hall, Normanby Road, Clayton North 3168. It is located on the 1990 Melway Street Directory on Map 70, G9. Your key will be available at the office and you will be directed to particular Halls of Residence. The Office will be open till 9.00 p.m. Sunday 7th July. During the week it will be open 9.00 a.m.–9.00 p.m. The cost is $35.60/night (non-students) and $31.15/night (students).

All participants who have not yet paid must do so at the Registration desk.

For more information contact: Viji Krishnapillai, Local Organizing Secretary.

PROGRAM

MONDAY, 8TH JULY, LECTURE THEATRE R1

9.00 – 9.15 a.m. Introduction

Chair: Jim Peacock

9.15 – 10.15 a.m. GSA Guest Speaker's Address: Analysis of homeotic genes controlling floral organogenesis in Antirrhinum majus *H. Saedler, H. Sommer and P. Huijser*

10.15 – 10.45 a.m. Tea/coffee in R1/R4 foyer

Symposium: Plant developmental genetics – Chair: David Hayman

10.45 – 11.15 a.m. DNA methylation and floral development
W.J. Peacock, D. Bagnall, R.D. Brock, J. Burn, J. Davidson and E. Dennis

11.15 – 11.45 a.m. Genetic control of flower formation in Arabidopsis
D.R. Smyth

11.45 – 12.15 p.m. Gene expression during petal senescence
E.A. Cornish, M.W. Graham, S.C. Baudinette, M.Z. Michael and K. Savin

12.15 – 12.45 p.m. Polyamines, Agrobacterium rhizogenes and plant development
J.D. Hamill and M.Z. Moran

12.45 – 2.00 p.m. Lunch

Session 1A, Lecture Theatre R1

Plant genes and chromosomes – Chair: David Smyth

2.00 – 2.20 p.m. Mutations affecting incompatibility in Phalaris coerulescens
D.L. Hayman and J. Richter

2.20 – 2.40 p.m. Setting up a transposon tagging system to isolate rust resistance genes from flax
J.G. Ellis, E.J. Finnegan, and G.J. Lawrence

2.40 – 3.00 p.m. Heavymetal – sensitive mutants of Arabidopsis thaliana
C. Cobbett, C. Hanrahan and R. Howden

3.00 – 3.20 p.m. A sequence specific to B chromosomes of Brachycome dichromosomatica
C.R. Leach, U.P. John and J.N. Timmis

3.20 – 3.40 p.m. Gene flow and level of outcrossing in a seed orchard of Eucalyptus regnans.
Y. Fripp and A.R. Griffin

3.40 onwards Tea/coffee and Posters in Main Dining Room of Union

Session 1B, Lecture Theatre R4

Theoretical and population genetics – Chair: Ross Crozier

2.00 – 2.20 p.m. Use of DNA fingerprints in management to conserve eastern barred bandicoot in Victoria
N.A. Robinson, N.D. Murray and W.S. Sherwin

2.20 – 2.40 p.m. Modelling conservation genetic problems using captive Drosophila populations
R. Frankham, S.C. Borlase, D.A.F. Loebel, R.K. Nurthen, D.A. Briscoe, and G.E. Daggard

2.40 – 3.00 p.m. Fisher's variance formula in genetic analysis
A.E. Stark

3.00 – 3.20 p.m. Reduced genetic variation and species borders
M.W. Blows, M. Watson and A.A. Hoffmann

3.20 – 3.40 p.m. Interpretation of genetic distances based on highly polymorphic loci
M. Goddard

3.40 onwards Tea/coffee and Posters in Main Dining Room of Union

TUESDAY, 9TH JULY, LECTURE THEATRE R1

Symposium: Modelling in genetics – Chair: John Sved

9.00 – 9.30 a.m. Gene mapping using YACs and RFLPs
W.J. Ewens

9.30 – 10.00 a.m. Fitting Kacser – Rapoport metabolic control theory into quantitative genetics
O. Mayo

10.00 – 10.30 a.m. A model of gene conversion
R.C. Griffiths

10.30 – 11.00 a.m. Evolutionary genetics of host specialization in insect/plant interactions
J. Thompson

11.00 – 11.20 Tea/coffee in R1/R4 Foyer

Session 2, Lecture Theatre R1

Population and evolutionary genetics — Chair: Oliver Mayo

11.20 – 11.40 a.m. A stochastic analysis of three viral sequences
G.A. Watterson

11.40 – 12.00 noon Deficiency of the CpG dinucleotide in the vertebrate genome
J. Sved and A. Bird

12.00 – 12.20 p.m. Moments, cumulants and polygenic dynamics
R. Burger

12.20 – 12.40 p.m. Biochemical correlates of heritable variation in ethanol tolerance in Drosophila melanogaster
S.W. McKechnie and B.W. Geer

12.40 – 1.00 p.m. Cytoplasmic incompatibility in Drosophila simulans: recent results
A.A. Hoffmann, M. Turelli and S.W. McKechnie

1.00 – 2.00 p.m. Lunch

Session 3A, Lecture Theatre R1
Cytogenetics – Chair: Chris Gillies

2.00 – 2.20 p.m. Synaptonemal complex analysis in three different Robertsonian translocations in sheep
K. Dai, C.G. Gillies and A. Dollin

2.20 – 2.40 p.m. Mapping by linkage analysis in the tammer wallaby (Macropus eugenii): Lack of close linkage between mannose-phosphate isomerase and nucleotide phosphorylase
L. McKenzie, J.A. Donald, W.E. Poole, G.L. Woodlee, C. Collet, T.K. Bell and D.W. Cooper

2.40 – 3.00 p.m. Hybridization between local and introduced populations of Delena cancerides: chromosomal evidence
A. Hancock

3.00 – 3.20 p.m. Chromosomal fusion and chiasma position in Delena cancerides (Sparassidae:Arachnida)
D. Rowell

3.40 – 4.00 p.m. Tea/coffee in R1/R4 Foyer

Session 3B, Lecture Theatre R4
Microbial Molecular Genetics – Chair: Jean Mayo

2.00 – 2.20 p.m. Cloning and analysis of genomic regions associated with virulence in the causative agent of ovine foot rot reveals similarities to N. gonorrheae and E. coli
M.E. Katz, and J.I. Rood

2.20 – 2.40 p.m. Functional analysis of the transcriptional regulator amdR of Aspergillus nidulans
L. Parsons, M. Davis and M. Hynes

2.40 – 3.00 p.m. Structural and functional comparison of amdR from two Aspergillus species
X.W. Wang, M.A. Davis and M.J. Hynes

3.00 – 3.20 p.m. The effect of rec–2 on repeat–induced point mutations (RIP) and recombination events that excise DNA sequence duplications at the his–3 locus of Neurospora crassa
F.J. Bowring, and D.E.A. Catcheside

3.20 – 3.40 p.m. Localization of the ribosomal RNA operons in Pseudomonas putida PPN
R. Saffery, and A. Morgan

3.40 – 4.00 p.m. Tea/coffee in R1/R4 Foyer

Session 4A, Lecture Theatre R1
Human population genetics – Chair: Des Cooper

4.00 – 4.20 p.m. Alternative splicing of CD46 mRNA and association with RFLPs
A. Wilton, D.W. Cooper, D.F.J. Purcell, R.J. Johnstone, S.M. Russell and I.F.C. McKenzie

4.20 – 4.40 p.m. HLA-DP amplified fragment length polymorphism in human populations in the Asian Pacific region
S. Easteal

4.40 – 5.00 p.m. Studies on the VNTR region 3' to the C-Harvey-RAS oncogene in Caucasians and Australian aborigines
J. Roberts-Thomson, and B. Boettcher

5.30. p.m. Annual general meeting of GSA (lecture theatre R1)

7.30 p.m. Conference dinner (Banquet room, Union first floor)

Session 4B, Lecture Theatre R4
Gene structure – Chair: Tony Howells

4.00 – 4.20 p.m. Interactions at the stoned locus of Drosophila melanogaster
J. Merakovsky, T.Z. Petrovich and L.E. Kelly

4.20 – 4.40 p.m. Cloning the stoned gene of Drosophila melanogaster: A neurological gene at the base of the X-chromosome
J. Andrews, and L.E. Kelly

4.40 – 5.00 p.m. Molecular characterization of a second Drosophila glutamate decarboxylase gene
A.M. Phillips, L.B. Salkoff and L.E. Kelly

5.30 p.m. Annual general meeting of GSA (lecture theatre R1)

7.30 p.m. Conference dinner (Banquet room, Union first floor)

WEDNESDAY, 10TH JULY, LECTURE THEATRE R1

Session 5, Lecture Theatre R1

General genetics – Chair: Jenny Marshall-Graves

9.00 – 9.20 a.m. Mutation detection by chemical cleavage – improvements
J. Saleeba and R. Cotton

9.20 – 9.40 a.m. The lozenge gene(s) affect pattern formation in the developing retina of Drosophila melanogaster
P. Batterham, S. Benzer and J.A. Pollock

9.40 – 10.00 a.m. Fatal association, a phenomenon of dieldrin resistance in Lucilia cuprina
A. Davies,, and J.A. McKenzie

10.00 – 10.20 a.m. The evolution of phosphoglycerate kinase (PGK) sequences in marsupials and their relation to X-inactivation
T. Feferman, and D.W. Cooper

10.20 – 10.40 a.m. Unequal crossingover at a rust resistance gene (Rp) in maize
A. Pryor

10.40 – 11.00 a.m. Tea/coffee in R1/R4 Foyer

Chair: Michael Hynes

11.00 – 12.00 noon M.J.D. White address: P elements of Drosophila
J. Sved, L. Blackman and R. Colless

Session 6, Lecture Theatre R1

General genetics (continued) – Chair: Chris Moran

12.00 – 12.20 p.m. Mutation and evolution: lifespan reduction by transposons and premeiotic clusters of mutation
R.C. Woodruff

12.20 – 12.40 p.m. Molecular analysis of the hairless (hr) mutation of the mouse
C. Moran and J. Stoye

12.40 – 1.00 p.m. Gene silencing in mammalian cells by uptake of 5-methyldeoxycytidine–triphosphate into DNA
R. Holliday and T. Ho

1.00 p.m. Barbecue

POSTERS

1. 5S ribosomal RNA: when did it change addresses? *G. Adcock, A.G. Brownlee, B.J. Howlett and G.J. McFadden.*
2. Molecular conservation genetics of the helmeted honeyeater. *H.B. Allen, N.D. Murray and W.B. Sherwin.*
3. Terminal flower: a gene affecting inflorescence development in Arabidopsis thaliana. *J. Alvarez and C. Guli.*
4. Modifier genes and modifiable lozenge (lz) mutant phenotype in Drosophila melanogaster. *P. Batterham.*
5. Conflicting phylogenetic hypothesis for marsupials tested by mitDNA sequencing. *P.R. Baverstock, N. Higgs and A.M. Johnson.*
6. Expression of a recombinant Drosophila cystatin. *M. Brown*
7. RFLP phenotypes using an 18S ribosomal gene probe discriminate between breeding systems. *M. Byrne, M. Waycott and S. James.*
8. Cloning a sex influenced protein gene in chironomids. *Z.Z. Chen, S.A. Fabb, J. Martin and B.T.O. Lee.*
9. A diet responsive mutation system in Drosophila? *C. Driver.*
10. Synaptenemal complex analysis of hybrids between Australian Rattus species differing widely in chromosome number. *C.B. Gillies, and M. Mahony.*
11. Mitochondrial genome of the marsupial, Sminthopsis crassicaudata: partial DNA sequence and its analysis. *C. Hefford, N. Dear, H. Scott and R. Hope.*
12. Excision repair of pyrimidine dimers in mouse epidermal DNA. *R. Lan, G.E. Greenoak and C. Moran.*
13. Cloning of amdR, a regulatory gene from Aspergillus nidulans. *R. Lints, M.A. Davis and M.J. Hynes.*
14. Human chromosome 21 genes map in two conserved autosomal clusters in marsupials and monotremes. *P. Maccarone, J.M. Watson, D. Francis, I. Kola and J.A. Marshall-Graves.*
15. A polymorphic 37 bp insert within the first intron of Adh from D. melanogaster. *P. Mathew and S.W. McKechnie.*
16. Arabinose metabolism mutants of Arabidopsis thaliana. J. Medd, O. Dolezal and C. Cobbett.
17. Analysis of a DNA amplifying region in the salivary gland chromosomes of Rhynchosciara americana. *L.O. Penalva, A.J. Stocker, J.M. Amabis and F.J.S. Lara*
18. Molecular characterization and organization of the glycerol-3-phosphate oxidase gene (Gpo) in Drosophila melanogaster. *J. Ross and S.W. McKechnie.*
19. Characterization of genetic and morphological differentiation between hybridizing taxa of the varied Sittella complex (Aves: Neosittidae). *I. Scott.*
20. Localization of cis – acting sequences affecting nitrogen control of amdS in A. nidulans. *J. Sharp, M. Davis and M. Hynes.*
21. Distribution of a taq 1 mitDNA polymorphism amongst populations of the koala,Phascolarctus cinereus. *A. Taylor, N. Yahki, J.A. Marshall-Graves, N.D. Murray, W.B. Sherwin and S.J. O'Brien.*
22. A PCR genotyping test for the causal site of the A and B electrophoretic variants of Cattle B – lactoglobulin. *M.K. Tee, C. Moran, F.W. Nicholas and A.G. MacKinlay*
23. Evolutionary genetics of the HLA region. *G. Thomson.*
24. Genes affecting nuclear division in Drosophila melanogaster which are located in chromosome region 89A1–89A10. *M. Webster, P. Moretti and N. Brink.*
25. Genetic variation in tammar wallaby whey proteins. *G.L. Woodlee and D.W. Cooper.*

Talks in order of appearance on program

revertants of def do not give stamen revertants without petal revertants

Tam1–11
Tam7 def gli

① Isolation of new def alleles

def gli null × Tam1 ▽ CHS Transposon

↓

F1 100,000 plants screened

15 candidate intermediate alleles, 12 alleles for sure

(1 in 100,000)

② Isolation of new loci

Tam1 ▽ CHS × WT

↓

8 candidate new loci

(1 in 100)

- deficiens protein does not bind to SRE * → likely to be genes regulated by defA i.e. in filaments
- other cDNAs which show coexpression with defA

fil2 (62G10) one identified: leucine rich repeats transmembrane region ≡ Tell
upstream has SRE motif.

→ * but deficiens / globosa } cotranslated in vitro heterodimer does bind to SRE

sterilis
floricaula (EC)

squamosa (MADS) ↑ cloned Peter Huijser
expressed in all parts except stamens (≡ deficiens)

ap3 — deficiens (MADS)
pi — fimbriata / globosa (MADS)
viridiflora
sympetalla

ag — plena / petaloidea / pleniflora

ap2 — ovulata / macho — allelic (enrico)

macho dominant / plena recessive — allelic

globosa expressed in petal & stamen

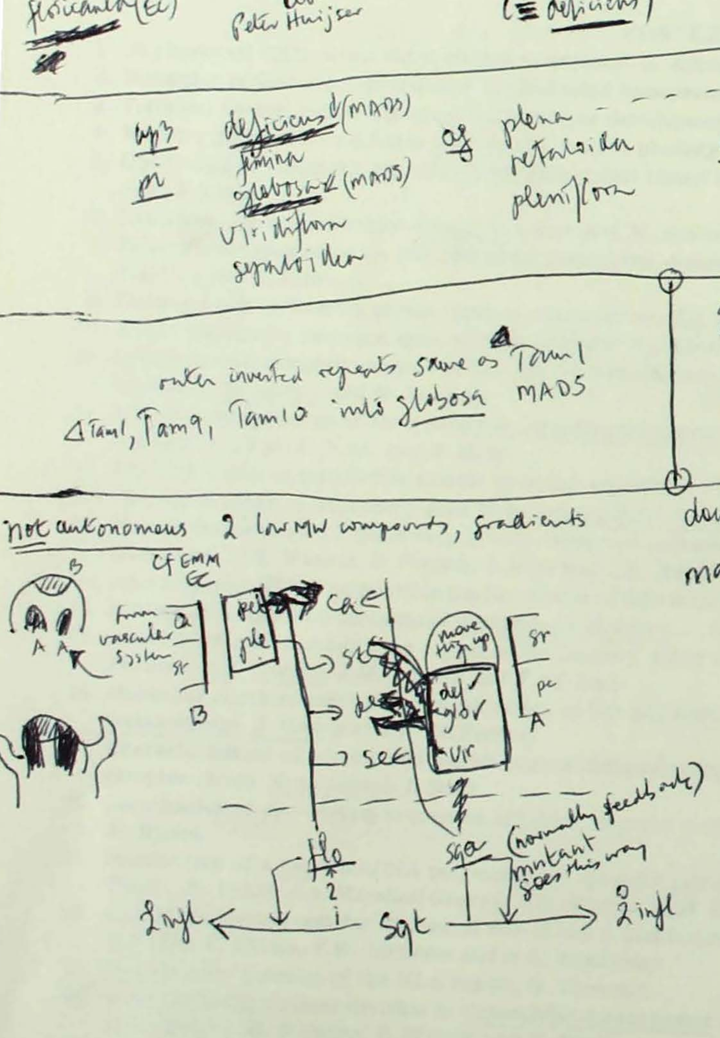
squamosa expressed in bract/sepal/petal (infl → inside inflorescence)
from MADS
very early in flower primordia + receptacle

outer inverted repeats same as Tam1
ΔTam1, Tam9, Tam10 into globosa MADS

double mutant
macho/def ca ca ca ca

can set flower of even in deletion mutant — squamosa
wt sets up concn in early flower primordia

not autonomous 2 low MW compounds, gradients



fimbriata petals only affected

fim/def double: only 3 whorls of sepals then gyno

ANALYSIS OF HOMEOTIC GENES CONTROLLING FLORAL ORGANOGENESIS IN *Antirrhinum majus*

Heinz Saedler, Hans Sommer, Peter Huijser,
Jose-Pio Beltran*, Wolfgang Nacken, Wolf-Ekkehard Lönnig,
Heike Pape, Peter Flor, Rolf Hansen, Zsuzsanna Schwarz-Sommer

Max-Planck-Institut für Züchtungsforschung,
5000 Köln 30, FRG

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Floral morphogenesis in *Antirrhinum majus* seems to be controlled by a set of homeotic genes (1). Three of these genes [*deficiens* (2), *globosa*, *squamosa*] have been isolated and their structure and expression pattern partially characterized by us. Mutations in *deficiens* and *globosa* lead to homeotic transformation of petals to sepals and stamens to carpels, while mutational inactivation of *squamosa* causes the production of "shoots" instead of flowers in the axils of the bracts, thus affecting an early step in flower morphogenesis.

Analysis of the temporal and spatial expression patterns of the three genes by *in situ* hybridization revealed that *deficiens* and *globosa* are turned on after the sepal primordia have appeared, at positions of the floral meristem where later the petal and stamen primordia arise. In contrast, *squamosa* is expressed much earlier, at the time when the flower primordium appears in the axils of bracts. Thus it is possible that *squamosa* is controlling early expression of *deficiens* and *globosa*.

The proteins encoded by the three genes are putative transcription factors: they have a conserved domain at the N-terminus in common that displays extensive homology to two known transcription factors, SRF of mammals and MCM1 of yeast. The conserved putative DNA-binding domain [the so-called MADS-box (1)] was found to be present in twelve aditional genes, the majority of which is expressd only in floral organs, in a specific manner. We speculate that specific combinations of these MADS-box genes, expressed in the respective organs, specify organ identity in the process of organogenesis.

1) Schwarz-Sommer,Zs., Huijser,P., Nacken,W., Saedler,H. and Sommer,H. (1990) Genetic control of flower development by homeotic genes in *Antirrhinum majus*. Science **250**, 931-936

2) Sommer,H., Beltran,J.P., Huijser,P., Pape,H., Lönnig, W.-E., Saedler,H. and Schwarz-Sommer,Zs. (1990) *Deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: the protein shows homolology to transcription factors. EMBO J. **9**, 605-613

DNA Methylation and Floral Development

Jim Peacock, D Bagnall, R D Brock, J Burn, J Davidson, E Dennis, CSIRO Division of Plant Industry, Canberra

It is important that plants flower at a time of the year which optimises the production of seed and makes provision for future generations. Many plants have evolved to respond to particular environmental cues which control the time of flowering in the annual cycle of seasons. Some respond to daylength, others respond to temperature and many plants that live in a temperate environment require an exposure to a period of cold temperature in order to flower weeks later in the spring. Some key observations have been made about vernalization, this period of cold requirement. Firstly, that the apex, the meristem of the plant, must experience the cold treatment and the cells in the apex have to be actively dividing at the time of cold treatment. A vernalization treatment given to an imbibed germinating seed can result in flowering several weeks later in many kinds of plants. The message received in the germinating seedling apex is apparently transmitted faithfully through much of the vegetative development time of the plant and finally leads to triggering of the development of the floral meristem. There appears to be a mitotic memory of the cold treatment resulting in the subsequent floral initiation. This epigenetic effect has been further proven by treatment of secondary meristems in leaves or other plant material which can subsequently give rise to a plant using tissue culture. The plants arising from a cold treated secondary apex can enter floral development while those that have not been treated cannot. Mitotic memory of vernalization parallels the behaviour of methylation of DNA. We propose that the methylation state of critical genes could determine the ability to flower or not. Vernalization would result in the demethylation of these critical genes and allow their expression. We have gained support for this hypothesis in experiments using 5 Azacytidine which we have found can substitute for vernalization treatments in at least some plants. This has led us to develop concepts of the control of flowering and has suggested a new mechanism of control of gene expression in plant development.

Genetic control of flower formation in *Arabidopsis thaliana*

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Four different genes are known in *Arabidopsis* which specifically disrupt the development of floral organs when in mutant form. Some of the changes are homeotic in that organs may develop in a manner appropriate to a different location within the flower. A pattern emerges from these changes in that two adjacent whorls of floral organ are usually affected, and the direction of change is mostly towards the adjacent unaffected organ type:

Organ whorl	1	2	3	4
Wild type	sepals	petals	stamens	carpels
Mutant				
apetala2	carpels	stamens	stamens	carpels
pistillata	sepals	sepals	carpels	carpels
apetala3	sepals	sepals	carpels	carpels
agamous	sepals	petals	petals	flower
Fields	---------A---------			
		---------B----------		
			----------C---------	

We have proposed that this pattern arises through the action of three overlapping fields in the early flower primordium (Bowman, J.L., Smyth, D.R. & Meyerowitz, E.M. 1991 Development **112**, in press). Sepals are specified by field A, petals by A and B in combination, stamens by B and C together, and carpels by field C. In *ap2* mutants field A is absent, in *pi* and *ap3* field B is missing while in *ag* field C does not arise. In addition if field A is absent, field C is present throughout the primordium, and conversely. Together these proposals can account for all the homeotic changes produced by mutations at the four loci singly and in multiply mutant combinations.

GENE EXPRESSION DURING PETAL SENESCENCE

E.C. Cornish, M.W. Graham, S.C. Baudinette, M.Z. Michael and K.Savin

Calgene Pacific Pty Ltd, 16 Gipps Street, Collingwood, Australia 3066

The process of petal senescence is associated with well defined biochemical and physiological changes. For both carnation and petunia flowers the onset of petal senescence is controlled by ethylene. Treatments which prevent ethylene biosynthesis by the corolla delay senescence, while exogenous application of ethylene accelerates the process.

cDNA clones of genes that are differentially regulated during petal senescence have been isolated from both carnation and petunia. These include cDNAs that show extensive homology to ACC synthase (1) and the tomato ethylene-related gene TOM13 (2). In addition, a petal-specific, senescence-associated gene has been isolated from carnation. The promoter region of this gene includes three 17bp sequences that have marked similarity to tomato sequences that have been shown to be involved in ethylene-mediated gene expression (3).

1. Van Der Straeten *et al* (1990). *Proc. Natl. Acad. Sci.* USA 87, 4859-4863.
2. Holdsworth *et al* (1987). *Nucleic Acids Res.* 15, 10600.
3. Cordes *et al* (1989). *The Plant Cell.* 1, 1025-1034

Polyamines, *Agrobacterium rhizogenes* and plant development

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Monash University, Clayton 3168, Victoria

Plants regenerated from tissues transformed with the bacterium *Agrobacterium rhizogenes* show alterations in development due to expression of Ri T-DNA. Typically these involve plagiogeotropism in roots, wrinkled leaves, a delay in the onset of flowering, lower fertility, short internodes and reduced apical dominance. Although the T-DNA of *A. rhizogenes* has been sequenced, and the individual ORFs responsible for the transformed phenotype have been identified, it is not clear how the expression of these genes has such profound effects upon plant development. Biochemical studies involving tobacco plants transformed with Ri T-DNA suggest that polyamine levels are reduced relative to non-transformed parental lines and that this reduction may be responsible for the alterations in development. We are investigating this putative link between polyamine metabolism and Ri T-DNA gene expression using tobacco and *Arabidopsis* transformed with *A. rhizogenes*. Experiments are being conducted using a biochemical approach, involving suicide inhibitors of polyamine metabolism and also using a genetic approach involving transformation with genes in the pathway leading to polyamine biosynthesis.

Mutations affecting incompatibility in *Phalaris coerulescens*.

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Phalaris coerulescens is a highly self-incompatible diploid perennial grass. Incompatibility is determined by two non-allelic genes *S* and *Z* each with a series of alleles. A pollen grain is incompatible if both the *S* and *Z* genes it contains are also present in the style. A system which has isolated self-incompatible mutants will be described, as will be the procedures adopted to detect the genetic change responsible for the mutation. The implications of the results will be discussed.

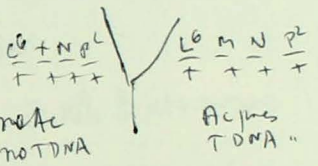
SETTING UP A TRANSPOSON TAGGING SYSTEM TO ISOLATE RUST RESISTANCE GENES FROM FLAX

Jeff Jean Greg
J.G. Ellis, E.J. Finnegan and G.J. Lawrence

Resistance to rust, a fungal pathogen of flax, is determined by dominant resistance genes that occur in 5 clusters or "loci" in the plant genome. The resistance gene products have not been identified and the biochemical basis of disease resistance is unknown. We are employing a transposon tagging approach to clone resistance gene from flax. This involves introducing the maize transposon Ac into flax by transformation with T-DNA vectors and isolating mutant disease susceptible plants caused by transposon insertion. Analysis of transgenic plants and their progeny indicates that Ac transposes in flax. Frequency of transposon movement has been increased by expression the Ac transposase gene *in trans* of a transposition deficient Ac element. Several disease resistance mutants have been isolated affecting the L6 resistance gene and are being analysed to determine whether they are caused by the presence of Ac.

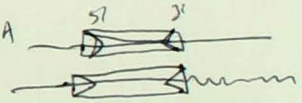
137 000 F1 plants screened.

Mutants: 1. M loci



2 L^6 loci T257 family 7 mutants /7,880
other families 11 " / 130 000

3' deletions from Ac → flax DNA



X224 deletion of Ac+M? Yes, <5 map units so far from M
X26 " " 3' DNA and L^6 No,
30
29

Heavy metal - sensitive mutants of Arabidopsis thaliana

Chris Cobbett, Cathy Hanrahan and Ross Howden

Department of Genetics
The University of Melbourne

We have adopted a genetic approach to investigate the mechanism/s by which plants respond to heavy metals by using the small crucifer, Arabidopsis thaliana. A screening procedure to identify mutants more sensitive to heavy metals has been devised. Mutagenised (M2) seeds are allowed to germinate in the absence of the heavy metal ions and then transferred to medium containing a partially inhibitory level of the heavy metal. The seedlings are examined for subsequent root growth and sensitive mutants are recovered. About 15,000 M2 seedlings have been examined for growth in the presence of cadmium and two independent cadmium-sensitive mutants have been isolated.

Both mutants display the same degree of cadmium-sensitivity and in addition are significantly more sensitive to mercury than the wildtype but are only slightly more sensitive to copper and zinc. This suggests that there is some level of specificity in the detoxification mechanism affected in these mutants.

Genetic studies have shown that the two mutations are allelic and that the phenotype segregates as a single recessive Mendelian character. The locus has been mapped close to the tt3 gene on chromosome 5. Further mapping studies using RFLP markers in this region have identified flanking RFLP clones which we are currently using to initiate a chromosome walk to isolate the gene involved.

tt3

tt3

291
247 close linkage on this side of 247
423
225

A SEQUENCE SPECIFIC TO *B* CHROMOSOMES OF *BRACHYCOME DICHROMOSOMATICA*.

Carolyn Leach, Ulrik John and Jeremy Timmis.
Department of Genetics, University of Adelaide.

Supernumerary *B* chromosomes represent one of many causes of numerical chromosome variation which exist in higher plants and animals. Sequences of DNA unique to *B* chromosomes of *Brachycome dichromosomatica* were enriched prior to cloning and resultant clones hybridising only to plants containing *B* chromosomes were further investigated. DNA sequences which were further characterised include members of a family of 176 bp tandem repeats which are specific to the *B* chromosomes of *B. dichromosomatica*, an annual Australian native plant species with only two pairs of *A* chromosomes and up to three dispensable *B* chromosomes. Sequence analysis of six related clones indicated that some regions of the sequence are more highly conserved than others or that some adenine residues at the *Nde* II site are methylated. The repeat is homologous to DNA from *B. ciliaris* var. languinosa but not to DNA from other closely or more distantly related taxa growing in the vicinity of the *B. dichromosomatica* populations.

NaeI CTAC pB3049
170 bp 2 cloned
176 " 4 "
Tandem repeats
1.8×10^5 copies ≡ 10% B chromosome

GENE FLOW AND LEVEL OF OUTCROSSING IN A SEED ORCHARD OF *EUCALYPTUS REGNANS*

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Current Address: Shell International Petroleum
(Tree Improvement)
Shell Centre London SE1 7NA

Samples of progeny from a number of trees in a seed orchard of *Eucalyptus regnans* have been collected over five years (1985 -1989) and assayed for their genotypes at a number of allozyme loci. This orchard, owned by APM Forests and located at Silver Creek, Gippsland, contains trees from both Victorian and Tasmanian provenances. Flowering time differences occur between some provenances. Before flowering commenced in 1987, the orchard was thinned heavily, from 185 to 42 trees per hectare. The data collected in this study will be used to investigate the effects of years, stand density and flowering time differences among trees on population parameters such as the level of outcrossing and the pattern of gene flow through pollen dispersal. The results currently available will be presented.

Use of DNA fingerprints in management to conserve eastern barred bandicoot in Victoria.

Nicholas A. Robinson [A], *Neil D. Murray* [A] *and William B. Sherwin* [B].

[A] Department of Genetics and Human Variation, La Trobe University.
[B] School of Biological Sciences, University of N.S.W.

Numbers of eastern barred bandicoot *Perameles gunnii* continue to decline in Victoria and population viability analysis suggests that wild Victorian populations of *P. gunnii* will be extinct within the next 11 years. Reduced genetic variability is likely to be accelerating the decline of *P. gunnii* in Victoria. Uncertainty exists over the current conservation status and protection afforded to *P. gunnii* in Tasmania. For these reasons, increasing emphasis is being placed on captive propagation with genetic management in efforts to maintain the species. Previous attempts to compare genetic variability amongst *P. gunnii* in Victoria with *P. gunnii* in Tasmania were uninformative as no protein polymorphism was detectable (27 loci, 74 individuals). Using DNA fingerprint and mitochondrial RFLP data, this project aims to: (1) determine relative levels of variability existing in wild populations of *P. gunnii* (2) promote genetic variability and minimise inbreeding amongst captive bred *P. gunnii* .

Blood samples for DNA analysis were collected from over 100 *P. gunnii* in the north and south of Tasmania and 100 *P. gunnii* derived from Hamilton Victoria. Samples were also collected from captive bred *P. gunnii* to genetically monitor a breeding program initiated by the State Department of Conservation and Environment in Victoria. Average percent differences (APD's) and proportions of fixed restriction fragments have been calculated for the DNA fingerprint profiles of individuals between and within sampled populations.

Relatively lower APD's and a greater proportion of fixed restriction fragments were found amongst *P. gunnii* in Tasmania than amongst *P. gunnii* in Victoria. These findings most likely reflect lower genetic variability and greater homozygosity in Tasmania relative to Victoria. APD's were particularly low amongst *P. gunnii* populations in the Huon Valley Tasmania. The results highlight: (1) the intrinsic importance of Hamilton *P. gunnii* as a source of species genetic variability for captive breeding purposes, (2) the need to thoroughly re-evaluate the conservation status and protection afforded to *P. gunnii* in Tasmania.

MODELLING CONSERVATION GENETIC PROBLEMS USING CAPTIVE POPULATIONS OF *DROSOPHILA*

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The biological diversity of the planet is rapidly being depleted through loss of habitat, hunting, pollution, etc. Further, many species now have fragmented distributions, such that they are susceptible to inbreeding depression. Consequently, many species require human intervention to ensure their survival and to optimise their genetic management.

The central issue in the genetic management of captive populations and endangered species are (i) maintenance of genetic variation (as it represents evolutionary potential) and (ii) avoidance of inbreeding depression. Relevant theory exists as a guide to managements, but it is predominantly simplistic single locus neutralist theory. In spite of the simplistic nature of the assumptions, most of it has not been subjected to experimental evaluation.

We have begun a program to evaluate underlying theories and problems in conservation genetics using captive populations of *Drosophila*. The effects of maintaining small (4 pairs) replicate populations with equalisation of family sizes (EFS) versus random choice of parents (RM) have been compared for their effects on inbreeding, allozyme heterozygosities, heritabilities for abdominal bristle number and reproductive fitnesses. Clear benefits over 10-11 generations from using EFS versus RM were found in terms of lowered inbreeding coefficients, higher heterozygosities, higher effective population sizes, and higher reproductive fitnesses, as expected. A surprisingly small difference in heritability was found between EFS and RM. Heritabilities in both were lower than in the outbred base population. Contrasts between results with different measures points to the need to evaluate theories in living organisms.

Fisher's Variance Formula in Genetic Analysis

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School of Community Medicine, University of NSW

R.A. Fisher (1928, *SMRW*, 2nd edition) gave a general formula for calculating the variance of an estimate T of a parameter ϑ, in relation to categorical data, when the expected frequencies are expressed in terms of ϑ (see Fisher, 1958, page 309). A simple algorithm based on this formula, which uses only the estimation (of T) procedure, that is without an explicit formula for the variance, gives good approximations to the variance of T. The algorithm, which resembles the jackknife estimate of variance, can cope with complex sampling and indeed demands that the sampling process be made explicit. It can be used for gene frequency estimation, segregation analysis, *inter alia*.

FISHER, Ronald A. (1958) *Statistical Methods for Research Workers*, 13th edition. Edinburgh: Oliver and Boyd.

STARK, Alan E. (1990) An algorithm for computing standard errors from categorical data. *Computational Statistics & Data Analysis* **10**:293-296.

Reduced Genetic Variation and Species Borders

Mark W. Blows, Marcus Watson and Ary A. Hoffmann

The question of species borders is fundamental to evolutionary biology: Why don't species continually evolve and expand their ranges? Much of the previous work on genetic differences between central and marginal populations has concentrated on comparing allozyme and inversion frequencies. The relevance of the observed reduction in inversion heterogeneity in marginal populations and, in general, the lack of such a reduction in heterogeneity in presumably neutral allozymes to the issue of range expansion is doubtful. However, very few attempts at measuring the levels of quantitative variation in central and marginal populations for ecologically important traits that may be partly responsible for the present position of a particular species border have been reported. Here we report realized heritabilities for desiccation resistance after 10 generations of selection in four populations of Drosophila serrata originating from the centre of its geographic distribution (Townsville) down to the southern most limit (Forster, 200km north of Sydney). The marginal population displayed no additive genetic variance for desiccation tolerance ($h^2 = 0.01 \pm 0.01$; mean $\pm$ SE) while the other three more northern populations displayed heritabilities between 0.08 $\pm$ 0.03 and 0.28 $\pm$ 0.03. Heritabilities calculated for body size and preliminary estimates of allozyme heterogeneity suggest that the marginal population does not generally have low levels of genetic variation. It is proposed that desiccation resistance has been selected for in the past in the marginal population and may be partly responsible for D. serrata not extending its range further south.

Interpretation of Genetic Distances based on Highly Polymorphic loci

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Recombinant DNA techniques have discovered classes of genetic markers which are highly polymorphic due to their high mutation rate. The B blood group locus in cattle is also highly polymorphic and considerable population data already exist on it. Therefore it may be a useful model for the newer highly polymorphic loci. An advantage of studying the evolution of cattle breeds is that the results of genetic analysis can be compared with the known history of the breeds.Two genetic distance measures, Wright's Fst and Nei's D, were calculated for pairs of American cattle breed using B phenogroup frequencies provided by Dr.H.C.Hines and Prof. C.Stormont.

Distances based on Fst and D do not correlate well. For instance, the distance between Hereford and Shorthorn are Fst=0.14 , D=0.68 and Holstein and Jersey are Fst=0.10 and D=3.65.

In the absence of mutation Fst is approximately e**(-t/2N) where t is generations since divergence and N is effective population size. The large Fst for Hereford-Shorthorn is due to the small N during the formation of these breeds. The importance of N and the high mutation rate means that more distant ralationships are difficult to estimate from Fst. However when Fst was used to estimate recent N the results were in reasonable agreement with historical evidence.

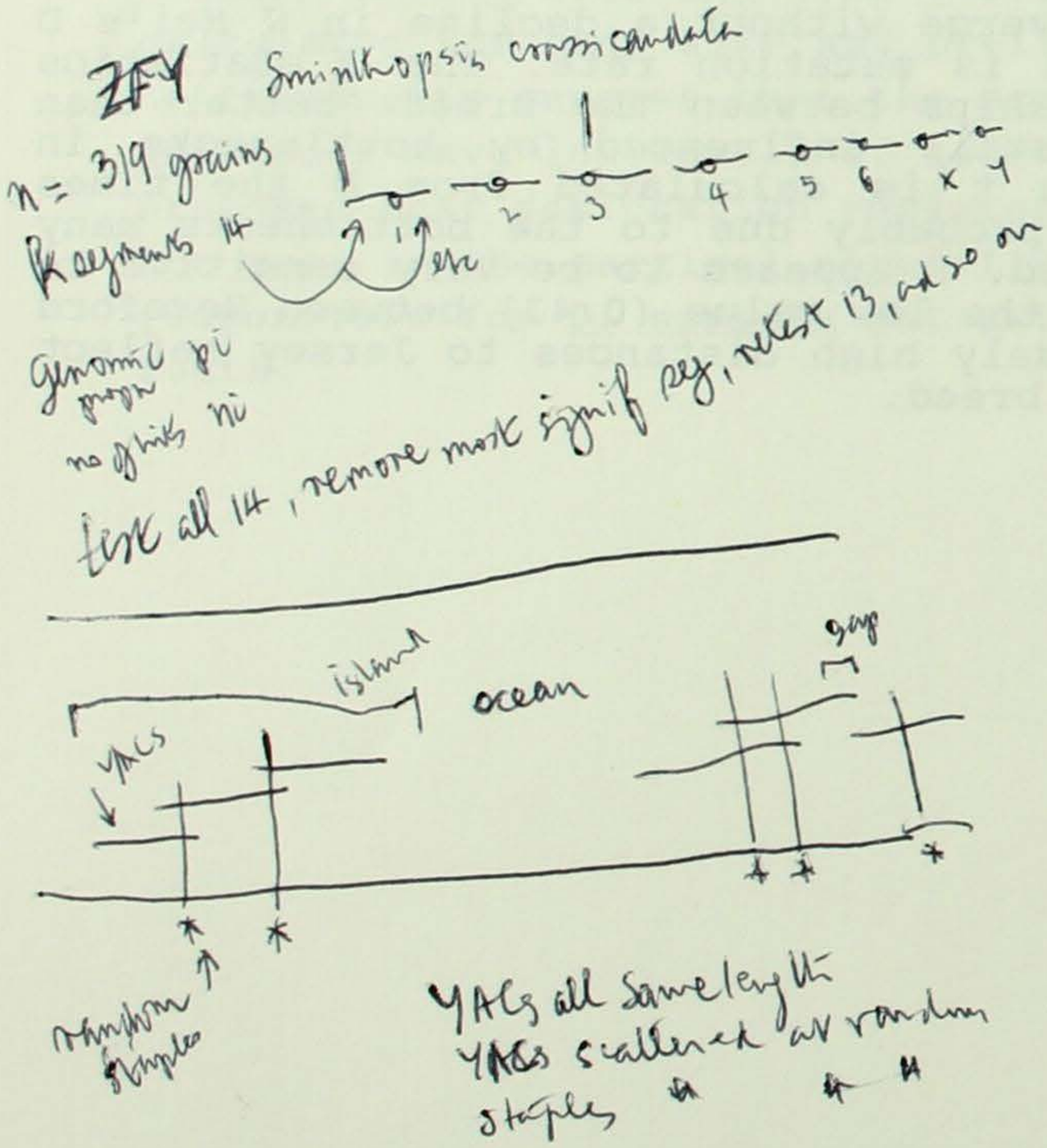
If two populations diverge without a decline in N Nei's D estimates 2ut where u is mutation rate. The D statistics estimate the relationships between the breeds better than Fst. However D is still influenced by bottlenecks in population size. When t is calculated from D the times appear a little long probably due to the bottlenecks many breeds have experienced. D appears to be very sensitive to migration as shown by the low value (0.43) between Hereford and Charolais. Conversely high distances to Jersey reflect the isolation of this breed.

Gene mapping using YACs and RFLPs

W.J.Ewens, Department of Mathematics, Monash University.

The theory associated with the construction of a physical map of the genome by using YAC clones will be discussed. These clones are "stapled", or "anchored", together by using RFLPs (or by other methods). The YACs thus "stapled" together form "islands", or "contigs". Theoretical questions arise concerning the mean island size, the mean inter-island size, the mean number of islands, etc., for which formulae will be given. These formulae contain, as parameters, the number of RFLPs and YACs available. An example will be given using *Arabidopsis* data.

Further theoretical problems arise in the procedure of *in situ* hybridization of DNA probes to sites of metaphase chromosomes. Hybridization with homologous gene probes usually produces a sharp peak in the distribution of locations of hybridization, and statistical analysis of hybridization site distribution is not really necessary. For more ambiguous data with secondary peaks, which could arise from pseudogenes or related sequences, a more sophisticated statistical technique is needed: this is true also when probe and target sequence are derived from different species, where base sequence homology will be reduced. The recently developed z_{max} procedure, which is the appropriate statistical technique to be used in such cases, will be described, and an example using *Macropus eugenii* will be given.



FITTING KACSER-RAPOPORT METABOLIC CONTROL THEORY INTO QUANTITATIVE GENETICS

Oliver Mayo, CSIRO Division of Animal Production, Prospect, NSW 2149

Two models have been proposed for the genetical determination of flux through a metabolic pathway as a quantitative trait. These models will be compared and contrasted.

A proposal will be presented for testing the utility of the theory for the understanding of selection response.

A model of gene conversion

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This is a model for the evolution of a population of chromosomes, where each chromosome has a multigene family at n loci. Allele types may be repeated along the loci. In the evolution of the population mutations occur which produce new allele types, and a gene conversion event occurring between locus i and locus j within a multigene family on a chromosome converts the allele type of the gene at locus j to the allele type of the gene at locus i.

Questions of interest about the population of chromosomes are:

- the probability that genes at two loci from a multigene family on a chromosome are of the same allele type,
- the probability that genes at two loci from multigene families on different chromosomes are of the same allele type,
- the probability distribution of the number of alleles on r chromosomes.

EVOLUTIONARY GENETICS OF HOST SPECIALIZATION IN INSECT/PLANT INTERACTIONS

John N. Thompson (Departments of Botany and Zoology, Washington State University, Pullman, Washington 99164, USA)

Much of the extraordinary diversity of phytophagous insects seems to have arisen through speciation onto different host plants. Genetic models of host shifts and speciation in these insects are often built upon the assumption that host selection and larval performance are under simple genetic control, perhaps even a single locus for host selection and another locus for larval survival on the host. Some alternative models are based upon the assumption that oviposition preference and larval performance may be pleiotropic effects of the same locus or loci. These assumptions have been tested using swallowtail butterflies in the *Papilio machaon* species complex in North America.

Interspecific crosses between two species that differ in the plant families on which they feed have shown that the differences in oviposition preference between these species are due primarily to one or more loci on the X chromosome, which are modified by one or more autosomal loci.

Larvae of these species are able to survive on a broader range of host plants than that used by females when ovipositing. The ability of larvae to survive on potential host plants appears to be controlled by a different set of loci from those controlling oviposition preference. Autosomal loci and maternal effects influence survivorship, development rate, and pupal mass achieved on different hosts. Consequently, oviposition preference and larval performance in these species are not simple pleiotropic effects of one gene locus or group of closely linked loci.

The localization to one chromosome of major interspecific differences in oviposition preference and the finding of different modes of inheritance in oviposition preference and larval performance make this species complex particularly useful for future studies of the origins of insect diversity through host shifts.

A Stochastic Analysis of Three Viral Sequences

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The paper analyses the nucleotide sequences of three viruses, Kunjin, West Nile, and Yellow Fever. We use the sequence for Kunjin virus as established by Coia *et al.*(1988), and the West Nile and Yellow Fever sequences which they used for comparison purposes. In each virus, one long open reading frame, of over 10,200 nucleotides, codes for four structural and seven non-structural genes.

The Kunjin and West Nile viruses are clearly the most closely related pair, when assessed on the basis of matches between their nucleotide sequences. As would be expected, the matching is least for bases at third position codon sites and greatest for second position sites.

To each of the 33 virus–gene segments, non-homogeneous Markov chain models have been fitted to describe the sequences of nucleotide bases. The models allow for different transition probabilities, and different degrees of dependency, at the three sites in the codons. Reasonably satisfactory fits can be obtained for many of the genes using models which are 1st order, for both first and second position sites in the codon, but which are 2nd order for third position sites. One consequence of such a model is that the correlation, between one amino-acid and the next, is limited to the correlation of the last base of the former with the first base of the latter. Another consequence is that the model can (and does) prohibit the occurrence of stop codons within a gene.

Stationarity of nucleotide base distributions can be interpreted in either of two ways, spatially along the sites, or temporally at each site. These interpretations must often be inconsistent, when the former allows for Markov dependence between adjacent sites whereas the latter assumes independence between sites. The inconsistency may effectively vanish if sub-sequences at different codon positions are analysed separately.

DEFICIENCY OF THE CpG DINUCLEOTIDE IN THE VERTEBRATE GENOME

John Sved, School of Biology, University of Sydney; Adrian Bird, Institute of Cell and Molecular Biology, University of Edinburgh

Vertebrate genomes show a systematic depletion of the CpG dinucleotide, to around 20% of expected levels. It is generally accepted that this depletion is a result of continued mutation from methyl-C to T. Calculations by Sved and Bird (PNAS 87: 4692, 1990) have shown that it is likely that currently observed levels of CpG represent a balance between mutation to and from this dinucleotide.

The calculations made by Sved and Bird assumed that all transition probabilities other than CpG to TpG and CpA are equal. However it is known that mutation from CpG to TpG results in the formation of a mismatched T/G nucleotide pair, and that repair of this nucleotide pair usually results in the restoration of the C/G nucleotide pair rather than the formation of T/A. (The excess of mutation from CpG occurs in spite of this mechanism). It is not known whether the biased repair mechanism occurs in all cases of T/G mismatching or whether it occurs only in cases where the mutated T occurs 5' to G.

It might therefore be expected that when T mutates to C, which presumably occurs at the "normal" transition rate, restoration of the original T occurs at much less than 50%. Any other transition mutation, however, is expected to be repaired back to the original condition 50% of the time. By this mechanism,the T to C mutation rate is expected to be about twice as great as other transition rates, excepting the methyl-C to T rate.

Calculations will be presented which show that this change in the back-mutation rate to CpG makes some, but not a great deal, of difference to the expected equilibrium. This conclusion is true irrespective of whether the enlarged back-mutation rate occurs in the restricted or more general case.

Moments, Cumulants, and Polygenic Dynamics

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Abstract. Several attempts have been made to establish the dynamics of the distribution of a polygenic character under selection (eg. Lande, 1975; Barton and Turelli, 1987; Turelli and Barton, 1990; Frank and Slatkin 1990). In this talk, a new approach for describing the evolution of polygenic traits subject to selection and mutation is presented. Differential equations for the change of cumulants of the allelic frequency distribution at a particular locus and for the cumulants of the distributions of genotypic and phenotypic values are derived. The derivation is based on the assumptions of random mating, no sex differences, absence of random drift, additive gene action, linkage equilibrium, and Hardy-Weinberg proportions. Cumulants are a set of parameters that, like moments, describe the shape of a probability density. Compared with moments, however, they have properties that make them a much more convenient tool for investigating polygenic traits. Applications to directional and stabilizing selection are given.

Biochemical correlates of heritable variation in ethanol tolerance in *Drosophila melanogaster*

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To help elucidate mechanisms of ethanol tolerance isochromosomal lines with different second chromosomes were fed ethanol and examined for associations between growth traits and biochemical characteristics previously implicated in the determination of tolerance variation. We measured levels of enzyme activities, flux from ethanol to lipid and characteristics of fatty acids. A high degree of positive association occurred among the variables. A partial correlation analysis controlling for performance on ethanol-free medium revealed a strong association between the degree of long-chain fatty acid content and line survival when ethanol was fed. Using the Canton S strain growth and ethanol tolerance characteristics were measured on cultures previously raised on diets supplemented with particular fatty acids. These supplements caused modifications to body lipid content. Results were consistent with the hypothesis that the lipid content of body tissues, especially the levels of long-chain fatty acids in cell membranes, may influence spatial and interspecific variation in ethanol tolerance.

CYTOPLASMIC INCOMPATIBILITY IN *DROSOPHILA SIMULANS*: RECENT RESULTS

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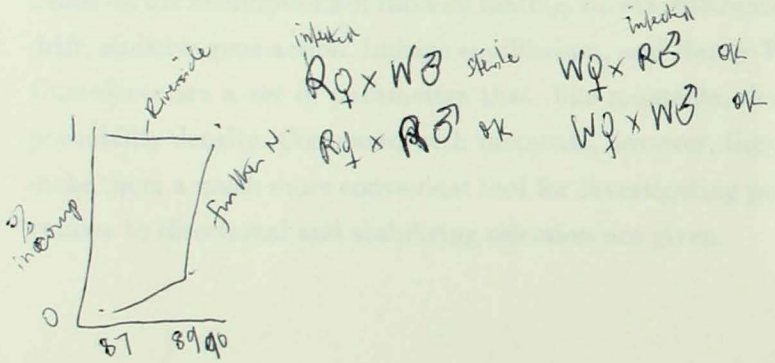
Drosophila simulans harbours an endoparasite that enhances its spread by manipulating the fertility of *D. simulans* females. When females from an infected strain are mated with males from an uninfected strain, few progeny are produced, whereas the reciprocal mating and crosses within infected (type R) and uninfected (type W) strains are fertile. The infection is vertically transmitted and is widespread because type R strains have been collected from many parts of the world. The endoparasite is expected to spread rapidly in a population and all members of a species are eventually expected to become infected, although laboratory and field work with *D. simulans* show that segregation and deleterious fitness effects can slow the rate of spread. Repeated sampling from several sites in California indicate that the infection is spreading rapidly northwards, providing indirect estimates of dispersal rates in *D. simulans*. This process is also causing the rapid spread of a mitochondrial DNA variant associated with the infection. These results suggest that endoparasites might be useful for rapidly introducing deleterious cytoplasmic factors into insect populations.



SYNAPTONEMAL COMPLEX ANALYSIS IN THREE DIFFERENT ROBERTSONIAN TRANSLOCATION SHEEP

I. SYNAPTONEMAL COMPLEX ANALYSIS IN NORMAL DOMESTIC SHEEP (*OVIS ARIES*)

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Abstract. Synaptonemal complexes have been analysed in normal domestic sheep (*Ovis aries*) spermatocytes with microspreading and settling techniques. The total complement lengths obtained by the two techniques are not significantly diffferent. The mean total length is 338 μm (n=73, S.D.=53.87). The SCs tend to increase in length from early to late pachytene although the increase is not significant. In 13 nuclei where the X and Y axes are measurable the relative lengths of the X and Y axes are 19.03% (S.D.=3.15) and 3.72% (S.D.=0.7) respectively. Based on synapsis and desynapsis of X and Y axes, six morphological types of XY pairs have been described. Type 0 occurs during late zygotene; types I and II occur during early pachytene; types III and IV occur from mid to late pachytene. Type V XY pairs are observed only in early diplotene nuclei. XY-SC is characterised by (1) short arm telomeric ends pair first, (2) the entire Y can take part in pairing, (3) length varies as stage progresses. Autosomal acrocentric bivalents sometimes have a drum stick configuration with the proximal end bulging at late pachytene. The bulging regions are probably the sites at which desynapsis commences. NORs have been observed on telomeric ends of 2 to 5 autosommal bivalents from early to mid pachytene. These bivalents include the 3 metacentric pairs and one large and one small acrocentric pair. At zygotene and late pachytene the NOR number may be over 5 because the NOR-bearing axes are unpaired or separated.

MAPPING BY LINKAGE ANALYSIS IN THE TAMMAR WALLABY (*MACROPUS EUGENII*): LACK OF CLOSE LINKAGE BETWEEN MANNOSE-PHOSPHATE ISOMERASE AND NUCLEOSIDE PHOSPHORYLASE

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The tammar wallaby can be bred in captivity in colonies of several hundred and its reproduction manipulated so that each female can produce at least 4-5 offspring a year (1). It has a 2n = 16 karyotype and an XX/XY female/male sex chromosome dimorphism. The male map distance is probably about 1150 cMs (2). The generation time is one year for females and nearly two for males. We are surveying the species for genetic variation so that markers can be found to do gene mapping. We have crossed two geographically distant races of the species, one from Kangaroo Island in South Australia and the other from Garden Island in Western Australia. These two races readily hybridise and the F1s of both sexes are fertile. This cross is similar in its power for performing linkage analysis to interspecific crosses between *Mus musculus* and *Mus spretus* (3). Over 70 backcross offspring have been produced from matings between F1 males and Kangaroo Island females and we plan to bring the total to about 200. To date twelve large gene frequency or fixed differences for allozyme, protein and RFLP variation have been defined between the races: the autosomal markers are transferrin (TF), protease inhibitor (PI), late lactation protein (LLP), mannose phosphate isomerase (MPI), purine nucleoside phosphorylase (NP), several phosphoglycerate kinase pseudogenes (PGKps), albumin (ALB), slow alpha-2 casein (CASAS2), and plasma esterase (EST). The sex linked marker is ribosomal RNA (RNR) and the mitochondrial marker is cytochrome C oxidase (COX1). The Nei genetic distance between the two races is approximately 0.15. In addition, fourteen loci have been found to vary at polymorphic frequency within the Kangaroo Island population.

Since MPI and NP are syntenic in a number of eutherian species i.e. rhesus monkeys, baboons, owl monkeys, mouse, lemurs, marmosets, domestic cats and pigs (4) and closely linked in the baboon (5), we investigated linkage between these two loci in a marsupial species. We have estimated the male recombination fraction between MPI and NP using the tammar backcross progeny. It is 0.39, n = 76 and chi-squared = 3.37, $p > 0.05$. Thus the two loci are not closely linked.

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"Hybridization between local and introduced populations of *Delena cancerides*: chromosomal evidence."

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Abstract

The social huntsman spider *Delena cancerides* consists of four chromosomal races, each differing in the nature of Robertsonian fusions present in the karyotype. Two of these races, the Victorian telocentric race TII and the central New South Wales chain of nine race CIX have come into contact in the Canberra area, following a suspected introduction of a number of Victorian spiders. Evidence for hybridization between these races comes from the electrophoretic data and chromosomal morphologies. Current work on the Canberra population has revealed breakage of chromosomal chains, caused by replacement of fusion metacentrics of the CIX race by the ancestral telocentrics found only in the telocentric race, TII. Furthermore, karyotypes beyond the 10 trivalents expected in the F1 hybrids suggests that hybrid derivatives (F2 and backcross) are also present in the population, and therefore that the F1 hybrid is fertile. It is hoped that a suitable statistic can be determined to enable mapping of both hybrids and hybrid dispersal patterns, using chromosomal morphology as an indicator of parentage.

Chromosomal Fusion in *Delena cancerides* (Sparassidae ; Arachnida)

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Delena cancerides consists of a number of chromosomal races which are characterised by the presence or particular combination of chromosomal fusion products in the karyotype. Races exist which are entirely homozygous either for fusion metacentrics or the ancestral telocentrics, or consist of a mixture fusions, some homozygous and others in a fixed heterozygous condition.

In a study of chiasma position in the various races it was found that, when in the homozygous condition, chromosomal fusion had little or no effect on chiasma position, nor was there any detectable chiasma interference across the centromere. However, in chromosome multiples resulting from heterozygosity for fusions there was a tendency for the chiasmata to form, on average in more distal positions.

Chromosomal fusion has been strongly linked to the speciation process as a result of the fact that complex fusion multiples rarely segregate correctly. It is suggested that here that temporary shifts in chiasma position through transient fusion polymorphism or hybridisation may also play a role in speciation.

CLONING AND ANALYSIS OF GENOMIC REGIONS ASSOCIATED WITH VIRULENCE IN THE CAUSITIVE AGENT OF OVINE FOOTROT REVEALS SIMILARITIES TO *N. gonorrhoeae* AND *E. coli*

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Footrot in sheep is caused by the anaerobic bacterium, *Dichelobacter nodosus*, formerly known as *Bacteroides nodosus*. The virulence of *D. nodosus* isolates varies. Under favourable climatic conditions infection with virulent strains results in severe debilitation. In contrast, benign strains cause only mild lameness. In order to study the genetic basis of virulence in *D. nodosus* and to develop a diagnostic method to identify virulent isolates we have attempted to isolate segments of the *D. nodosus* genome found only in virulent strains. Three recombinant plasmids containing regions associated with virulence were identified by using a differential colony hybridization procedure to screen a *D. nodosus* gene bank. Southern blot analysis shows that one of the plasmids, pJIR318, contains sequences which are present in multiple copies in the genome of a virulent *D. nodosus* strain. The DNA sequence of the pJIR318 insert has been determined. Four small open reading frames are present. One of the ORFs, designated *vapD*, shows 46% amino acid identity with an ORF present on the *N. gonorrhoeae* cryptic plasmid. This plasmid is found in nearly all clinical isolates of *N. gonorrhoeae*. Adjacent to *vapD* is a 700bp segment which is homologous to the *trbH* region of the *E. coli* F factor plasmid.

Functional analysis of the transcriptional regulator *amdR* of *Aspergillus nidulans*.

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The *amdR* gene of *Aspergillus nidulans* encodes a positively acting regulatory protein. In response to omega amino acids, AmdR activates the transcription of five structural genes.

The N-terminal region of the AmdR protein contains a DNA binding domain . This domain is predicted to form a C6 zinc finger structure. To demonstrate the functional requirement of the cysteine residues within the predicted zinc finger region, in vitro mutagenesis was performed. The fourth cysteine of the zinc finger, (*amdRcys?0*) was converted to a glycine residue (*amdRgly?0*). When plasmids which contain the *amdRgly?0* gene are transformed into an *amdR* loss of function strain they fail to restore wildtype *amdR* activity. This indicates that a functional DNA binding domain is essential for the activity of the AmdR protein. However, the expression of the *amdRgly?0* gene in a *amdR* wildtype background results in decreased *amdR* mediated activation of the structural genes. The molecular basis of this competition phenotype is unknown.

The mechanism by which small molecular weight molecules control the activity of regulatory proteins is a poorly understood aspect of gene regulation. In the presence of omega amino acid inducers, GABA and ß-alanine, the *amdR* gene product causes an increase in structural gene expression. Mutants have been isolated which show an altered response to the presence or absence of inducer molecules . Determination of the DNA sequence changes in these mutants may help to define specific regions of the AmdR protein involved in mediating the response of the AmdR to inducer molecules. In addition various C-terminal regions of AmdR have been fused to a heterologous DNA (facB) binding domain to determine which regions of the AmdR protein interact with, and respond to, the presence of inducer molecules.

STRUCTURAL AND FUNCTIONAL COMPARISON OF AMDR FROM TWO ASPERGILLUS SPECIES

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In the ascomycete fungus Aspergillus nidulans, amdR mediates omega-amino acid induced expression of amdS, gabA/gatA and lamA/B genes, involving acetamide, omega-amino acid and lactam catabolism respectively. Evidence, in vivo, suggests that another ascomycete species, Aspergillus oryzae, has an amdR gene in its genome.

To investigate and compare the structures and related functions of both amdR genes and their products, a programme was initiated to clone and characterize the amdR gene of A. oryzae. Comparison of the amdR genes will allow us to locate the functional domains for both amdR genes, and to explore the mechanism of transcription regulation and the evolutionary variations between two species.

A lambda genomic library of A. oryzae (A1560) DNA was probed with the amdR gene of A. nidulans. A 5.8-Kb SacI-SacI fragment of A. oryzae DNA was cloned into the pB/S SK$^+$ vector to give pORSK-11 plasmid. pORSK-11 was shown to partially complement the amdR44$^-$ mutant of A. nidulans. Southern analysis indicated that two amdR genes are highly homologous to each other at corresponding regions, which helps to locate and orientate the amdR <A. oryzae> gene within pORSK-11. Up to date, a 2.4-Kb HindIII-SacI fragment of pORSK-11 has been sequenced from both strands. Both amdR genes have multiple ATG translational start codons before the major ATG codon, which suggests that amdR may be translationally regulated by the upstream reading frames, or their possible products. There are three putative introns in the amdR <A. oryzae> gene, the sizes and positions of which resemble those of amdR <A. nidulans>. However, the second intron has 5'GC dinucleotide pair instead of the conservative 5'GT splice site and lacks an in-frame stop codon, which implies that this region might be included in the open reading frame. The predicted amdR <A. oryzae> protein sequence contains a C_6 "zinc-finger" DNA-binding motif, two putative nuclear localization sequences at the N-terminal end and two acidic amphipathic alpha-helical motifs, which are probably transcription activation domains in the C-terminal half. All these functional motifs are extremely homologous to those of amdR <A. nidulans> at the corresponding positions. An 80-amino acid region of amdR <A. oryzae> which follows the second nuclear localization domain shows the largest variation from amdR <A. nidulans>, the homology of which is 23.9% at amino acid level. With respect to the known amdR <A. oryzae> sequence, the overall homology between two amdR proteins is 77.8%.

Determination of the remaining amdR <A. oryzae> sequence and mRNA transcript mapping will be done in the near future.

The effect of rec-2 on repeat-induced point mutation (RIP) and recombination events that excise DNA sequence duplications at the his-3 locus in Neurospora crassa.

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ABSTRACT

Selker and colleagues have recently found that duplicated DNA is subjected to high levels of repeat-induced point mutation (RIP), during the dikaryotic phase of the Neurospora crassa life cycle that precedes karyogamy and meiosis. Recombination events that excise duplicated DNA are also reported to occur during the dikaryotic phase. We describe here experiments designed to test the effect of rec-2, a gene that modulates the level of meiotic recombination at his-3, on RIP and recombination events that excise duplications carrying his-3 and a marker, hyg^r, that confers hygromycin resistance. In the presence of the dominant allele rec-2^+, excision of hyg^r was substantially reduced in comparison to the excision frequency in crosses homozygous rec-2. A small but significant rec-2^+ mediated reduction in RIP frequency was also observed but this is likely to be an artefact of the assay used to quantify RIP. Thus, whilst meiotic recombination and recombination leading to excision of duplications are both under the control of rec-2, RIP appears to be independent. Since RIP and recombination both involve recognition of homologous sequences, this suggests that rec-2 regulates a step in recombination after sequence pairing.

Localization of ribosomal RNA operons in *Pseudomonas putida* PPN

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A macrorestriction map of the genome of *Pseudomonas putida* PPN is currently being constructed using the rare cutting restriction enzyme *Spe*1 in conjunction with Pulsed Field Gel Electrophoresis (PFGE). In conjunction with this work the location and organization of rDNA in *P.putida* PPN is being investigated.

Using cloned *Pseudomonas aeruginosa* 5s, 16s and 23s rDNA as probes, 7 distinct regions containing rDNA have been identified by PFGE analysis of *P.putida* genomic DNA. In addition, 20 clones from a *P.putida* cosmid genomic library have been characterized. Using specific genes to probe genomic digests of PPN1 it was found that the 5s, 16s and 23s genes are located in an operon like cluster with one of each gene present per cluster.

All seven PPN 23s genes contain a *Spe*1 site which has facilitated their physical mapping. This was accomplished by probing PFGE separated genomic DNA with DNA flanking the rDNA cluster in specific cosmid clones. The orientation of the clusters on the physical map was found to vary in different locations.

Alternative splicing of CD46 mRNA and association with RFLPs

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MCP (membrane cofactor protein) which is also known as CD46 and TLX (trophoblast-lymphocyte cross-reactive) antigen is a cell surface glycoprotein for factor I mediated cleavage of complement components C3b and C4b. A 1.66 kb cDNA probe, pm5.1, which is missing exon 7 was used to detect RFLPs. Three enzymes, PvuII, HindIII and BglII, gave simple two allele polymorphisms. Fourteen other enzymes that were tested on at least 15 unrelated subjects and were monomorphic.

Enzyme	Allele 1			Allele 2				
	Name	Size	Frequency	Name	Size	Frequency	N	PIC
HindIII	H1	4.3	.48	H2	2.3	.52	153	.37
PvuII	P1	16.5	.40	P2	14.8,1.9	.60	300	.36
BglII	B1	10.0	.08	B2	8.3,1.8	.92	220	.14

No B1B1 homozygotes were found. There are strong linkage disequilibria between these RFLPs. Only two exceptions were found to the associations of H1 with P1 and H2 with P2 (N=147) and the rare BglII variant, B1, was only found in the presence of the H1 or P1 alleles (N=89 and N=124 respectively). In the subjects typed for all three RFLPs (N=83) the genotypes are consistent with the presence of 3 common haplotypes of the possible 8, H2/P2/2, H1/P1/B2 and H1/P1/B1 at frequencies 0.54, 0.37 and 0.08 and a single case of haplotype H2/P1/B1 (PIC = .49). There is also a strong association between the RFLPs and the protein polymorphism for predominance of MCP forms α and β of 66 kD and 56 kD detected on peripheral blood lymphocytes by SDS-PAGE. The association between H2 and protein from α was confirmed but H1 was not completely associated with protein from β. In 23 subjects typed for all four polymorphisms genotypes are consistent with haplotype H2/P2/B2 (0.39) producing more protein α, H1/P1/B1 (0.07) also producing more of α and H1/P1/B2 (0.54) producing more β in 80% of cases and more α in 20%. The protein polymorphism is caused by alternative splicing of an exon. The splicing preference is probably not directly dependent on the alleles at the RFLP sites but is a separate site in linkage disequilibrium with the RFLPs. The expected frequencies of the four common haplotypes in the population is 0.60, 0.08, 0.06 and 0.26 for H2/P2/B2/α, H1/P1/B1/α, H1/P1/B2/α and H1/P1/B2/β respectively (PIC = .50). Based on these the expected frequencies of protein genotypes $\alpha\alpha$, $\alpha\beta$ and $\beta\beta$ are 0.55, 0.38 and 0.07 which is close to the observed proportions.

This work was supported by the NHMRC.

HLA-DP AMPLIFIED FRAGMENT LENGTH POLYMORPHISM IN HUMAN POPULATIONS IN THE ASIA PACIFIC REGION

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HLA-DP is the least investigated of the highly polymorphic human major histocompatibility complex genes. There are 19 WHO recognized alleles at the HLA-DP locus and at least four more that have been recently described. The locus has particular potential use in studying the historical relationships among human populations as HLA-DP alleles are, with few exceptions, in gametic phase equilibrium with alleles at the other HLA ioci. A new method of distinguishing HLA-DP genotypes based on digestion of PCR amplified second exon sequences at allele-specific combinations of restriction sites is used to determine the distribution of allelic variation in populations in the Asia-Oceanic region. The results are interpreted in the context of the evolutionary history of these populations.

Studies on the VNTR region 3' to the c-Harvey-*ras* oncogene in Caucasians and Australian Aborigines.

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The c-Harvey-*ras*-1 oncogene contains a hypervariable region 3' to the fourth exon. This locus is highly polymorphic because of variation in the number of repeats of a 28 base pair sequence. Over twenty different allelic fragments have been defined for this locus. The presence of multiple alleles at a hypervariable locus can be used in genetic analysis and DNA profiling.

A preliminary study on Caucasians and Australian Aborigines revealed variability in fragment sizes in both populations. The presence of common alleles and different allelic fragments suggests that selection does occur at this locus. From the pattern of allelic variation observed, possible mechanisms for the generation of new alleles at this locus are discussed.

Interactions at the *stoned* locus of *Drosophila melanogaster.*

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The interaction of *stoned* mutations with various neurological mutations has been examined. The phenotype of the *stoned* $^{t.s.}$ mutants can be described as a temperature-sensitive debilitation, the flies being extremely sedentary even at permissive temperatures. These mutations are actually viable alleles of a lethal complementation group in division 20 of the X chromosome. In order to verify the distinct nature of these behavioural and lethal mutations, heteroallelic combinations were characterised with respect to viability and electrophysiology.

Previous studies have demonstrated alterations in cAMP-dependent protein phosphorylation in *stoned* $^{t.s.}$ head extracts. We have identified mutations at the *dunce* locus as enhancers of the *stoned* phenotype. The *dunce* mutations carry lesions in a cAMP phosphodiesterase isozyme and thus elevate cAMP levels. In a *dunce* background, *stoned* $^{t.s.}$ flies are lethal. As *dunce* itself is viable, the reduction in viability of the double mutant would suggest synergism as opposed to additional mutational load. The *stoned* $^{t.s.}$ mutations also result in an increased off-transient amplitude of the electroretinogram as compared to wild-type. A few *dunce-stoned* escapers showed an even further increase in this amplitude. Another allele ,*stoned* ^{C}, causes sensitivity to mechanical shock. The *stoned* ^{C} electroretinogram lacks both on and off transients. Furthermore, the *stoned* ^{C}*-dunce* double mutant is viable, and as such appears to act in a manner opposite to the temperature sensitive alleles.

Cloning the *stoned* gene of *Drosophila melanogaster*: A neurological gene at the base of the X-chromosome

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Previous studies of *stoned* mutations indicate that the wild type stoned gene product is required for the correct functioning of the *Drosophila* nervous system. We are cloning this gene with the aim of identifying it's cellular role in neural function.

The *stoned* locus is located in polytene chromosome Division 20 at the base of the X-chromosome. This region is composed of ß-heterochromatin and contains a large number of repeated sequences. Using quantitative Southern blots and deletion mapping we screened a mini-library, microdissected from Division 20 (Miklos *et al.* 1988, Proc. Natl. Acad. Sci. USA 85: 2051-2055.), to identify unique clones from the *stoned* region. Two of the clones identified detect altered banding patterns on Southern blots of *stoned* mutants. These clones were used to initiate a chromosome walk which,to date, spans 23 kb. The alterations associated *stoned* mutations map to within a 6.5 kb region, which, when used to probe Northern blots, detects a single 8.5 kb transcript that is preferentially expressed in adult heads. We believe that this transcript is from the *stoned* gene and are continuing the molecular characerisation of this gene.

Molecular Characterization of a Second *Drosophila* Glutamate Decarboxylase Gene.

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Glutamate decarboxylase, GAD, is the rate-limiting enzyme in the biosynthesis of the inhibitory neurotransmitter γ- amino butyric acid, (GABA). Studies on GABA and its biosynthesis are central to an understanding of seizure induction, auto immune diabetes and questions of nervous system function and development. The GABAergic receptors are also an important target site for insecticides.
Cross species hybridisation has been used to isolate a neurally expressed *Drosophila* gene with 42% identity to a feline cDNA (Kaufman *et al.*, 1986 *Science* 232 1138-1140). The *Drosophila* gene DGAD $_2$, differs in chromosomal location, sequence and spatial expression from a *Drosophila* GAD gene (DGAD$_1$) isolated by Jackson *et al* ., (1990, *J. Neurochem* . 54 1068-1078). DGAD$_2$ is expressed in the adult fly in the first optic ganglion, the lamina, and in presumed inhibitory motor neurons innervating the thoracic muscles. This tissue expression is consistent with neurological effects seen in flies treated with picrotoxin, a potent GABA antagonist. Preliminary experiments with head extracts from flies heterozygous for a deletion of the DGAD$_2$ gene, indicate a significant reduction in GAD enzyme activity compared with wild-type flies. The presence of isozymic forms of GAD in *Drosophila* suggests that tissue specific expression of multiple GAD genes may account for the different forms of GAD reported in other organisms.

MUTATION DETECTION BY CHEMICAL CLEAVAGE - IMPROVEMENTS

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Detection of mutations by the chemical cleavage method relies on the principle that mismatched base pairs in double stranded DNA have a greater reactivity with hydroxylamine and osmuim tetroxide than double stranded DNA, in which all residues obey Watson-Crick base pairing rules. A DNA heteroduplex is formed by annealing wild-type and mutant DNA of up to 2 kb. The heteroduplex is reacted with hydroxylamine and osmium tetroxide and cleavage of DNA at mismatched residues takes place by reaction with piperidine. Cleavage products are resolved by denaturing gel electrophoresis allowing the position of mismatches, small insertion and deletions to be identified. The method has found a range of applications including, for example, the assessment of variation in viral populations, confirmation of *in vitro* mutagenesis and detection of new mutations in a wide variety of systems. Improvements in the method will be outlined including presentation of the new non-radioactive technique.

The *lozenge* gene(s) affect pattern formation in the developing retina of *Drosophila melanogaster*.

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The wild type adult retina of *D. melanogaster* is a compound eye consisting of approximately 700 ommatidia. Each ommatidium is composed of an inner core of 8 photoreceptor cells which are flanked by secondary and tertiary pigment cells. We have previously reported that in *lozenge (lz)* mutants these cell types from a bilayer beneath the the lens layer; photoreceptor cells laying beneath pigment cells. The photoreceptor cells are abnormal in morphology and number per cluster. Our recent investigations suggest that in some *lz* mutants the number of primary pigment cells and cone cells is reduced and the basement membrane is absent. Studies of temperature sensitive period using ts mutants suggested that the *lz* product(s) is required in late third instar larvae - early pupae for normal eye development. Analysis of eye imaginal discs at this stage using cobalt staining revealed irregularities in pattern formation consistent with the adult eye defect.

Fatal association, a phenomenon of dieldrin resistance in *Lucilia cuprina.*

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A novel phenomenon of dieldrin resistance in the Australian sheep blowfly, *Lucilia cuprina*, was investigated. This phenomenon is observed as the mortality of susceptible flies exposed to dieldrin-resistant flies which have been raised on media containing dieldrin. The mechanism of the effect was examined in a variety of experiments. The concentration of dieldrin on which the resistant flies were raised was found to be a significant factor in the mortality of the exposed susceptible flies. The sex of the flies involved was not important. It was shown that homozygous resistant flies were capable of inducing the mortality of heterozygous resistant flies. Direct contact between resistant and susceptible flies was not required as containers exposed to treated resistant flies could produce the effect. Chemical analyses revealed the presence of dieldrin at high levels in the treated resistant flies and at lower but still toxic levels in the exposed containers. The relevence of these results will be discussed with regard to the nature and evolution of dieldrin resistance.

THE EVOLUTION OF PHOSPHOGLYCERATE KINASE (PGK) SEQUENCES IN MARSUPIALS AND THEIR RELATION TO X-INACTIVATION

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Compensation mechanisms have evolved in organisms with XX/XY sex dimorphisms in order to equalize the dosage of X-linked gene products between the sexes. Like eutherian mammals, marsupials also have dosage compensation for the extra X in females. However, unlike eutherians, the marsupials X-inactivation is paternal instead of random, and is incomplete. The molecular basis of X-chromosome inactivation (XCI) in marsupials needs to be elucidated. The current hypothesis is that methylation of cytosine residues is important in this kind of gene regulation. Methylation in mammalian cells usually occurs at CpG sites which are under-represented in the mammalian genome but frequently found clustered together in islands situated near the 5' end of genes.

In order to study the phenomenon of XCI, in marsupials, the X-linked gene PGK-1 was cloned from liver cDNA of the tammar wallaby using PCR techniques, and then sequenced. Based on the sequence information the evolution of PGK sequences in mammals has been examined.

Hybrization with the cDNA clone reveals multiple bands in genomic DNA, indicating that there is a large family of PGK pseudogenes.

In order to distinguish the coding PGK-1 gene from the processed and non-processed pseudogenes, sequences immediately 5' to the gene have been cloned by PCR. In addition we are obtaining intron sequences. With these probes we will examine the methylation status of PGK genes on active and inactive chromosomes.

Unequal crossing over at a rust resistance gene (*Rp*) in maize.

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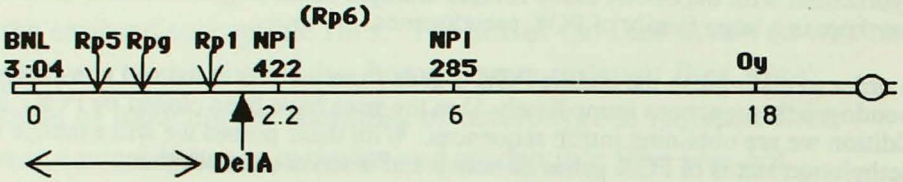
Resistance against rust ,*Puccinia sorghi*, is specified by genes at 5 different loci in maize. Three loci *Rp1 Rp5* and *Rp6* map together in a gene complex at the tip of the short arm of chromosome 10. Genetic tests show that 8 supposed *Rp1* alleles are all unstable producing susceptible progeny at allele specific frequencies ranging from 0.01--> 0.0001. The susceptible progeny have an exchange of outside markers indicating a crossover event.However since this event occurs in the homozygote it must at least be an unequal crossing over.

Further studies of this event have been made using the most unstable gene, *Rpg*, which has been shown not to be an allele of the *Rp1* locus but to map distally between *Rp1* and *Rp5*. Several RFLP markers have been mapped in this region and the approximate locations are given in the following map of the short arm of chromosome 10.



BNL3:04 and *NPI 422,285* are RFLP markers. *Oy* is oil yellow seedling . *DelA* marks the approximate site of a terminal deletion which deletes *BNL3:04*, *Rp5* and *Rp1* but not *NPI422*. *DelA* homozygotes are lethal giving a defective kernel or *dek* phenotype. The EMS induced mutant *dek14* is known to map in this region.

Rpg produces about 1% susceptible gametes from either a homozygous parent or when heterozygous with the *Rp1* gene. There is something inherent in the structure of the *Rpg* gene that causes the susceptibles. In a heterozygote with *DelA* there are no susceptible progeny. The event requires the presence of the homologue. In one cross, 2/9 recovered *rpg* susceptibles had a *dek* phenotype and could represent deletions produced by an unequal crossing over event. It is suggested that these events are part of a genetic reassortment mechanism involved in generating new rust resistance specificities.

P ELEMENTS OF *DROSOPHILA*

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The study of P elements in Drosophila has been revolutionised by the availability of genetically engineered elements. We have used the P(CaSpeR) and P(ry^+ Δ2-3) (99B) system to study male recombination. The combination of these two elements leads to levels of recombination which are of the same order of magnitude as those seen in interstrain crosses of *D. melanogaster*. This recombination occurs preferentially in the chromosome region containing the P(CaSpeR) element. When homologous P(CaSpeR) elements are used, in the presence of P(ry^+ Δ2-3) (99B), levels of recombination are an order of magnitude higher.

Recombinant chromosomes from male recombination using single P(CaSpeR) elements have been made homozygous by use of balancer chromosomes. These chromosomes have been classified into those which, by phenotype, contain P(CaSpeR) and those which do not. Chromosomes were then crossed in pairs to re-create genotypes which, on the assumption that an original P(CaSpeR) element had been retained at the site, should contain homologous elements. Most such stocks showed recombination at the levels of the original homologous stock. The presence of P(CaSpeR) elements at the original site was also monitored in recombinant chromosomes using Southern hybridization. The pattern of elements at the site as shown by Southern hybridization was consistent with that shown by recombination.

These results suggest that the process of recombination in many cases leaves behind a copy of the element at the recombination site. However other results, in which excisions are repaired from a homologous site, suggest a model for recombination in which there should be no element at the site. Resolution of this potential paradox will be discussed.

Mutation and evolution: Lifespan reduction by transposons and premeiotic clusters of mutation. R. C. Woodruff, Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio and J. N. Thompson, Jr., Department of Zoology, University of Oklahoma, Norman, Oklahoma.

Although mutations (genic and chromosomal) are the source of genetic variation, the role of newly arising mutations in adaptive evolution is unclear. For example, many spontaneous mutations occur in meiotic or postmeiotic stages and change only a single gamete. However, premeiotic genetic changes, including those caused by transposable DNA elements, can lead to multiple mutant products which may have a larger impact than single mutations in determining population genetic make-up. We draw upon data from almost two million tested chromosomes in *Drosophila melanogaster* to document that premeiotic mutations occur much more often than generally assumed. From tests of 22,589 parental males, 3,585 recessive sex-linked lethal mutations were recovered among 1,950,335 total chromosomes scored (0.18%). If all males in this study had about the same mutation rate of 0.18%, then the probability of two independent mutations occurring per male would be less than 0.000005. Hence, with the highly conservative definition that three mutations must occur per male for the event to be called a premeiotic cluster, a total of 20.9% of the 3,585 mutations did not occur as independent events. These data bring into question estimates of how often gonadal mosaicism will occur to produce multiple progeny affected with a rare condition and the role that clusters of mutations may play in evolution.

In addition to premeiotic clusters of mutation, the movement of P DNA elements in somatic cells of *D. melanogaster* significantly reduces lifespan. This is true for flies that have as few as three P elements. Since the frequencies of mutation and chromosome breakage were also significantly increased in males that have active P elements, it is proposed that the reduction in lifespan is due to genetic damage and to changes in gene regulation due to P-element transpositions.

MOLECULAR ANALYSIS OF THE HAIRLESS (hr) MUTATION OF THE MOUSE.

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Hairless is a recessive mutation of the mouse, located on chromosome 14. Mutant mice grow a juvenile haircoat. However they are unable to initiate a second round of the hair cycle, so this is progressively shed leaving the adults without any hair except for vibrissae. Histologically the defect can be seen as a breakdown of the connection between the hair follicle and the dermal papilla. Contact between the dermal papilla and the follicle is beiieved to be essential for activation of stem cells in the "bulge" and initiation of a new cycle of hair growth. Additionally mutant mice are qualitatively more sensitive to the induction of skin tumours by either UV light or chemical mutagens, such as DMBA, than shaven congenic control mice. The mechanism of this susceptibility is unknown, but does not appear to involve pyrimidine dimer repair (Lan, pers. comm.).

The hr mutation is due to insertion of a polytropic Murine Leukemia virus in an intron towards the 5' end of the gene, following the first two exons. The splice junction between these exons, which account for about 15% of the coding sequence, has been confirmed by PCR on a cDNA template and sequencing of the double stranded product. Despite extensive efforts, coding sequences downstream of the insertion have not yet been cloned as chromosome walking is impeded by repetitive sequences. Further conventional cDNA cloning and RACE have not succeeded because of large message size and low levels of expression. Given the two important phenotypes associated with the mutation, efforts are continuing to clone the remainder of the gene in order to reveal its sequence and function and to permit detailed analyses of its expression.

Gene silencing in mammalian cells by uptake of 5-methyl deoxycytidine-triphosphate into DNA

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DNA methylation has been found to be associated with gene inactivity in many biological contexts, including X chromosome inactivation, latent viruses, transposable elements and a variety of genes in somatic cells. It has been commonly concluded that these results show no more than a correlation between DNA methylation and gene inactivity. Experiments with mammalian cell lines suggest a more direct relationship, since many housekeeping genes can be inherited in a silent non-expressed form and they can be reactivated at very high frequency by the demethylating agent 5-aza-cytidine (5AC). Both silent and reactivated genes stably maintain their phenotypes through cell division, and this strongly indicates that the pattern of methylation is stably transmitted by a maintenance DNA methyl transferase.

To demonstrate a direct relationship between DNA methylation and gene silencing we have introduced 5-methyl deoxycytidine-5'-triphosphate (5mdCTP) into CHO K1 cells by electroporation and have shown that housekeeping genes frequently become inactivated. Under normal conditions DNA or nucleotides are not taken up by cells, but collaborative experiments by J. Nyce (East Carolina University) have shown that labelled nucleotide triphosphates are incorporated into cellular DNA after electroporation. This treatment increases by 10-100 fold the frequency of cells lacking thymidine kinase, hypoxanthine-guanine phosphoribosyl transferase or adenine phosphoribosyl transferase. The inactivation of the genes coding for these enzymes is thought to occur following the direct incorporation of the methylated dCTP into DNA. The enzyme deficient clones were stable, but almost all were reactivated at high frequency by the demethylating agent 5AC to produce derivatives with enzyme activity. The results indicate that methylation of DNA is a primary cause of gene silencing.

POSTERS

5S Ribosomal RNA: When did it change addresses?

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During evolution of eukaryotes from prokaryotes, the genes encoding cytoplasmic ribosomal RNAs have undergone several structural modifications. The 16S-like ribosomal RNA (rRNA) and the 23S-like rRNA have acquired many extra nucleotides increasing their typical "size" to 18S and 28S respectively. The 5' end of the 28S has become detached to produce a separate transcript, the 5.8S rRNA. Another modification has been the relocation of the 5S rRNA genes. Whereas the 5S rRNA gene lies downstream of the 23S gene in a single operon (16S-23S-5S) in bacteria, the 5S genes of most eukaryotes are organised in one or more tandem clusters at loci remote to the 18S and 28S gene cluster. It is assumed that the 5S genes have been excised from the cistron containing the other rRNAs and relocated to new loci during the transition from prokaryotes to eukaryotes.

In some lower eukaryotes (eg certain fungi) 5S genes are linked to the other rRNA genes. These organisms perhaps represent an ancestoral intermediary in the reorganisation of rRNA genes. We have been localising 5S genes in fungi and algae by PCR. Using primers complementary to the 5S gene and the 3' end of the 28S gene we amplified and partially sequenced a spacer region linking the two genes in selected fungi and algae. The 5S genes are located downstream of the 28S genes, and are apparently transcribed in the same direction. Using a rightwards primer from 5S with a leftwards primer from the 5' end of the 18S gene we amplified across the spacer region into the adjacent rRNA operon. The PCR can thus be used to rapidly localise 5S genes in various lower eukaryotes and help determine when the genes were relocated during evolution.

MOLECULAR CONSERVATION GENETICS OF THE HELMETED HONEYEATER

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The Helmeted Honeyeater *Lichenostomus melanops cassidix* is an endangered subspecies within the Yellow-tufted Honeyeater complex. There is, however, debate as to its taxonomic status, and there is possibly introgression from other subspecies within the complex.

To clarify these questions, and to assist in genetic management of the captive breeding colony of *L.m. cassidix*, DNA fingerprinting and mitochondrial RFLP techniques are being applied. DNA has been extracted from small blood samples from *L.m. cassidix*, *L.m. gippslandicus* and *L.m. meltoni*.

DNA fingerprints so far indicate that *L.m. cassidix* is distinct from nearby *L.m. gippslandicus* populations. The implications of these data, and of others on the inheritance of fingerprint restriction fragments, are discussed in the context of future management strategies for the Helmeted Honeyeater.

terminal flower: A gene affecting inflorescence development in _Arabidopsis thaliana_

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Flowers are produced indefinitely on the flanks of the growing inflorescence apex in wild type *Arabidopsis*. Seven recessive mutant alleles of a gene affecting inflorescence development have been isolated and characterized. Four of these alleles result in a severe mutant phenotype whereby the main inflorescence meristem becomes determined early in its development as do the secondary branches. A few normal flowers may be produced before the growing point is "invaded" by flower primordia. The other mutant alleles result in a greater number of normal flowers being produced before determination occurs. The locus has been named *terminal flower* (*tfl*) and has been located at the extreme top of linkage group 5 by morphological and RFLP mapping.

Modifier genes and modifiable *lozenge* (*lz*) mutant phenotype in *Drosophila melanogaster*.

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In *Drosphila melanogaster* a number of modifier mutations have been identified which enhance or suppress the phenotype of retrotransposon induced mutants. The phenotype of four *lz* mutants (lz^1, lz^{34}, lz^{37} and lz^k) is modified by these genes. The lz^1 mutation has been demonstrated to have been caused at least in part by the insertion of the retrotransposon gypsy. Molecular analysis of lz^1 the other mutations is continuing.

This poster reports the further characterization of three additional modifiers. These mutations $su(lz^1)$, $su(lz^{34})$ and $E(lz^1)$ map to three distinct loci on the third chromosome. Their interaction with known retransposon induced mutations at other loci will be discussed.

Conflicting phylogenetic hypotheses for marsupials tested by mtDNA sequencing.

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[b]Dept. of Microbiology & Infectious Diseases, School of Medicine, Flinders University of South Australia, Bedford Park, 5042, Australia.

The phylogenetic affinities of Tarsipes rostratus have remained uncertain. Molecular data have provided conflicting views with one indicating a relationship with Diprotodonta but no special relationships with any Diprotodont family, while the other confirmed Diprotodontid affinity and indicated close affinity with Acrobatidae and a relationship of this clade to Petauridae. Here we use direct sequencing via the polymerase chain reaction (PCR) to compare 238 bases of mitochondrial (mt) DNA (12S rRNA gene) of eight diprotodontid taxa and three out-group taxa, in an attempt to test the conflicting hypotheses. Our data are showing yet again that the radiation of the families of Diprotodonta occurred over a very short period of time.

Expression of a recombinant *Drosophila* cystatin .

Michael Brown, Department of Genetics, University of Melbourne.

A cDNA from *Drosophila melanogaster* encoding for a cystatin-like protein has been cloned and sequenced in our laboratory. It has amino acid sequence homology to the type 2 family of the cystatin gene superfamily. This family comprises small (molecular weight approx. 14 kDa), predominantly secreted, enzymes with two internal disulphide bonds which inhibit papain-like cysteine proteases in a "pseudo-irreversible" manner.

We nave used the Biolabs Protein Fusion and Purification System to express *Drosophila* cystatin as a fusion protein. A 420 b.p. EcoR1 fragment of the cystatin cDNA was cloned into the EcoR1 site of the pMal-cR1 expression vector in the same reading frame as the malE gene which encodes for a maltose binding protein (MBP).

The MBP-cystatin fusion protein, found mainly in the crude soluble extract of transformed cells, was affinity purified on an amylose resin column. SDS-PAGE has shown that it co-purifies with an active E.coli protease. Biochemical studies of the fusion protein and its ability to inhibit digestion of casein by papain are underway.

RFLP PHENOTYPES USING AN 18S RIBOSOMAL GENE PROBE DISCRIMINATE BETWEEN BREEDING SYSTEMS.

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Isotoma petraea is a diploid autogamous self-pollinating species which has evolved complex hybridity in response to inbreeding. It exhibits high levels of genetic differentiation between its geographically isolated populations. *Macrozamia riedlei* is a diploid dioecious wind pollinated cycad characterised by elevated levels of gene flow throughout its population system. A wheat 18S ribosomal gene probe yields RFLP phenotypes which are characteristically single banded in *Isotoma* and multiply banded in *Macrozamia*. The occasional two or three banded phenotypes occurring in *Isotoma* probably derive from rare migrations and interpopulational hybridisations that are involved with the dispersal and elaboration of complex hybridity throughout its population system. These phenotypes evidently become replaced by single banded phenotypes within derivative lineages. Such homogenisation may be attributed to homologous recombination events within the ribosomal gene aggregation at meiosis, and autogamy. We suggest that the single banded versus multiple banded contrast between 18S induced RFLP phenotypes may have general application as an indicator of the breeding system in plants.

CLONING A SEX INFLUENCED PROTEIN GENE IN CHIRONOMIDS

by
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A protein with the properties and size of a dimeric haemoglobin was found in only the female larvae of *Chironomus tentans*, where it appeared at about phase 6 of the fourth instar. Using a polyclonal antibody raised to this protein, it was shown that the protein was ultimately taken up into the vitellogenic eggs. The sex influenced nature of this protein and its involvement with vitellogenesis suggested that it may differ from previously studied chironomid haemoglobins.

The polyclonal antibody was therefore used to screen a λgt11 expression library of North American *Chironomus tentans*. A 2kb clone (SF#13) was isolated which contains part of a gene, believed to be that for the sex influenced protein. The clone was sequenced and an open reading frame of 89 amino acids (AA) identified. The putative AA sequence shows little DNA or AA homology to other characterized haemoglobins.

SF#13 was used to probe a Southern blot of genomic DNA from a species belonging to a sister genus, *Kiefferulus 'cornishi'*, and, since a strong signal was obtained, to probe a genomic library. Four clones, all apparently from the same region, were isolated and a subclone of one (pBS4.4) sequenced.

Comparison of the AA sequence in the two clones indicated an overall <80% homology but including one region of 44 AA in which there were only two AA changes and a further four base changes which did not lead to an altered AA.

Northern analyses, while suggestive that the gene is transcribed in phase 7-9 female larvae but not in males or earlier phase females, have given equivocal results. Therefore other approaches are being followed to confirm the identity of the gene.

The two clones *in situ* hybridize to the same regions on polytene chromosomes - to 9A on arm D of *C. tentans*, and the tip of arm 2L in *K. 'cornishi'*.

A DIET RESPONSIVE MUTATION SYSTEM IN *DROSOPHILA*

Christopher J.I. Driver, Victoria College, Vic.

The somatic mutation model of ageing has several problems that preclude ready acceptance. One such problem is that diet is known to alter the rate of ageing, but there is a lack of a demonstrated relationship between diet and mutation. The virus like nature of transposable elements means that they potentially are able to respond to intracellular signals and metabolic changes. Thus if somatic mutation is induced by transposable elements this difficulty associated with the model may be resolved.

A somatically active P element carrying a White marker was constructed using a strain carrying a CaSpeR element in a region of the chromosome where it is poorly expressed, producing peach coloured eyes. This element contains an intronless version of the White gene flanked by the P element terminal repeats. When mobilised by a somatically active transposase from the $P_{\Delta}2.3$ element and on a white background, transposition can be monitored by the appearance of darker red patches on the eyes, or the appearance of red eyes, the colour of the wild type. The latter flies with wild type colored eye transmit the characteristic to their progeny with an average frequency of about 50%.

Adults of this type were fed and bred on a high fat diet that previously has been shown to accelerate ageing, and compared to the control low fat diet. The frequency of offspring showing colored patches on their eyes and the frequency of red eyed offspring was increased on the high fat diet. The suggestion is that transposition is increased by the ageing accelerating diet. This will be verified by genetic analysis of the red eyed offspring.

This system provides a possible link between diet and somatic mutation, and a system with which to study mechanisms.

(Poster Abstract)

Synaptonemal complex analysis of hybrids between Australian *Rattus* species differing widely in chromosome number

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The eight Australian species of the genus *Rattus* show a considerable amount of karyotype repatterning. Most of the changes involve Robertsonian fissions and fusions, but a pericentric inversion has also been identified (Baverstock et al, 1977). Homology of the chromosome arms between species has been determined by G banding (Baverstock, 1983). Workers at the South Australian Museum have produced hybrids in various combinations between species with 2n = 50, 42, 38 and 32. Males of these hybrids show various levels of reduced fertility, probably as a result of meiotic failure. Synaptonemal complex spreads have been prepared from spermatocytes of some hybrids in an attempt to confirm from their pachytene pairing behaviour the postulated chromosomal rearrangements. Pachytene spreads of three different hybrids with respectively two trivalents, 8 or 9 trivalents, and five trivalents plus a quadrivalent will be illustrated. The pachytene association of the XY pair with translocation multivalent synaptonemal complexes is being investigated as a possible cause of differences in fertility between male and female hybrids.

MITOCHONDRIAL GENOME OF THE MARSUPIAL, *SMINTHOPSIS CRASSICAUDATA*: PARTIAL DNA SEQUENCE AND ITS ANALYSIS

Chris Hefford, Neil Dear, Hamish Scott and Rory Hope, Department of Genetics, University of Adelaide.

A 16kb BamHI fragment representing approximately 95% of the mitochondrial genome of *S. crassicaudata* was cloned into pRB322. A 2.8kb EcoRI sub-fragment encoding the 12S and 16S rRNAs, $tRNA^{Phe}$, $tRNA^{Val}$ and part of the D-loop region was subsequently sub-cloned and sequenced from both strands. Preliminary analyses of this DNA sequence have enabled us to:

- show that the gene arrangement in this region of the mitochondrial genome is similar to that reported for eutherian mammals, namely (light strand): 5' D-loop - $tRNA^{Phe}$ - 12S rRNA - $tRNA^{Val}$ - 16S rRNA 3'.
- use a region of the 12S rRNA gene to carry out a phylogenetic analysis of selected marsupial species, including *S. crassicaudata* and the extinct marsupial wolf, *Thylacinus cynocephalus*. This analysis confirmed the close relationship of the marsupial wolf to other Australian marsupial carnivores (Thomas *et al* 1989) and indicated that *S. crassicaudata* and *T. cynocephalus* are monophyletic, to the exclusion of the other marsupial taxa examined.
- show that the $tRNA^{Phe}$ and $tRNA^{Val}$ anti-codons are (from 5' to 3') GAA and TAC respectively, as is the case for all other animals.
- examine the likely secondary structures of *S. crassicaudata* mitochondrial $tRNA^{Phe}$ and $tRNA^{Val}$ and compare these structures with those of a range of other organisms. These comparisons suggest that marsupial mitochondrial tRNAs adopt a secondary structure containing a single major stem and anti-codon loop. Such an arrangement is atypical of species in the Phylum Chordata, which tend to have multiple stem/loop structures, but is similar to the tRNAs of non-chordate animals, protista and fungi.

Reference: Thomas, R.H., Schaffner, W., Wilson, A.C. and Paabo, S. (1989) DNA phylogeny of the extinct marsupial wolf. Nature 340: 465-467.

Excision repair of pyrimidine dimers in Mouse epidermal DNA

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Pyrimidine dimers are the most abundant type of DNA damage induced by ultraviolet light of wavelength between 280-320nm (UV-B). There are two major pathways to repair the damage: photoreactivation and excision repair. Mouse epidermis does not have photoreactivation capacity. However, the reports on excision repair in mouse skin are conflicting; some claim that the excision repair rate is rapid while others show the rate is slow. Three strains of mice: HRA/SKH hairless(hr/hr) and its congenic hairy revertant ($hr/+^{rev}$); HRS/J hairless (hr/hr) and its hairy congenics (hr/+); and BALB/c hairy (+/+) mice were used in this study. Mice were irradiated with low dose of UV (280-400nm). The frequency of pyrimidine dimers was measured in the epidermal DNA, using dimer specific endonuclease of *micrococcus luteus* and alkaline denaturing gel electrophoresis. The results demonstrate that most of pyrimidine dimers remained 24 hours after irradiation in all three strains of mice. No difference in repair was found between the hairless and its hairy counterpart in both the HRA/SKH and HRS/J strain. The results are in agreement with the reports that mouse epidermal cells *in vivo* have little capacity for excision repair of pyrimidine dimers. The results also suggest that the difference in susceptibility to UV carcinogenesis between the hairless and hairy mice in the HRA/SKH strain (Greenoak, personal communication) is not due to the difference in the ability to repair pyrimidine dimers.

Cloning of *amdA*, a regulatory gene from *Aspergillus nidulans*

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

The *amdA* gene has been mapped to Linkage Group VII of the *A. nidulans* genome between the genes *gatA* and *alcC* (6). A chromosome walk from both of these flanking markers has been carried out in a lambda Gem11 *amdA*+ library. The location of *amdA* within this series of overlapping clones will be determined by transformation and selection for complementation of the *amdA* loss of function mutant.

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HUMAN CHROMOSOME 21 GENES MAP IN TWO CONSERVED AUTOSOMAL CLUSTERS IN MARSUPIALS AND MONOTREMES

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We have used a comparative mapping approach to investigate the origin of the human chromosome 21. We used in situ hybridization with human cDNA probes to four conserved HSA21 genes SOD1 (superoxide dismutase), CR (carbonyl reductase), ETS2 (cellular homologue of viral protooncogene) and INFAR (interferon alpha receptor) to locate homologous sequences in the metaphase chromosomes of two distantly related marsupials, the tammar wallaby (Macropus eugenii), the dasyurid marsupial Sminthopsis macroura, and the monotreme Ornithorhynchus anatinus (the platypus). These four genes mapped in two distinct autosomal clusters in all three species, with SOD1 and CR located together on chromosome 7 in the tammar wallaby, 1p in S. macroura and 4q in the platypus, and ETS2 and INFAR together on 3q in both marsupial species and 2q in the platypus.

Since marsupials and monotremes diverged independently from the eutherian lineage, this suggests that HSA 21 genes were originally located in two separate autosomal blocks, and have come together in the eutherian lineage.

In another marsupial species Sminthopsis crassicaudata, SOD1 is linked to the HSA3 marker TRF (Bennett et al 1986, Nature 233:59). Since HSA 21 and HSA 3q markers are syntenic in bovine, and most also in mouse, this suggests that an ancestral SOD region, including HSA3 markers, was fused to an ancestral INFAR-ETS2 region early in the eutherian radiation. This fused chromosome has remained intact in artiodactyls, but was independently disrupted in the primate and rodent lineages.

A polymorphic 37bp insert within the first intron of *Adh* from *Drosophila melanogaster*

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Estimates of alcohol dehydrogenase enzyme quantity in larval and adult extracts from isogenic second chromosome substitution lines indicated larval differences between lines with and without this insert. Strong linkage disequilibrium with other polymorphic sites around the *Adh* gene made it impossible to attribute this difference to the presence of the insert. Here we report the results of transient ADH expression assays on *Adh-null* larvae injected with *Adh* constructs which differ only by the presence or absence of the insert. An absence of tissue-specific expression differences and total enzyme activity differences in larvae with the different constructs indicates that the enzyme quantity variation is due to a variable site closely linked to this 37bp insert.

Arabinose metabolism mutants of Arabidopsis thaliana

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

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

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

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

ANALYSIS OF A DNA AMPLIFYING REGION IN THE SALIVARY GLAND CHROMOSOMES OF *RHYNCHOSCIARA AMERICANA*.

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The C3 region of the salivary gland chromosomes of the sciarid fly, *R. americana*, contains a band, C3d, that has been shown to undergo amplification during the prepupal period when its gene is active (Glover *et al* 1982. PNAS 79: 2947-2951). This is one of several amplifying regions in the *Rhynchosciara* salivary gland chromosomes which we have been studying in order to gain an understanding about their origins of amplification (which should also be origins of replication) and how they are controlled. In order to achieve this aim, we must isolate fragments containing possible origins, which entails chromosome walks from known sequences in each region.

As a prerequisite to these walks, puff stage cDNA and genomic DNA libraries were constructed using Stratagene Lambda Unizap™ and Lambda Dash™ II vectors respectively. The cDNA library was screened using a previously isolated genomic probe (Millar *et al* 1985. Gene 34: 81-88) which hybridized *in situ* to the C3d puff. Several positive recombinants were obtained and the one containing the longest insert was selected for further analysis. It contains most of the C3d mRNA sequence and hybridizes *in situ* unambiguously to the C3d puff.

This cDNA was used as a probe for screening the genomic library. A number of positive recombinants were obtained from this library. Three of these have been analysed by restriction mapping, Southern hybridization to the cDNA and *in situ* hybridization to polytene chromosomes. A restriction fragment from one of the above recombinants was used to isolate two other genomic clones which are in the process of being analyzed. These five clones correspond to more than 30 kb of DNA in the C3 region. The cDNA hybridizes to a 5.6 kb fragment bordered by a Hind III and a Sac I site within them. Two of the five clones hybridized *in situ* unambiguously to C3d. Two others hybridized to additional chromosome bands and are being analyzed further. This latter result emphasizes the need to monitor chromosome walking by *in situ* hybridization.

This work was supported by FAPESP, CNPq and the Volkswagen Foundation. A.J. Stocker was the recipient of a FAPESP grant.

Molecular characterisation and organisation of the glycerol-3-phosphate oxidase gene (*Gpo*) in *Drosophila melanogaster*.

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The *Gpo* gene has not been previously sequences in any organism other than *E. coli*. Considerable early research effort located the chromosomal position of the *Gpo* gene in *Drosophila* (MacIntyre and Davis, 1987). A 5.3 kb genomic region corresponding to the *Gpo* gene from the Canton S strain was sequenced along with three cDNA clones. Following comparison of the genomic and cDNA sequences a model for the structure and organisation of the *Drosophila Gpo* gene is presented. Analysis of the derived amino acid sequence revealed putative protein domains for a mitochondrial import sequence, a hydrophobic transmembrane segment, an iron-sulphur center and a FAD binding site, all consistent with a gene encoding a GPO enzyme.

MacIntyre, R.J., and Davis, M.B., 1987. *Current Topics in Biological and Medical Research, Molecular and Cellular Biology*. **14:**195-227.

CHARACTERISATION OF GENETIC AND MORPHOLOGICAL DIFFERENTIATION BETWEEN HYBRIDISING TAXA OF THE VARIED SITTELLA COMPLEX (AVES: NEOSITTIDAE)

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The Varied Sittella, Daphoenositta chrysoptera, is a small, aboreal passerine bird, endemic to the Australo-Papuan region. Within Australia, this polytypic species consists of five morphologically distinct subspecies which are distributed peripherally. Hybrid zones form wherever the subspecific populations meet. These can involve two, three and in one case all five forms.

This unusual situation provides a unique opportunity to study dynamics of simple and complex hybrid zones as well as providing insights into the nature of species and their origins. A combination of morphological, allozyme, mitochondrial and nuclear gene sequences will be used to characterise the five subspecies. This will provide definitive markers with which to study gene flow, isolation etc at the hybrid zones. Preliminary results will be presented here.

Localization of *cis*-acting sequences affecting nitrogen control of *amdS* in *Aspergillus nidulans*.

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The *amdS* gene of *A.nidulans* is regulated by multiple independent regulatory circuits. Genetic analysis has shown that the positively acting *areA* gene product is required for *amdS* expression under nitrogen limiting growth conditions.

A variety of *amdS*-*lacZ* fusion constructs have been used to define the *cis*-acting binding sites of the *areA* protein. These constructs have been integrated at the *amdS* genomic location by a two-step gene replacement method. The results of these studies suggest that sequences between -157 and -131 upstream of the start point of translation, are necessary for full response to *areA* control but that sequences further 3' to this site are also involved. Attempts to identify *areA* binding sites *in vitro* by mobility shift assay are currently underway.

DISTRIBUTION OF A Tag I mtDNA POLYMORPHISM AMONGST POPULATIONS OF THE KOALA, *PHASCOLARCTOS CINEREUS*

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Koala (*Phascolarctos cinereus*) populations in south eastern Australia have a history of bottlenecks - earlier this century the species became extinct in South Australia, and almost so in Victoria - as well as translocations of large numbers of animals from over-productive island populations, to sites around Victoria, and Kangaroo Island in South Australia. Some mainland sites may also have genetic lineages from animals surviving the crash. It is possible, therefore, that some populations have higher levels of genetic variability than others.

As part of a study of the genetic structure of koala populations in south eastern Australia, we have undertaken a survey of mitochondrial DNA (mtDNA) variability. Total DNA from 93 koalas from 5 populations was examined using 23 restriction enzymes, both 4 and 6-base cutters. The mtDNA fragments were detected using a domestic cat full-length mtDNA clone, Fca mt3-2. Of the 23 restriction enzymes, only one, Tag I, detected variability, allowing us to assign koalas one of two mtDNA haplotypes. All of the Victorian populations (South Gippsland, French Island, Stony Rises and Brisbane Ranges) possessed both haplotypes, while Kangaroo Island in South Australia showed only haplotype B in our sample. Haplotype frequencies were not homogenous amongst all populations, even with Kangaroo Island removed from the analysis.

A PCR GENOTYPING TEST FOR THE CAUSAL SITE OF THE A AND B ELECTROPHORETIC VARIANTS OF CATTLE β-LACTOGLOBULIN

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Abstract

The A and B variants of cattle β-lactoglobulin (BLG) are polymorphic at an *Hph*I site in exon III and a *Hae*III site in exon IV. The two polymorphic sites correspond to positions 64 and 118 of the mature polypeptide respectively: where variant A has an Asp-64 (negatively charged) while B has a Gly-64 (neutral), and A has a Val-118 (neutral) while B has an Ala-118 (neutral).

Conventional BLG genotyping involves amino acid charge separation of the milk protein variants. Therefore, this method detects only the amino acid substitution at position 64 (exon III polymorphic site). Using this method, previous studies have shown that cows with the B allele produce better milk for cheesemaking, attributable to higher contents of total solids, fat and casein.

A Polymerase Chain Reaction (PCR) genotyping test for the exon IV polymorphic site was reported by Medrano and Aguilar-Cordova (1990), but its effectiveness as a marker for BLG genotyping depends on strong linkage disequilibrium between the exon IV polymorphic site and the electrophoretic causal site in exon III. Although we have shown complete disequilibrium exists in Holstein-Friesian and *Bos taurus* x *Bos indicus* crossbred cattle, it would be useful to have a PCR test based on the electrophoretic causal site.

Based on the cattle BLG genomic sequence of L.J. Alexander and A.G. Mackinlay (manuscript in preparation), we have developed a PCR genotyping test for the electrophoretic causal site. The size of the PCR product is 240 bp. When cleaved with *Hph*I, the A variant produces a 229 bp fragment, while B produces 164 and 65 bp fragments. A PCR-genotyping method is more suitable for routine genotyping application because of its speed and technical ease. Genotyping using the *Hph*I RFLP can be carried out without prior knowledge of linkage disequilibrium in any dairy cattle population, as it detects the same variants as protein electrophoresis.

Genes affecting nuclear division in *Drosophila melanogaster* which are located in the chromosome region 89A1 - 89A10

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We have identified a recessive maternal effect gene, *supernova*, in *Drosophila melanogaster* which perturbs nuclear divisions in the cleavage embryo. Mutant embryos exhibit a terminal phenotype characterised by many variable sized nuclei scattered throughout the cytoplasm of the egg. When attempting division these nuclei produce ring like arrangements of chromosomes and/or DNA bridges. Nuclear divisions occurring in the first two to four hours after fertilization are characterised by mitotic asynchrony as well as defects in spindle organization.

Using deficiency mapping and in situ hybridization to polytene chromosomes we have mapped *supernova* to 89A1/2 on chromosome 3. The original mutation was recovered from a P-element screen and we have subsequently generated a number of P-element and EMS mutants in the region 89A1 - 89A10 which are currently being analyzed. DNA from 89A1/2 is being cloned to further characterize *supernova* and other genes in the region, some of which appear to be involved in cell division.

GENETIC VARIATION IN TAMMAR WALLABY WHEY PROTEINS

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Electrophoretic analysis of tammar wallaby whey proteins reveals both developmental and genetic variation. The appearance of the late lactation protein (LLP) after the young has suckled for 180 days accounts for the developmental variation. In addition, the LLP is polymorphic, with two different mobilities observed. Another genetically polymorphic protein is β-lactoglobulin (β-lac), with a four allele system being present in the 90 animals typed at present. The results also reveal that the heterozygous LLP is always associated with the heterozygous β-lac, suggesting that the genes for these two proteins may be linked. In order to investigate this further, and be able to type males and non-lactating females, Southern blots have been done. The membranes probed with β-lac exhibit a two allele RFLP which, with the animals typed so far, completely corresponds with the protein polymorphism. Several enzymes have been used for probing with LLP, but no RFLPs have been found as yet. However, a fixed difference between two races (Garden Island and Kangaroo Island) of tammar wallaby does exist, which will allow linkage studies to be done using hybrid animals.

"*Arabidopsis thaliana* and the Molecular Basis of Plant Biology"

A Robertson Symposium to be held at the Australian National University, Canberra, September 30 and October 1, 1991 in conjunction with the annual meeting of the Australian Society of Plant Physiologists (September 30 to October 4). The program will feature a wide range of topics showing the increasing value of this species to plant research. The program consists of invited speakers, offered contributions and a poster session.

Overseas speakers:

Tony Bleecker, Madison, *ethylene responses*
John Browse, Pullman, *lipid biosynthesis*
Xing-Wang Deng, Albany, *photomorphogenesis*
Ken Feldman, Tucson, *insertional mutagenesis using T-DNA*
Jerome Giraudat, Gif-sur-Yvette, *gibberellin response*
Gerd Jurgens, Munich, *morphogenetic mutants*
Maarten Koornneef, Wageningen, *floral initiation*
Richard Meagher, Athens, *actin genes*
Ry Meeks-Wagner, Oregon, *genetic regulation of the floral transition*
Elliot Meyerowitz, Pasadena, *homeotic genes in flowering*
Kyotaka Okada, Okazaki, *root growth direction mutants*
Renate Schmidt, Norwich, *floral genes*
Ronald Suzek, San Diego, *photomorphogenesis*

Further details and registration (A$75) from Dr R.E. Williamson, Research School of Biological Sciences, Australian National University, PO Box 475, Canberra, ACT 2601, Australia. Fax (06) 248 9995. Tel. (06) 249 5087. E-mail: Williamson@rsbs0.anu.edu.au

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ALLOTOPIC EXPRESSION OF YEAST MITOCHONDRIAL ATP SYNTHASE MEMBRANE SUBUNITS: ENHANCEMENT OF PROTEIN IMPORT BY DUPLICATION OF CLEAVABLE MITOCHONDRIAL TARGETING SEQUENCE

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The biogenesis of mitochondrial membranes requires the coordinated expression of both the mitochondrial and nuclear genomes. In our laboratory, the main focus of our studies has been the structure/function analysis of the mitochondrial ATP synthase complex (mtATPase). Of particular interest to us are the mitochondrially encoded subunits which constitute the F_o sector. We have been able to directly manipulate the genes of two of these F_o subunits (8 and 9) via allotopic expression (Nagley and Devenish, 1989). This method involves the reprogramming of the mitochondrial genes, via chemical gene synthesis, for expression in the nucleocytosolic system concomitant with delivery of the now cytosolically synthesised proteins back to the mitochondria by means of a suitable N-terminal leader. Subunit 8 (48 amino acids), when furnished with the N-terminal cleavable leader of *N. crassa* mtATPase subunit 9 (66 amino acids), is capable of being imported into isolated mitochondria *in vitro* and, when expressed *in vivo*, is able to complement a yeast mutant lacking endogenous subunit 8 (Nagley and Devenish, 1989). On the other hand, allotopically expressed subunit 9 (76 amino acids), when furnished with the same leader, is targeted to isolated mitochondria *in vitro* (Farrell, *et al.*, 1988) but conditions have not yet been found for successful complementation of yeast mutants lacking endogenous subunit 9.

Two complementary strategies have been devised in our laboratory to achieve both efficient delivery of subunit 9 to the mitochondria together with functional rescue of subunit 9 mutants. The first strategy involves the duplication of the leader sequence used to target the subunit 9 to the mitochondria. *In vitro* import expperiments indicate that such a duplication greatly enhances the efficiency of import of subunit 9. Yet thsesdouble leader constructs, when expressed *in vivo do not lead to complementationof a* subunit 9-deficient mutant. The failure of this imported protein to functionally rescue the appropriate yeast mutants may be due to aberrant processing tof the precursors to yield non-functional subunit 9 molecules within the mitochondrial matrix. To remedy this situation a second strategy is now being applied in the laboratory using a saturation mutagenesis technique centred upon the matrix protease cleavage site region of the chimaeric precursor proteins. The introduction of a synthetic gene sequence derived by the annealing of oligonucleotides incorporating regions of high variability, and their subsequent extension using PCR is also being developed as a system for the introduction of random mutations in the region of the gene spanning the matrix protease cleavage site of the leader and the N-terminus of the subunit 9 gene.

Nagley, P. and Devenish, R.J. (1989) *Trends Biochem. Sci.* **14**, 31-35
Farrell, L.B., Gearing, D.P. and Nagley, P. (1988) *Eur. J. Biochem.* **173**, 131-137.

SYSTEMATIC MUTATIONAL ANALYSIS OF YEAST MITOCHONDRIAL ATP SYNTHASE SUBUNIT 8

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The proton-translocating F_o sector of the mitochondrial ATP synthase complex (mtATPase) in yeast is constituted by three hydrophobic integral membrane proteins, subunits 6, 8, and 9. Subunit 8 (Y8), a 48 amino acid polypeptide encoded by the mitochondrial *aap1* gene, spans the inner membrane with its positively-charged C-terminal tail protruding towards the matrix and its N-terminus facing into the intermembrane space. Allotopic expression of the chimaeric subunit 8 precursor N9L/Y8-1 (Y8 fused to a powerful N-terminal mitochondrial targeting signal denoted N9L) in strain M31, an *aap1 mit*⁻ mutant lacking endogeneous Y8, leads to restoration of mtATPase function (Nagley *et al.*, 1988) and provides a means of systematically analyzing the structure/function relationships of Y8. To facilitate the cloning of DNA fragments specifying variants of N9L/Y8-1 generated by site-directed *in vitro* mutagenesis, a uniform cassetting strategy has been developed. Mutagenesis is carried out on M13 templates incorporating *Bam*HI and *Not*I recognition sites at the 5' and 3' ends of the N9L/Y8-1 sequences, respectively. Following mutagenesis and confirmation of the desired alteration by nucleotide sequencing, *Bam*HI-*Not*I digestion is used to release gene cassettes that can be inserted directly into a series of different vectors for expression *in vivo* or *in vitro*.

A focal point for systematic variations in Y8 is the C-terminal positively charged tail, a conserved feature of fungal and metazoan Y8 counterparts. Previous work by Grasso *et al.* (1991) indicated that the allotopic expression of a variant truncated at the penultimate residue Lys47 leads to only partial restoration of function in the transformed M31 cells, due to a reduction in the rate of assembly of Y8. We have made an additional set of variants in which each of the three positively charged residues (Lys47, Arg42, Arg37) has been replaced with the neutral residue (Ile) maintaining the full length of Y8. A second thrust concerns the probing of the N-terminal and C-terminal boundaries of the transmembrane stem, and the examination of flexibility with which amino acid substitutions in the transmembrane stem can be tolerated. We have constructed a set of Y8 variants where charged residues are introduced at intervals along the length of Y8. All these variant constructs are currently being introduced into M31 host cells, and the ability of allotopically expressed variant Y8 to restore growth of the transformant strains on non-fermentable substrates assessed at various temperatures. Parallel *in vitro* studies of import and assembly will provide further details on the properties of the Y8 variants.

Grasso, D.G., Nero, D., Law, R.H.P., Devenish, R.J. and Nagley, P. (1991) *Eur. J. Biochem.* (in press)
Nagley, P., Farrell, L.B., Gearing, D.P., Nero, D., Meltzer, S., and Devenish, R.J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2091-95

EVOLUTIONARY GENETICS OF THE HLA REGION

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A number of observations indicate that strong selection is acting on the HLA region, including its extensive polymorphism with very even allele frequencies, the preferential occurrence of high levels of variability at positions critical to antigen recognition, the great age of alleles and the patterns of linkage disequilibrium among loci. Mutation, recombination and gene conversion all contribute to the generation of HLA variability. The overall pattern of normalized linkage disequilibrium values for all HLA A-B and B-DR haplotypes indicates clear departures from that expected under neutrality. Two methods have been developed to detect the haplotypes (disequilibrium pattern analysis - DPA) or alleles (constrained disequilibrium values - CDV) on which selection has acted, and created the deviations from neutrality expectations. DPA identifies recent selection events from the pattern of the array of two-locus haplotypes in the disequilibrium space subdivided on the basis of all haplotypes sharing one allele. The CDV method uses the fact that three locus systems impose additional constraints on the range of possible disequilibrium values for any pair of loci. Both methods identified six HLA haplotypes showing disequilibrium patterns strongly suggestive of selection. In each of these cases the CDV method identified the B locus allele as the one most likely subject to selection. The CDV method further identified strong evidence for selection on two rare Bf locus alleles and weak evidence for selection on DR4.