34th ANNUAL CONFERENCE OF THE
GENETICS SOCIETY OF AUSTRALIA

26th - 29th August 1987

Ti

at the Australian National University and CSIRO, Canberra

Programme and Abstracts

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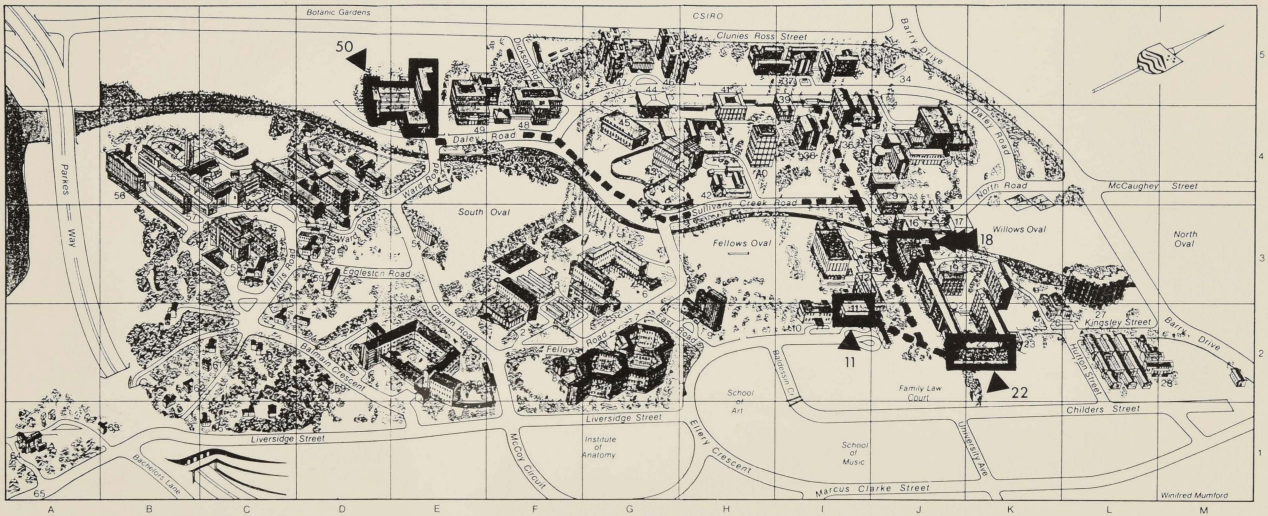

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GENERAL INFORMATION

WELCOME

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

John Oakeshott (Local Secretary)
Rudi Appels
Abigail Elizur
Robyn Russell
Mike Arnold

REGISTRATION

Registration is $28.00 for non-students and $15.00 for students, except for students giving a paper or poster, for whom the registration fee will be waived. Non-financial members of the Society are encouraged to pay their membership fees ($22, non-students; $7, students) with their registration.

The registration desks will be in the main foyer of Burgmann College from 7.00 pm to 10.00 pm on Wednesday, 26 August, and in the foyer of the Copland Lecture Theatre from 8.30 am to 9.00 am on Thursday, 27 August. John Oakeshott or Rudi Appels will be available in Melville Hall during the morning and afternoon tea breaks for those wishing to register after this time.

ACCOMMODATION AND MEALS

Single rooms have been allocated in Burgmann College to all those who have indicated their need for College accommodation. Keys are available at registration or from Denece Bayliss in the College Office (Tel: 486018). Those who have not paid in advance must pay for their rooms at registration ($20 a night students, $35 a night for others). Rooms should be vacated by 10.00 am on the day of departure but baggage can be left at the College Office until final departure.

Those staying at Burgmann College are entitled to breakfast, which is served between 7.45 am and 8.45 am in the main dining room of the College. Room keys will serve as "tickets" for breakfast.

Lunch is available at the University Union which is closer to the Conference venue. The Union includes a Hamburger & Snack Bar (which also serves tacos and roasts), a Sandwich & Coffee Bar, Steak Bar, Pancake Bar, Health Food Buffet and, upstairs, an Asian Bistro.

Several of the Union facilities also serve evening meals. Other moderately priced restaurants off-campus but in or near Civic include:

Imperial Court (Chinese) 40 Northbourne Avenue (Tel: 48 5547)	Malaysian Restaurant 71 London Circuit (Tel: 498736)	Mama's Trattoria 7 Garema Place (Tel: 480936)

Pepper's Bistro Canberra Arcade East Row (Tel: 491120)	Sinbad's Lebanese 25 East Row (Tel: 474068)	Shamrat Indian Restaurant 39 Northbourne Avenue (Tel: 475724)

SCIENTIFIC SESSIONS

The Conference Opening and Plenary Sessions will be held in the Copland Theatre. Concurrent Sessions will be held in the Copland Theatre and Theatres G7 & G8, also on the ground floor of the Copland Building. Tea, coffee, posters and the Trade Display will be in Melville Hall in the adjacent A.D. Hope Building. The locations of the Copland, A.D. Hope and Union Buildings are indicated on the campus map on the inside front cover of this booklet.

PAPERS

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

POSTERS

Posters will be on view in Melville Hall over afternoon tea on both Thursday, 27th, and Friday, 28th, and over morning tea on Friday, 28th. Presenting authors are asked to set up their posters by 1.30 pm on Thursday at the latest and to be in attendance at the poster sessions on both afternoons. Please contact Abigail Elizur and Robyn Russell at the registration desks for directions to set up posters.

STUDENT SUBSIDY

Student members of the Society who present a paper or poster are eligible for a travel subsidy, pro-rated on the distance travelled to the meeting. Cheques will be available from Mike Arnold during afternoon tea in Melville Hall on Friday, 28th. Cheques not collected then will be delivered by mail subsequently.

TELEPHONES AND MESSAGES

Public pay 'phones are located in Burgmann College, the University Union and next to the Post Office in the plaza adjacent the Union. During normal office hours messages for delegates can be received on (062) 479811. Messages will be posted on a designated poster board in Melville Hall.

SHOPPING AND BANKS

Within the University Union or adjacent plaza there are a Union Shop, Pharmacy, Post Office, Book Shop, a branch of the Commonwealth Bank and agencies for the National and Westpac Banks. Extensive shopping facilities are available 1 km east in Civic. Civic shops close at 5.30 pm on Wednesday and Thursday, 9.00 pm on Friday, and 12 noon on Saturday.

Petrol can be purchased in Cooyong or Lonsdale Streets, Braddon, adjacent Civic.

Taxis can be contacted on 460444.

EMERGENCY

Emergency medical assistance can be obtained from the campus Health Service, in the Union complex (493598), or at the Royal Canberra Hospital in Acton (432111).

SOCIAL FUNCTIONS

MIXER

7-10 pm on Wednesday, 26 August, in the Common Room of Burgmann College. A certain (reasonably large) quantity of drinks and light snacks will be provided free to registered delegates. When this supply is exhausted, the bar will remain open but delegates will have to pay for their own drinks.

ANNUAL SOCIETY DINNER

7.10 for 7.30 pm on Friday, 28 August, in the main dining room of Burgmann College. Tickets are $22 and can be obtained at the registration desks on Wednesday evening and Thursday morning. The tickets cover pre-dinner drinks, a three course meal and some drinks at the table. A bar will be open for the purchase of additional soft drinks, beers, wines and spirits. There will also be a dance band.

BARBEQUE

1.00 pm - ? on Saturday, 29 August, in the garden at Burgmann College (or the dining room if the weather is unfriendly). Tickets are $8.50 and can be obtained at the registration desks on Wednesday evening and Thursday morning. The tickets will not include drinks but the bar will be open for their purchase.

ACKNOWLEDGEMENTS

On behalf of the GSA, the organising committee wishes to thank the CSIRO Divisions of Entomology and Plant Industry and the Australian National University for hosting the Conference. Ansett Airlines of Australia also provided financial and other assistance. Staff of the secretarial, copying and mailing services of the two CSIRO Divisions are also to be thanked for their invaluable contributions. Of those many other individuals who helped at different times we particularly thank Dave Shaw, who worked hard on the committee but was unable to attend the Conference, Anne Game and Dave Smith, who assisted with the preparation of the circulars and this booklet, and Peter Cooke, who supervised the projection facilities. Denece Bayliss of Burgmann College is gratefully acknowledged for coordinating the accommodation and social functions.

The committee also thanks the following Sustaining Members for supporting the Conference with their trade displays:

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YOUNG,	Ian	ANU

PROGRAMME

Names of presenters are underlined

WEDNESDAY 26 AUGUST

REGISTRATION AND MIXER
7.00-10.00 pm Burgmann College

THURSDAY 27 AUGUST

REGISTRATION
8.30 am Copland Theatre Foyer

OPENING ADDRESS
9.00 am Copland Theatre
Chairman, Des Cooper
The Hon. Barry Jones, Minister for Science

PRINCIPAL GUEST SPEAKER
9.15 am Copland Theatre
Chairman, Des Cooper
Projectionist, Peter Cooke
Dan L. Hartl (Washington University, St Louis)
Transposable elements and molecular evolution.

MORNING TEA
10.15-10.45 am Melville Hall

SESSION 1A Copland Theatre
Chairman, Jim Peacock
Projectionist, Philip England

10.45 am Neil Willetts (invited speaker)
Genetically engineered vaccines.

11.25 am J.D. Murray, C.D. Nancarrow & K.A. Ward
Expression of sheep growth hormone fusion genes in transgenic animals.

11.45 am W.L. Gerlach, D. Llewellyn & J.P. Haseloff
Molecular synthesis of a plant virus resistance gene.

12.05 pm J.P. Haseloff & W.L. Gerlach
RNA with enzymatic activity; autolytic cleavage sites in a satellite RNA.

SESSION 1B Theatre G7
Chairman, John McKenzie
Projectionist, David Rowell

10.45 am J.W. James
Standard errors of heterosis estimates.

11.05 am B. Oldroyd
Are queen or worker honeybee characters more important to honey production?

11.25 am J. Daly & J. Fisk
When does selection for insecticide resistance occur in *Heliothis*?

11.45 am N.D. Murray
Electrophoretic variation in *Cactoblastis cactorum* : estimating the effective size of introduced populations in Australia and Hawaii.

12.05 pm J. Tomasov
Electrophoretic comparisons between ancestral and derived populations of the biocontrol agents *Chyrosolina quadrigemina* and *C. hyperici*.

SESSION 1C Theatre G8
Chairman, David Smyth
Projectionist, Harvey Perkins

10.45 am C.M. Merritt, G.C. Webb and P.G. Board
Characterisation and chromosomal localisation of the human α-1 acid glycoprotein genes.

11.05 am R.T. Baker, G.C. Webb and P.G. Board
The human ubiquitin gene family : chromosomal location and molecular characterisation of the UbB subfamily.

11.25 am G.B. Peters, J.H. Ford and J.K. Nichol
Trisomy 21 and the maternal age effect.

11.45 am V. Korolik and V. Krishnapillai
Development of an RNA probe for *Compylobacter jejuni* (a human enteropathogen).

12.05 pm S.P. Davies and V. Krishnapillai
DNA sequence of the replication region of the *Pseudomonas* plasmid R91-5.

LUNCH
12.25-1.30

SESSION 2A Copland Theatre
Chairman, Wayne Gerlach
Projectionist, Lynne McIntyre
SYMPOSIUM ON GENOME ORGANIZATION AND REPEATED SEQUENCES

1.30 pm Art Hilliker (invited speaker)
Chromosome organisation in *Drosophila*.

2.30 pm A. Lohe and C. Moran
Repression of a white P element transposon by flanking satellite DNA sequences in *Drosophila*.

2.50 pm C. Moran and A. Lohe
Genetics of an unstable white transposon containing satellite DNA in *Drosophila*.

SESSION 2B Theatre G7
Chairman, Stuart Barker
Projectionist, Alex Bortoli
SYMPOSIUM ON POPULATION GENETICS

1.30 pm Tim Prout (invited speaker)
Some theory on the "Balance of Forces".

2.30 pm K. Jahromi, H-H.M. Dahl, J.F.B. Merler, K.N.P. Mickelson and R.J. Trent
Study on phenylalanine hydroxylase gene restriction fragment length polymorphisms in polynesians.

2.50 pm G.F. Moran and O. Muono
Multiple paternity in acacias.

AFTERNOON TEA, POSTERS AND TRADE DISPLAY
3.10-4.10 pm Melville Hall

SESSION 3A Copland Theatre
Chairman, Wayne Gerlach
Projectionist, Lynne McIntyre
SYMPOSIUM ON GENOME ORGANIZATION AND REPEATED SEQUENCES (cont.)

4.10 pm M.E. Matthews, A.E. Lord and K.L. Reed
Y-specific repeated sequences conserved in ruminants.

4.30 pm C.E. May and R. Appels
The extent of variation in the *Nor* loci in wheat and some implications forthe function of the rDNA spacer region.

4.50 pm R. Appels, L. Moran and O. Frankel
The *Ter* loci in the grasses: regions at the termini of chromosomes defined by repetitive DNA sequence families.

5.10 pm J.W. Sentry and D.R. Smyth
Structure of *del*, an abundant dispersed repeat from the genome of *Lilium henryi*.

5.30 pm I. Kola, A. Trounson, G. Dawson and P. Rogers
Tripronuclear human oocytes: altered cleavage patterns and subsequent karyotypic analysis of embryos.

SESSION 3B Theatre G7
Chairman, Stuart Barker
Projectionist, Alex Bortoli
SYMPOSIUM ON POPULATION GENETICS (cont.)

4.10 pm C. Jiang, J.B. Gibson, A.V. Wilks and A.L. Freeth
Comparison of restriction endonuclease variation surrounding *Adh* null and normal alleles from natural populations of *Drosophila melanogaster*.

4.30 pm A.L. Freeth, J.B.Gibson and A.V. Wilks
Molecular analysis of alcohol dehydrogenase null alleles from natural populations of *Drosophila melanogaster*.

4.50 pm M.A.M. Meredith and F.Y.T. Sin
Genetic variation of four populations of the Little Blue Penguin, *Eudyptula minor*.

5.10 pm A. Torkamanzehi, C. Moran and F.W. Nicholas
Another test of the role of transposable elements in generating quantitative genetic variation, using the P element system of *Drosophila*.

5.30 pm P.D. Christian
The distribution of *Drosophila* A virus in Australian populations of *Drosophila melanogaster* and *D. simulans*.

FRIDAY 28 AUGUST

SESSION 4A
Copland Theatre
Chairman, John Oakeshott
Projectionist, Philip England

9.00 am W.R. Knibb, P. England and R.B. Saint
Genetic analysis of a homeobox-containing region of *Drosophila*.

9.20 am R.B. Saint, B. Kalionis, A. Elizur and J. Davidson
Molecular analysis of a homeobox-containing gene of *Drosophila*.

9.40 am A. Elizur, B. Wicksteed, C. Landsberg and A.J. Howells
The white and topaz genes of *Lucilia cuprina* : structure, function and analysis of mutants.

10.00 am W.D. Warren and A.J. Howells
The cloning and preliminary characterisation of the cinnabar locus in *Drosophila melanogaster*.

SESSION 4B
Theatre G7
Chairman, Brett Tyler
Projectionist, Jill Karotam

9.00 am I. Richardson
Characterisation of the *gatA* gene of *Aspergillus nidulans*.

9.20 am R. Van Heeswyck and M.I. Groncero
DNS mediated transformation of mucocircinelloides.

9.40 am J. Piskur, G.D. Clark-Walker and E. Wimmer
Intergenic sequences are involved in transmission of the yeast mitochondrial genome.

10.00 am D.J. Colgan and D.A. Willocks
Electrophoretic variation and plasmid diversity in the enteric bacteria of *Caledia captiva* and other grasshoppers.

SESSION 4C
Theatre G8
Chairman, Ross Crosier
Projectionist, David Rowell

9.00 am M.L. Arnold, N. Contreras and D.D. Shaw
Asymmetric introgression of rDNA between subspecies: evidence for the involvement of biased gene conversion.

9.20 am A. Marchant
Comparison of allozyme, mitochondrial and rDNA variation across a chromosomal tension zone.

9.40 am C. Moritz, S. Donnellan, M. Adams and P. Baverstock
Extent of clonal diversity of the parthenogenetic gecko *Heteronotia binoei*.

10.00 am D. Bedo
Cytogenetic studies in the mediterranean fruitfly, *Ceratitis capitata*.

MORNING TEA
10.20-10.50 Melville Hall

SESSION 5A Copland Theatre
Chairman, Tony Howells
Projectionist, Jill Karotam

10.50 am P.H. Cooke and J.G. Oakeshott
Nucleotide and amino acid polymorphisms within and between Esterase 6 electromorphs of *Drosophila melanogaster*.

11.10 am A.Y. Game and J.G. Oakeshott
Esterase 6 activity variation in *Drosophila melanogaster*: associations with restriction site polymorphism.

11.30 am E.A. Van Papenrecht and J.G. Oakeshott
Molecular basis of rapid evolutionary change in the structure and function of *Drosophila* Esterase 6.

11.50 am J. Bonsing, A.F. Stewart and A.G. Mackinlay
The 5′ flanking sequences of the bovine β casein genes : conserved elements in related genes.

SESSION 5B Theatre G7
Chairman, Kieran Scott
Projectionist, Mike Arnold

10.50 am A. Delves, B. Carroll and P.M. Gresshoff
The inheritance of supernodulation in soybeans.

11.10 am A. Mathews, B. Carroll and P.M. Gresshoff
Host genetics of non-nodulation mutants of soybeans.

11.30 am N.M. Upadhyaya, K.F. Scott and P.J. Dart
Bacterial genes essential for systemic pathogenesis in plants.

11.50 am T.J. Close
Regulation of *Agrobacterium* Ti plasmid virulence gene.

SESSION 5C Theatre G8
Chairman, Len Kelly
Projectionist, Bill Warren

10.50 am J.R. Andrews, G.M.F. Pasquini and P. Batterham
How complex is the lozenge locus of *Drososphila melanogaster*.

11.10 am M. Phillips, L. Salkoff and L. Kelly
Isolation and characterisation of *Drosophila* genes with homology to a feline glutamate decarboxylase cDNA.

10.30 am H.A. Mariath
Operant conditioning in *Drosophila melanogaster* wild type and mutants with defects in neurotransmitter synthesis and in cyclic AMP metabolism.

11.50 am G.G. Foster, G.M. Clarke and G.L.Weller
Crossing over in male *Lucilia cuprina*.

LUNCH
12.10-1.10

SESSION 6A — Copland Theatre
Chairman, Rudi Appels
Projectionist, Mike Arnold
SYMPOSIUM ON MOLECULAR EVOLUTION

1.10 pm Lynne McIntyre (invited speaker)
Evolutionary change in species of the *Triticeae* as measured using isozyme variation and DNA sequence change.

1.40 pm Luke Mitchell (invited speaker)
Evolutionary change at the *Adh* locus in wheat in its relatives.

2.10 pm P. Reddy
Variation at the DNA level among *Secale* species.

2.30 pm M.A. Ayliffe, N.S. Scott and J.N. Timmis
Homologies to chloroplast DNA in nuclear DNA of a number of plant species.

SESSION 6B — Theatre G7
Chairman, Mike Hynes
Projectionist, Mark Myers
SYMPOSIUM ON GENE REGULATION

1.10 pm Peter Molloy (invited speaker), F. Watt and D.J. Tremethick
DNA methylation and its possible roles in gene regulation.

1.50 pm M. Frommer and L. McDonald
Methylation in CpG islands.

2.10 pm B. van Leeuwen, J.D. Penschow, J.P. Coghlan and R.I. Richards
Differential response of mouse kallikrein genes to hormonal induction.

2.30 pm M.E. Katz and M.J. Hynes
The characterisation of *facB*, a gene in *Aspergillus nidulans* with properties of a structural and regulatory gene.

AFTERNOON TEA, POSTERS AND TRADE DISPLAY
2.50-3.50 Melville Hall

SESSION 7A — Copland Theatre
Chairman, Rudi Appels
Projectionist, Mike Arnold
SYMPOSIUM ON MOLECULAR EVOLUTION (cont.)

3.50 pm A. Gibbs
Origins and speciation in the viruses.

4.10 pm S. Easteal
Vertebrate β globin gene evolution and the molecular clock.

4.30 pm P.R. England, H.W. Stokes and R. Frankham
X-Y exchange and the coevolution of X and Y rDNA.
4.50 pm A. Johnson and P.R. Baverstock
Rapid sequencing of the small ribosomal RNA gene for phylogenetic analysis.

SESSION 7B Theatre G7
Chairman, Mike Hynes
Projectionist, Mark Myers
SYMPOSIUM ON GENE REGULATION (cont.)

3.50 pm C. Cobbett
Regulation of the *aroFtyrA* operon of *Eschericia coli*.
4.10 pm D. Llewellyn, J. Tokuhisa, B. Taylor, E.S. Dennis and W.J. Peacock
Anaerobic regulation of alcohol dehydrogenase from *Pisum sativum*.
4.30 pm T. Lockett and M. Ashburner
Alcohol dehydrogenase gene promoter usage during *Drosophila melanogaster* development.
4.50 pm P. East, K. Paigen and D. Schott
Molecular characterisation of two naturally occurring regulatory variants of *Adh* in *Drosophila melanogaster*.

BUSINESS MEETING
5.10 pm Copland Theatre

ANNUAL SOCIETY DINNER
7.10 for 7.30 Burgmann College

SATURDAY 29 AUGUST

GUEST SPEAKER Copland Theatre
Chairman, Des Cooper
Projectionist, Peter Cooke
9.00 am Ian Young
Abnormal expression of interleukin-3 and leukemia.
10.00 am John Shine
Genetics of multifactorial disease.

MORNING TEA
11.00-11.30 Melville Hall
M.J.D. WHITE ADDRESS
Copland Theatre
Chairman, Des Cooper
Projectionist, Peter Cooke
11.30 am Michael Hynes
The new fungal genetics.

BARBEQUE
1.00- Burgmann College

POSTERS

1. D. Rouch, J. Camakaris, G. Adcock and B. Lee
Genetic and biochemical analysis of copper transport in *Escherichia coli*.
2. L. Farmer and C. Cobbett
Translational coupling in the *aroFtyrA* operon of *Escherichia coli*.
3. A. Andrianopoulos and M.J. Hynes
Analysis of the positively-acting regulatory gene *amdR* of *Aspergillus nidulans*.
4. M.A. Davis and M.J. Hynes
Expression of the heterologous regulatory gene, *nit2* of *Neurospora crassa*, in *Aspergillus nidulans*.
5. T.G. Littlejohn and M.J. Hynes
Activation sequences of the *amdS* gene in *Aspergillus nidulans*.
6. J.A. Saleeba and M.J. Hynes
Investigation of *ariA* in *Aspergillus nidulans*.
7. R.A. Sandeman and M.J. Hynes
The regulation of genes involved in acetate utilisation in *Aspergillus nidulans*.
8. J. Piskur, G.D. Clarke-Walker and E. Wimmer
A site specific recombination through short direct repeats is involved in the generation of smaller *rho*$^+$ yeast mitochondrial genomes.
9. A.L. Bull and L.E. Kelly
Cloning calmodulin binding proteins from *Drosophila*.
10. M. Morgan and L.E. Kelly
Isolation and characterisation of lethal mutations at the *shibire* locus in *Drosophila melanogaster*.
11. T. Petrovich and L.E. Kelly
Characterisation of the suppressor of stoned mutant.
12. N. Brink and R.B. Saint
The phenotype of a presumptive homeobox gene in *Drosophila*.
13. J. Karotam and J. Oakeshott
Conservation and change in Esterase 6 nucleotide sequences among sibling *Drosophila* species.
14. P. Matthew and S. McKechnie
Characterisation of a polymorphic insert within the *Adh* gene of *Drosophila melanogaster*.
15. J. Ross and S. McKechnie
Sn-glycerol-3-phosphate oxidase studies in *Drosophila melanogaster*.
16. J. Martin and B.T.O. Lee
Cloning a sex determining gene in *Chironomus*.
17. S.A. Fabb, B.T.O. Lee and J. Martin
A sex-influenced protein in *Chironomus*-properties and cloning strategy.
18. S. Constable
Comparison of proteases produced by larvae of *Lucilia cuprina* and other blowfly species.

19. G.G. Foster and R.J. Mahon
Field trials of genetic control of *Lucilia cuprina*.

20. D. Bedo
Polytene chromosome map of the mediterranean fruitfly, *Ceratitis capitata*.

21. A. Wilton, J. Sved, H. Kai and F.J. Ayala
Fitness of half chromosome homozygotes of *Drosophila melanogaster*.

22. W.B. Sherwin, N.D. Murray and J.A.M. Graves
Genetic variation in threatened and secure bandicoot populations.

23. G.R. Bishop, O. Mayo and L. Beckman
The possible influence of major gene heterozygosity on variation in quantitative traits.

24. D.L. Hayman, H.D.M. Moore and E.P. Evans
Cytogenetic comparison between the American marsupial *Monodelphis domestica* and Australian marsupials.

25. K. Nicholas and C. Collet
The Tammar wallaby mammary gland: a model system to study prolactin dependent control of gene expression.

26. S. Cooper
The isolation of a β globin gene from *Sminthopsis crassicaudata*.

27. J.M. Wrigley, L.M. McKay and J.A.M. Graves
Genome instability in interspecific cell hybrids. I. Aminopterin resistance and gene amplification in lines arising from fusions of cells from divergent mammalian species.

28. R.A. Farrell, J. Camakaris, J.F. Mercer and D.M. Danks
Metallothionein mRNA levels in Menkes' disease lymphocytes.

29. A. Davey and I. Kola
Chromosomal localisation of the 70 kDa heat shock protein (hsp70) gene in humans.

30. C. Kirby, I. Kola, J. Shaw, A. Davey, S. Edmondson and A. Trownson
Vitrification inhibits the ability of mouse oocytes fertilised *in vitro* to form viable foeteses.

31. V.H.K. Low and J.A. Thompson
Cyanogenesis in Australian bracken (*Pteridium esculentum*): factors influencing induction of enzyme and substrate.

32. P.J. Mathews
Ribosomal DNA variation in taro.

33. R.G. Saini, G. Malhotra and A.K. Gupta
Inheritance of adult plant leaf rust resistance to race 77A- in cultivars WG138 and Teznos Precos Pintos.

PRINCIPAL GUEST SPEAKER

TRANSPOSABLE ELEMENTS AND MOLECULAR EVOLUTION

D.L. HARTL

Washington University, St. Louis

An unstable allele of the *white* locus in *Drosophila mauritiana* mutates at the rate of approximately 10^{-3} per generation. The allele, designated *white-peach* ($\underline{w}^{pch}$), results from the insertion of a transposable element *mariner* just upstream from the first *white* exon. The *mariner* element is 1286 nucleotides long, including 28 base-pair terminal inverted repeats, and contains a single open reading frame of 346 amino acids. The *mariner* element is present in approximately 20 copies in the genome of *D. mauritiana*, its copy number is much lower in *D. simulans* and other members of the *melanogaster* species group, and it is apparently absent from the genome of *D. melanogaster* and *D. erecta*. In certain strains, the *mariner* element in the $\underline{w}^{pch}$ allele undergoes a high rate of excision in somatic cells, which is indicated phenotypically by the occurrence of mosaic eye colour. The inherited mosaicism results from a single dominant genetic factor denoted *Mos*, which is located in chromosome number 3. The *Mos* gene may result from aberrant expression of a copy of *mariner* present at the site. In strains of *D. simulans* into which the *Mos* gene has been introduced, cytoplasmic transmission of substances also results in eye-colour mosaicism. This maternal effect is mediated by a product acting after fertilization, and it gives rise to somatic mosaics that are phenotypically distinct from those which inherit the *Mos* gene itself.

GENETICALLY ENGINEERED VACCINES

N. Willetts

Biotechnology Australia Pty Ltd, P.O. Box 20, Roseville, N.S.W. 2069

Vaccination provides a simple, inexpensive and effective means of protecting humans and animals against infectious disease. By providing the means to produce in pure form large quantities of protective antigens, recombinant DNA technology is expected to lead both to improvements in the quality of traditional vaccines against viruses and bacteria, and to the introduction of novel vaccines, particularly against parasite infections. Vaccines prepared from killed or attenuated organisms are not feasible for parasites because of the difficulty of growing sufficient quantities, and the complexity of their antigenic components. The route for development of genetically engineered sub-unit vaccines will be described, and their advantages and limitations discussed. It is important to elicit an immune response appropriate to the site of infection, and genetic engineering techniques may also provide novel immunomodulatory molecules, as well as novel presentation routes such as the use of attenuated live viruses or bacteria to carry the gene for a protective antigen. Finally, commercial considerations such as return on investment and product liability, which can have as profound an effect on vaccine development and marketing as scientific considerations, will be briefly mentioned.

EXPRESSION OF SHEEP GROWTH HORMONE FUSION GENES IN TRANSGENIC ANIMALS

J.D. Murray, C.D. Nancarrow, K.A. Ward,
N.W. Rigby, K.A. Raphael and C.T. Townrow

CSIRO, Division of Animal Production,
Great Western Highway, Prospect, N.S.W., Australia

Two sheep metallothionein Ia - sheep growth hormone fusion genes have been constructed. GH5 contains the SV-40 promoter 3' to the gene, while GH9 does not. The sheep metallothionein Ia promoter has an altered basal level expression element (BLE) when compared to the BLE found in other metallothionein (Mt) genes and the SV-40 promoter is known to compete with the Mt promoter for transcription binding factors such as SP1.

The GH5 and GH9 constructs have been transferred into L-cells and mice. Both constructs are expressed constitutively in L-cells and retain inducibility when challenged with zinc or cadmium. The GH5 construct is not expressed in transgenic mice even in the presence of zinc. However GH9, which lacks the SV-40 promoter, is induced by 25mM zinc sulphate in the drinking water and results in an increased growth rate with a final body size of ~1.8 times normal. GH5 is not expressed in transgenic sheep. The data will be discussed with respect to the application of the sheep MtIa promoter for use as a controllable promoter in transgenic animals.

MOLECULAR SYNTHESIS OF A PLANT VIRUS RESISTANCE GENE.

W.L. Gerlach, D. Llewellyn and J. Haseloff, CSIRO Division of Plant Industry, GPO Box 1600, Canberra. ACT 2601.

Tobacco ringspot virus (TobRV) is the type member of the Nepovirus group of plant viruses. It infects a range of dicotyledonous plants. A small RNA, termed the satellite RNA of tobacco ringspot virus (STobRV), which can replicate to high levels and be encapsidated by TobRV in infected plants, has been previously found during serial passages of virus isolates. STobRV is not required for virus propagation and has no extensive sequence homology with the TobRV genomic RNAs. It can be considered a parasite of the virus and its presence during virus infection ameliorates symptoms in plants.

We have prepared gene constructions based on cloned cDNA sequences of STobRV, and have expression of forms of the STobRV in transgenic tobacco plants produced by transformation with these gene constructions. Plants which express full length STobRV or its complementary sequence as RNA transcripts show phenotypic resistance when infected with TobRV. This is correlated with amplification of STobRV to high levels during virus infection of plants and reduced systemic spread of the virus in infected plants.

RNA WITH ENZYMATIC ACTIVITY; AUTOLYTIC CLEAVAGE SITES IN A SATELLITE RNA.

Jim Haseloff and Wayne L. Gerlach.
CSIRO Division of Plant Industry, GPO Box 1600, Canberra. ACT. 2601.

The satellite RNA of tobacco ringspot virus (STobRV) is a member of a class of small (300-400b), single-strand RNAs which are parasites of plant viruses. These RNAs do not encode polypeptide products, and for replication must contain signals for recognition by plant host or helper virus enzymes, or rely on RNA-encoded catalytic activities. Circular and concatameric forms of the satellite RNAs are found *in vivo*, and are thought to be intermediates in "rolling-circle" type mechanisms of RNA replication. Concatameric transcripts undergo spontaneous autolytic cleavage to generate unit-length RNAs *in vitro* and *in vivo*. We are using "reverse-genetics" approaches to define the activities and signals encoded by the STobRV RNA, including those involved in autolytic cleavage of (+) and (-) strand RNAs.

From cloned STobRV cDNA, a plasmid for *in vitro* STobRV transcription has been constructed and used to obtain a library of randomly mutagenized STobRV sequences. Screening of these mutants has allowed precise definition of the sequences required for (+) and (-) strand autolytic cleavage. The sequences and RNA structures associated with cleavage of each strand differ markedly. Cleavage of the (+) strand requires at least 52 bases surrounding the site of cleavage, and formation of a conserved structure similar to those found in other satellite RNAs and viroids. In contrast, cleavage of the (-) strand requires only a small region of around 12 bases at the site of cleavage, and a sequence of 55 bases which may act in *trans*. It may be possible to design simple, specific RNA ribonucleases.

STANDARD ERRORS OF HETEROSIS ESTIMATES

J.W. JAMES

Department of Wool Science
University of New South Wales

In animal breeding, heterosis is usually defined as the difference between the average of reciprocal crosses between two breeds of strains, and the average of the two parental genotypes. Estimates of heterosis are subject to sampling errors, mainly from variation in average breeding values of animals used in the matings. Accuracy can be increased by using the same parents to produce both purebred and crossbred progeny as far as possible.

In some circumstances, one purebred may be rare or absent, and heterosis must be estimated indirectly by fitting models to data on several genotypes. The accuracy of such estimates is greatly reduced.

When several generations are required for an experiment, much of the data will be on animals which are not contemporary. The design of such experiments raises problems which may not be easy to resolve.

ARE QUEEN CHARACTERS OR WORKER CHARACTERS MORE IMPORTANT TO HONEY PRODUCTION?

B.P. OLDROYD

Plant Research Institute, Department of Agriculture & Rural Affairs, Burnley Gardens, Burnley 3121

Most research into bee breeding has concentrated on improving the performance of worker bees. However, there is evidence that the performance of queen bees also influences honey production. For example, a genetic correlation between brood area and honey production of 1.30 has been reported. This correlation suggests that honey production is almost entirely determined by a queen's capacity to lay eggs, and that the performance of workers is irrelevant. On the other hand, it has also been demonstrated that workers vary in their ability to produce honey.

If worker performance is only marginally relevant to honey production, then queen rearers need not control matings for commercial queens. Queen bees selected for their ability to lay eggs, and mated to any available drones would be suitable for commercial colonies. On the other hand, a number of factors (e.g. disease resistance and longevity) are the sole determinants of honey production, then queen rearers should control matings, as half the performance of workers is determined by the breeding value of the drones which sired them.

Experiments will be described that test the relative importance of queen and worker characters to colony weight gain and brood area.

WHEN DOES SELECTION FOR INSECTICIDE RESISTANCE OCCUR IN HELIOTHIS?

Joanne C. Daly and Jennifer H. Fisk

CSIRO Division of Entomology
Black Mountain ACT

Evolution of insecticide resistance can be retarded or prevented if the alleles conferring resistance can be made recessive, so that heterozygous individuals are at no, or little, selective advantage compared with susceptible individuals. Traditionally, this is achieved either by increasing the dose of insecticide applied or by adding synergists with the insecticide. Little attention has been given to changing the fitness of heterozygotes by applying insecticides to a sensitive life-stage.

The noctuid moth, Heliothis armigera, is a major pest in summer of broad-acre and horticultural crops. Crops are sprayed regularly to control infestations of larvae. Resistance to synthetic pyrethroids (SPs) was first detected in 1983. Despite the increasing frequency of this resistance, lack of field control with the SPs is unusual.

We examined strains of H. armigera, either resistant or susceptible to the synethetic pyrethroids to determine at what life-stage selective mortality was occurring. Eggs or larvae up to ten days old were exposed to leaves sprayed commercially with SPs from 0-10 days before. Three genotypes were used, a susceptible strain, a resistant strain which is thought to be homozygous resistant for one allele and the F_1 cross between these. Laboratory results confirm that the resistance was expressed in neonate larvae.

In neonate larvae, the target age-class for pest control, no selective mortality occurred on leaves which had been sprayed between 0-6 days before. Selective mortality was observed in larvae older than 4 days old on freshly sprayed leaves or in larvae older than 2 days old as the insecticide degraded. These results suggest that selection for resistance occurs when older age-classes of larvae are present in the field at the time of insecticide application or from the exposure of these older larvae to degrading insecticide. Thus, the relative fitness of the heterozygotes differs in the different larval stages.

We propose a third strategy to change the relative fitness of resistance genes: to apply the insecticide to a sensitive age-class.

ELECTROPHORETIC VARIATION IN Cactoblastis cactorum: ESTIMATING THE EFFECTIVE SIZE OF INTRODUCED POPULATIONS IN AUSTRALIA AND HAWAII.

N.D. Murray

Department of Genetics and Human Variation

La Trobe University

Bundoora, Victoria, 3083

A number of successful biological control campaigns have been based, either deliberately or accidentally, on genetically small introductions of biocontrol agents. Genetic changes may therefore accompany such introductions, or they may follow them, as the introduced populations adapt to their new environments. In either case, analysis of genetic variation in ancestral and derived population could theoretically be used to assess what fraction of the originally introduced gene pool generally contributes to the established populations. This would be a valuable piece of information for the design of biocontrol programs. An approach based on the estimation of effective population size of derived populations is outlined in this and the following paper.

The phycitid moth Cactoblastis cactorum was introduced into Australia from Argentina in 1925 to control prickly pear (Opuntia spp.). The moth was subsequently taken from Australia to other countries for further prickly pear control. Within Australia C. cactorum quickly became widespread and some isolates were established. An introduction to the island of Hawaii in 1950 was followed by natural spread to other islands in the Hawaiian group.

Electrophoretic analysis of protein variation at 13 polymorphic loci has been applied to samples from within Australia and from Hawaii, Maui, and Oahu. The patterns of genetic differences thus revealed are compared with the recorded population histories of the introductions. Allele loss has accompanied some introductions, and the methods of Pollak and of Nei & Tajima have been used to estimate the effective sizes of the derived populations since establishment.

The value of similar electrophoretic and/or RFLP studies for the design of biocontrol programs will be emphasized, as will the advantages of approaching these questions at the time of introduction rather than a posteriori!

ELECTROPHORETIC COMPARISONS BETWEEN ANCESTRAL AND DERIVED POPULATIONS OF THE BIOCONTROL AGENTS CHRYSOLINA QUADRIGEMINA AND C.HYPERICI

J. Tomasov

Department of Genetics and Human Variation

La Trobe University

Bundoora, Victoria, 3083

The chrysomeliid beetles Chrysolina quadrigemina and C.hyperici are well known for their successes in controlling the noxious weed Hypericum perforatum (St John's wort) both in Australia and overseas. Imported to Australia during the 1930's from Europe, subsequent populations have served as a source for introductions to South Africa, United States, Canada, Chile and New Zealand. On occasions establishment of both or either species has followed the classical "post colonization adaptation" mode ie. initial disappearance for a period of years followed by a sudden reappearance and establishment. Such patterns of dissemination and colonization makes both species particularly suitable for investigating genetic changes associated with population bottlenecks.

Samples from within Australia and from South Africa, Canada and The United States were assayed for variation at a number of protein coding loci (28 for C.quadrigemina and 25 for C.hyperici). Overall the proportion of loci polymorphic was 0.32 for C.quadrigemina and 0.4 for C.hyperici. In general this proportion was greater in "ancestral" populations than "derived" ones as was the average level of heterozygosity eg. France (0.0855) v Australia (0.0516) for C.quadrigemina.

To characterise the degree of genetic differentiation between and within ancestral and derived populations Wright's F-statistics have been calculated. The differentiation observed correlates well with historical data on introduction and establishment of the species. In addition the methods of Pollak and of Nei and Tajima have been used to estimate effective sizes of various derived populations since introduction. These estimates will be contrasted with the numbers of individuals known to be released and subsequent population histories.

CHARACTERISATION AND CHROMOSOMAL LOCALIZATION OF THE HUMAN α1-ACID GLYCOPROTEIN GENES.

Catherine M. Merritt [1], Graham C. Webb [2] and Philip G. Board [1].

[1] Department of Human Genetics, John Curtin School of Medical Research. Australian National University, Canberra. A.C.T. 2601. Australia.

[2] The Murdoch Institute for Research into Birth Defects. Royal Children's Hospital, Parkville, Victoria. 3052. Australia.

Human α1-Acid Glycoprotein (α1-AGP, Orosomucoid) is a normal component of human plasma. Synthesized in the liver, its plasma concentration exhibits a characteristic increase in response to acute inflammation and hence it is a member of a class of plasma proteins known as the acute phase reactants. A definite function for human α1-AGP has not yet been established although there is evidence for an immunosuppressive role.

α1-AGP has a molecular weight of around 40,000 daltons, containing about 45% carbohydrate. The primary sequence of the polypeptide chain and the carbohydrate side chains of human α1-AGP have been determined. The amino acid sequence studies revealed that α1-AGP contains alternate amino acids at 21 different positions suggesting multiple forms of the plasma protein.

A previously isolated cDNA clone, pα1-AGP contained a deduced amino acid sequence corresponding to one of the published amino acid sequences. Southern blot analysis suggests only a small number of α1-AGP genes. To settle the question of the origin of the observed heterogeneity and confirm the presence of at least two α1-AGP genes we have cloned the gene coding for the alternative amino acid sequence and have determined the complete nucleotide sequence. In addition to the entire coding sequence for α1-AGP2, the genomic clone contained 3' sequence of the gene α1-AGP1 indicating that there has been a duplication event resulting in the tandem arrangement of the two genes.

In situ hybridization of the cloned human cDNA, pα1-AGP, to human chromosomes shows a localization for the human α1-AGP genes between bands 9q31 to 9q34.1. This is in accordance with previous linkage studies which assigned the α1-AGP locus to a linkage group with *ABO* and *AK* on the long arm of chromosome 9.

THE HUMAN UBIQUITIN GENE FAMILY: CHROMOSOMAL LOCATION AND MOLECULAR CHARACTERISATION OF THE Ub B SUBFAMILY.

Rohan T. Baker[1], Graham C. Webb[2] and Philip G. Board[1].

1: Department of Human Genetics, John Curtin School of Medical Research, Australian National University, Canberra.
2: The Murdoch Institute for Research into Birth Defects, Royal Children's Hospital, Parkville, Melbourne.

Ubiquitin (Ub) is a 76 amino acid protein present in all eukaryotic cells. It exhibits remarkable evolutionary conservation, with identical protein sequence from insect to man. Ub functions in several distinct processes via covalent attachment to free amino groups of other proteins. In the nucleus, it is conjugated to histone H2A and H2B and these complexes have been implicated in the regulation of gene expression. In the cytoplasm, Ub is required for ATP-dependent, non-lysosomal proteolysis of both native and foreign proteins, and is also a heat shock protein. Finally, Ub forms a part of branched chain cell surface receptor molecules - the lymphocyte homing receptor and the platelet-derived growth factor receptor.

Ub genes exist as a family of natural gene fusions. In humans there are three structural types. The Ub A class consist of a single Ub coding unit fused to a basic, DNA binding protein. The Ub B class consists of 3 tandem repeats of the coding unit, while the Ub C class contains 9 tandem repeats. We have isolated and sequenced a human cDNA and gene of the Ub B class (1). This gene consists of 3 Ub coding units followed by one extra cysteine codon, and a 715bp intron within the 5' non-coding region. Southern analysis suggests that this gene may be duplicated within the human genome. We have also isolated 3 processed pseudogenes of this Ub B gene (1,2).

Further sequence analysis of the region further upstream of the gene has revealed: (i) 3 copies of the heat shock promoter consensus, implying a role for this gene in the stress response, and (ii) two members of the ALU family of repetitive DNA elements in opposite transcriptional orientations. Detailed analysis of the gene and pseudogene 5' and 3' non-coding regions indicates that the gene's 5' flank has diverged more rapidly than its 3' flank.

In situ hybridisation of the Ub B cDNA clone to human chromosomes produced a major peak over band p12 on the short arm of chromosome 17. Several smaller signals were also observed, including peaks over 2q22, 12q24 and 6p12. Interpretation of these results may be difficult owing to: (i) the effect of coding unit copy number on hybridisation signal, (ii) the presence of (at least) three processed pseudogenes in the genome, and (iii) hybridisation of the Ub B cDNA to all classes of Ub genes/pseudogenes. In situ hybridisation using the Ub B intron as a probe is in progress to determine the location of the bona fide Ub B gene(s).

References:
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TRISOMY 21 AND THE MATERNAL AGE EFFECT

G.B. PETERS, J.H. FORD and J.K. NICHOLL

Genetics Dept, The Queen Elizabeth Hospital,
Woodville, SA 5044

The "relaxed selection" hypothesis[1] holds that incidence of trisomy 21 increases with maternal age because of the progressive failure of a post-fertilisation screening mechanism. This proposal rests on observations showing that the relative frequency of maternally-derived extra chromosomes may not increase with age. This absence of age dependence conflicts with predictions based on the commonly assumed increased rate of meiotic non-disjunction in older mothers[2].

In a recent paper[3], it is suggested that the screening mechanism may operate through either i) spontaneous miscarriage prior to recognition of pregnancy; or ii) elimination of the extra chromosome at mitosis of the first cleavage division. The present paper attempts to show that these post-fertilisation "elimination" hypotheses can be tested using data available from parents who are mosaic for trisomy 21[4]. The predictions of "elimination" hypotheses are contrasted against the conventional or "generative" hyposthesis, whereby it is differential rate of origin, rather than elimination, which is responsible for increased incidence in older mothers. The mosaic data is not consistent with any elimination hypothesis.

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DEVELOPMENT OF AN RNA PROBE FOR CAMPYLOBACTER JEJUNI (A HUMAN ENTEROPATHOGEN)

V. Korolik and V. Krishnapillai

Department of Genetics, Monash University

The bacterial enteropathogen Campylobacter jejuni is a cause of acute gastroenteritis in man. A 6.1 kb Sau3A chromosomal DNA fragment was cloned into pBR322. This fragment used as a DNA probe in Southern hybridizations hybridized specifically with C. jejuni chromosomal DNA from a number of clinical isolates. However, it showed weak cross-hybridization with a closely related species, C. coli, but not with any other Campylobacter species DNA.

In order to develop a specific probe for C. jejuni, five HindIII fragments of the 6.1 kb fragment were tested for cross-hybridization with C. coli. A 1.6 kb fragment showing the least cross-hybridization was then subcloned into a small transcriptional vector. This system allows the synthesis of a complementary RNA sequence to the 1.6 kb DNA fragment from a phage SP6 promoter. The transcripts were then labelled with ^{32}P-UTP and then used as a probe. The RNA probe hybridized specifically only with C. jejuni DNA and showed no cross-hybridization with any other species of Campylobacter.

The RNA probe should prove useful in the rapid and specific detection of C. jejuni especially from clinical material.

DNA SEQUENCE OF THE REPLICATION REGION OF THE PSEUDOMONAS PLASMID R9115

S. P. Davies and V. Krishnapillai

Department of Genetics, Monash University, Clayton, Victoria 3168

The bacterial plasmid R91-5 is unable to replicate or maintain itself stably in bacterial species other than Pseudomonas aeruginosa. The genetic basis of this narrow host range is currently under investigation in our laboratory. A 2.7 kb fragment of DNA which encodes all the necessary functions for plasmid replication and incompatibility has been sequenced by the Sanger method using plasmid templates. Northern blotting has identified two transcripts from within this 2.7 kb replication region. A large transcript, believed to encode the replication protein has been mapped and the corresponding open reading frame identified. The second transcript identified is much smaller, about 300 bp. This transcript may be involved in the control of plasmid replication and/or plasmid incompatibility. Sequences within the 2.7 kb involved in incompatibility are currently being identified by subcloning. These results will be valuable in terms of determining how replication is controlled in this plasmid, and also the possible role of the 300 bp transcript.

CHROMOSOME ORGANISATION IN DROSOPHILA

Arthur J. Hilliker
Department of Molecular Biology and Genetics
University of Guelph
Guelph, Ontario
Canada

I will present research I have undertaken on several aspects of chromosome organisation in Drosophila. Topics to be discussed include:

1) The localization and properties of genetic elements in heterochromatin;

2) The mapping of specific repeated DNA sequences within heterochromatin;

3) The arrangement of chromosomes in diploid interphase nuclei and

4) The analysis of the functional significance of linkage element conservation in Drosophila.

Repression of a *white* P Element Transposon by Flanking Satellite DNA Sequences in *Drosophila*

Allan Lohe and Chris Moran

CSIRO Division of Entomology, GPO Box 1700, Canberra, A.C.T. 2601

Position-effect variegation is an intriguing phenomenon of mosaic gene expression and results from the positioning of an active gene close to heterochromatin. The suppression of gene activity is believed to act at the time of the determination event in development. We are investigating the molecular basis for position-effect variegation by placing cloned segments of satellite DNA (the principal component of heterochromatin) adjacent to the wild-type *white* gene, and inserting this construct into *Drosophila via* the P-element system of DNA transformation. Our initial results with two transformants showed that small segments (0.3kb) of two satellite repeats did not induce variegation in the eye, and gave a wild-type phenotype. However, both stocks were unstable and produced many individuals with bleach white eyes. Investigation of lines derived from these exceptional flies has shown that the transposon has developed a unique cycle of instability. The results can be summarized as follows:-

1. The *white*-satellite transposon can become repressed spontaneously and by unknown mechanisms, with the phenotype changing from red to white eyes. Derepression of the *white* gene can occur subsequently, sometimes to yield the original phenotype, or different phenotypes.

2. The *white*-satellite transposon is a hot spot for chromosome breakage. About one third of all events giving red to white eyes show chromosome aberrations (inversions, deletions, transpositions), each with one breakpoint at the site of the transposon.

3. The *white* transposon can, on rare occasions, jump to other chromosomal sites. This is despite the use of the P-element helper "wings-clipped", which is unable to integrate into the chromosomal DNA.

4. Both initial *white*-satellite transformants respond to P element transposase normally, moving to different chromosomal locations at high frequency in P-M mutagenic crosses. However, transposons that have moved in a P-independent manner are resistant to mobilization in P-M mutagenic crosses. Nevertheless, they can still move further (at low frequency) to other chromosomal sites in the absence of P-elements.

5. Both initial *white*-satellite transformants have characteristic restriction digest fragments, reflecting the different sites of integration of the transposon. However, Southern analysis of stocks derived from these transformants by P-independent mobilization has not been possible, as the *white*-satellite transposon DNA simply "disappears". This is despite the fact that *white* DNA remaining at the deleted *white* locus on the X chromosome can be detected readily in these hydridizations. Furthermore, *in situ* hybridization to polytene chromosomes is successful in localization of the *white*-satellite transposons in all cases, both initial and derivative transformants.

The segment of satellite DNA is implicated as a cause for these unusual characteristics and our working hypothesis is that amplification of the satellite (by unequal crossing-over or *de novo* synthesis) is both capable of suppressing *white* gene transcription and destabilizing the transposon.

Genetics of an unstable white transposon containing satellite DNA in *Drosophila*

Chris Moran[1,2] and Allan Lohe[2]

[1]Department of Animal Husbandry, The University of Sydney, N.S.W. 2006
[2]CSIRO Division of Entomology, GPO Box 1700, Canberra City, A.C.T. 2601

A plasmid containing the white gene plus 300 bp of satellite DNA enclosed within P element terminal repeats has been used to transform the w^{111G} (white deletion) stock of *Drosophila melanogaster*. In contrast to previous situations where the transforming DNA lacked repetitive sequences, our transformed stock is unstable and reverts to white at a frequency of 0.16% per chromosome. Pigmented rerevertants have been obtained from these white revertants having new sites of integration of the transposon. This cycle of instability continues indefinitely. Reversion to white can be as high as 15.2% per chromosome. Rereversion to a pigmented phenotype occurs with a frequency from 0% up to 11%, with an average across stocks of about 0.6%. Reversion is due in some cases to deletion, in others to repression. A large proportion of reversion events generate recessive lethals. Complementation maps for these lethals are generally compatible with models of deletion or repression extending in either or both directions from the point of insertion of the transposon into flanking DNA. Chromosomal rearrangements, including inversions and deletions, also frequently accompany reversion.

All of these events occur in the absence of P element transposase. Indeed, the transposon has become resistant to P transposase in stocks derived from the original transformant. The role of the repetitive DNA in transposition is unclear. However, since the transposon can be suppressed and subsequently reactivated at the same site, it can be argued that we are observing position effect suppression of the white gene due to amplification of the satellite sequences in these cases. Deletion of part of the satellite sequence would then derepress the white gene.

DO OPPOSING FORCES ALWAYS BALANCE?

T. PROUT

University of California, Davis

Natural selection can act in opposite directions on different aspects of a population genetics system. Such opposing selection is of current interest in the study of quantitative characters where it is frequently invoked to explain the *status quo* (equilibrium). In classic one locus theory opposing selection can result in:

1. net neutrality
2. net directional selection
3. stable equilibrium, or
4. an unstable equilibrium when operating on different life history components.

The same four outcomes are possible with selection in opposite directions at different times or in different places. There is little by way of theory or empirical findings which says that, if there is an equilibrium, it will more likely be stable than unstable.

When considering polygenetically determined quantitative characters, the current focus is on opposing selection on different life history components. Opposing selection at this level cannot maintain genetic variance (stable equilibria at polygene loci) except by induced overdominance. However, the above four possible outcomes can occur when considering the behaviour of the population mean. The pattern of net selection can be determined and studied by multiplying together the component selection functions. If the opposing selection functions (one increasing and the other decreasing) are linear or convex upwards, net selection is always stabilising, resulting in a stable mean. However, concave upwards exponential functions never result in a stable mean, but the three remaining outcomes noted above can occur. If the opposing selection functions represent divergent optima then there can be either one stable equilibrium or three equilibria one of which is unstable (disruptive selection) depending on the position of the optima and the functions used. Simple gausian component optima always give net stabilising selection which it would seem is an unrealistic peculiarity of this widely used function.

The objective of this analysis is to point up the need for experimental determination of the form of component selection functions. It is suggested that the "balance of forces" metaphor has deterred from the pursuit of such experiments.

STUDY OF PHENYLALANINE HYDROXYLASE GENE RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN POLYNESIANS

K.Jahromi[1], H.H.M.Dahl[2], J.F.B.Mercer[2],

K.N.P.Mickleson [3] and R.J.Trent[1]

[1] Molecular Biology Laboratory, Clinical Immunology Research Centre, University of Sydney, N.S.W. 2006;

[2] Murdoch Birth Defects Research Institute, Parkville, VIC 3052;

[3] Middlemore Hospital, Auckland, New Zealand.

A number of restriction fragment length polymorphisms (RFLP's) at the phenylalanine hydroxylase (PAH) gene locus (chromosome 12q 22.1-22.4) were identified in DNA from Polynesians (Maoris, Cook Islanders, Tongans, Samoans & Niue Islanders), using the restriction enzymes EcoRI, Bg1II, PvuII, XmnI, MspI, EcoRV & HindIII, and standard gene mapping techniques. This was undertaken to determine the feasibility of using highly polymorphic gene markers to identify subgroups within Polynesians, since previous studies with globin gene markers had shown 3 distinct Polynesian groups, (i) Maoris and Cook Islanders, (ii) Tongans and Samoans, (iii) Niue Islanders.

A significant number of 200 randomly sampled Polynesians differed in their haplotype frequencies compared to the Danish populations described by Di Lella et al (1985). Approximately 4-5 hitherto unreported haplotypes were detected in Polynesians. Comparison of RFLP's associated with the PAH gene and globin gene markers in Polynesians (Trent et al '86) provides interesting data in terms of population structure, migratory patterns and forces responsible for directional and non-directional changes in gene frequencies.

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SESSION 2B

MULTIPLE PATERNITY IN ACACIAS

G.F. MORAN and O. MUONA[1]

Division of Forest Research, CSIRO, P.O. Box 4008, Queen Victoria Terrace, A.C.T. 2600

[1] Present address: Department of Genetics, University of Oulu, Oulu, Finland.

Pollen in Acacias is dispersed as a polyad with all individual grains being the product of a single meiotic event. The number of pollen grains per polyad per species is usually reasonably constant but can range from 4 to 32 between species. About 90% of Australian species of Acacias have 16 grain polyads. Often because of their size only a single polyad can fit on the stigma of each flower at any one time. It has therefore been hypothesised that within a single seed pod all the ovules may be fertilized by the same father.

Acacia melanoxylon has 16 grain polyads and 14 ovules per ovary. From a population of *A. melanoxylon* the seeds within 125 pods from 15 trees were assayed for 11 polymorphic loci. The maternal multilocus genotypes of these 15 trees were determined from open-pollinated progeny seed arrays and were confirmed by assays of the same loci in leaf samples. The multilocus genotypes of the other 131 trees in the population were determined from leaf assays. Within pods multiple paternity was detected by screening paternal genotypes of seed arrays for (1) more than 3 alleles at one locus, (2) more than 8 copies of one allelic type, and (3) low number of segregants even with supposedly heterozygous loci. The average number of seeds assayed per pod was 8.1. Only seven pods were detected to have more than one paternal contribution. From the gene frequencies of the entire population it was possible to calculate that about 60% of the cases of multiple paternity should be detected. Thus in 90% of the pods all the seeds have the same paternal parent and are, in fact, full-sibs.

These results make acacias rather good model organisms to study the population genetics of plants. For instance, direct estimates of such elusive parameters as gene flow, male fertility and effective population size should now be possible.

SESSION 3A

Y-SPECIFIC REPEATED DNA SEQUENCES

CONSERVED IN RUMINANTS

Margaret E. Matthews, Eric A. Lord, and Ken C. Reed
Department of Biochemistry, Faculty of Science
The Australian National University
Canberra A.C.T. 2601

We have recovered a short (307 bp) repeated DNA sequence (BRY.1: bovine repeat, Y-associated) from the genome of a domestic bull by enrichment cloning. Hybridization analyses with DNA samples isolated from an extensive range (78) of individual cattle, including representatives of a number of breeds, both unrelated and of known pedigree, confirmed our initial assignment of BRY.1 to the Y chromosome, since the DNA of every male but of no female animal included homologous repeats. The repeat frequency varies between unrelated males but is stably heritable through a single generation. Furthermore, BRY.1-related sequences were found to be represented in the genomes of male but not female sheep.

Screening an amplified male bovine genomic 'library' with BRY.1 led to the isolation of a recombinant phage containing not only BRY.1 elements, but representatives of two longer repeats associated universally with the bovine Y chromosome (BRY.2, BRY.3). Both of these elements are also represented in the genomes of male sheep, and both are present in *Bos* and *Ovis* at higher frequency and more consistent copy number than is BRY.1.

Subsequently, a high-complexity library was constructed in λEMBL3A from the genomic DNA of a ram and screened (without amplification) with BRY.2. Eleven independent, non-overlapping recombinants containing isolated BRY.2 elements have been recovered, many of which also contain BRY.1 and BRY.3 homologues. Hybridization analyses demonstrate that the inserts in all 11 recombinants are derived from the ovine Y chromosome. At least one of these recombinants has been found to contain an additional repeated element (OY1.1) which is present at high copy number exclusively and universally in the genomic DNA of male sheep, cattle, and goats. The full extent of its conservation is currently being investigated.

Studies in progress aim to define each of the Y-associated repeat units, and to establish their physical and evolutionary relationships. We anticipate that these four elements will offer unique insights into mechanisms by which DNA sequences are propagated in *cis* in the mammalian genome. Close inter-element linkage might suggest they have arisen as components of a larger unit, although we have been unable to detect any recurrent restriction sites in independently isolated representatives. Their interspersion with kilobases of DNA common to the genome of both males and females negates the possibility of simple tandem duplication mechanisms. However, the most fascinating implication follows from their exclusive Y-linkage throughout at least 15 million years. Such total suppression of recombination is unique for repeated elements, and argues strongly for their linkage to (or involvement in) the one functional imperative of the mammalian Y chromosome - testis differentiation.

VARIATION AT THE *Nor* LOCI OF WHEAT AND SOME IMPLICATIONS FOR THE FUNCTION OF THE rDNA SPACER REGION

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

1: Agricultural Research Institute, Wagga Wagga
2: CSIRO Division of Plant Industry, Canberra

The plasmid, pTA250.4, contains a 2.4kb fragment of DNA isolated from the spacer DNA which separates the highly repeated ribosomal-RNA (rRNA) genes of hexaploid wheat. Much of the DNA within this fragment is composed of 133bp sub-repeats. In our analyses of over 120 wheat cultivars and their hybrids, the lengths of the DNA fragments restricted by TaqI and labelled by pTA250.4, vary markedly. A 1.7kb fragment is found at the *Nor-D3* locus of chromosome 5D, fragments of 2.55-3.10kb are present at the *Nor-B1* locus of chromosome 1B, and the *Nor-B2* locus of chromosome 6B contains fragments with lengths from 2.05-4.30kb. Within a locus, the lengths of spacer DNA fragments usually differ by multiples of 133bp so that the differences in length can be ascribed to the addition or loss of discrete numbers of sub-repeats.

Allelic variants of each locus are comprised of one or more DNA spacer fragments, the amounts of which may also differ quantitatively. Presently, we have found five alleles of the *Nor-B1* locus and up to 15 alleles of *Nor-B2*. The various combinations of these alleles in the wheat cultivars analysed enable the cultivars to be placed into phenotypic groups.

It is apparent that the absolute number of rRNA genes within a particular cultivar are also variable. Comparative numbers can be determined by calculating quantitative differences in the total amounts of different DNA spacer fragments present. F_1 plants allow such allelic combinations to be compared. If the number of rRNA genes in the cultivar 'Chinese Spring' is taken as 100% (and estimates of the actual number of rRNA genes present in this cultivar vary between 4,000 and 9,200), other cultivars have from 40-100% of this number. So far, however, neither the allelic combinations nor the number of rRNA genes present within a cultivar have been shown to have any agronomic affects.

Interestingly, in the diploid species *Critesion bogdanii*, a distant relative of wheat, the spacer DNA is of the order of 1kb in length and no sub-repeats are present. We postulate that this length of spacer is the minimal amount for effective function and the presence of sub-repeats is not an absolute requirement. Consequently, the wheat spacer can be calculated to contain a minimum of five and a maximum of 25 sub-repeats. The presence of sub-repeats within the spacer DNA of the *Nor* alleles of wheat may indicate that these are necessary for a species to have a highly adaptive potential.

The *Ter* loci in the grasses: regions at the termini of chromosomes defined by repetitive DNA sequence families

R. Appels, L.B. Moran and O.H. Frankel

CSIRO, Division of Plant Industry, G.P.O. Box 1600, Canberra, A.C.T. 2601

The 350-family of DNA sequences were among the first of the DNA sequences cloned from the terminal regions of rye chromosomes and, in limited surveys, appeared to be specific to *Secale* species (R genome). Recently the 350-family has been found in *Agropyron*, sensu stricto, species (P genome, Xin and Appels, 1987) and in this abstract we now report that the family also exists in *Critesion* species (H genome), and *Pseudoroegneria* species (S genome). The terminal sequences in the S and H genome species do not cross-hybridize with a rye 350-family probe, but sequencing analysis clearly demonstrates they belong to the 350-family. Furthermore the sequences from a given genome resemble each other more than sequences from other genomes. The available data suggest that the 350-family may be widespread in the terminal regions of chromosomes in Triticeae species and that within a given genome a certain sequence variant has been amplified to dominate the terminal regions (*Ter* loci).

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Structure of *del*, an abundant dispersed repeat from the genome of *Lilium henryi*

John W. Sentry and D. R. Smyth
Department of Genetics, Monash University, Clayton, Vic. 3168

The genomes of *Lilium* species, the true lilies, are very large (>30 million kbp). We have been characterizing one repeated component of these genomes, a 9 kbp dispersed sequence named *del* (from dispersed element of lilies).

A genome library of *Lilium henryi* DNA was prepared using the phage lambda vector EMBL3. Around 4% of inserts, which were of 16 kbp average size, hybridized with *del* probes. Because only 40% of the inserted DNA had *del* homology overall, it seems *del* makes up about 1.5% of the *L. henryi* genome. Even so this is equivalent to 50,000 copies of the full repeat unit.

The usual structure of *del* involves long terminal repeats of 2 kbp which flank 5 kbp of internal sequence. The termini are in direct orientation such as is found in retroviruses, *copia*-like elements and *Ty* sequences. However, solo termini and tandem repeats of *del* have also appeared in some clones.

To learn more of the design and possible origin of *del* one full element, 1-46, is being sequenced. At least two open reading frames have been revealed in the internal sequence. One may have sequence motifs characteristic of reverse transcriptase. Thus *del* could be a retroid element which has amplified within *Lilium* genomes contributing significantly to their large bulk.

TRIPRONUCLEAR HUMAN OOCYTES : ALTERED CLEAVAGE PATTERNS AND SUBSEQUENT KARYOTYPIC ANALYSIS OF EMBRYOS

Ismail Kola, Alan Trounson, Garey Dawson and Peter Rogers.

Centre for Early Human Development, Monash University, Queen Victoria Medical Centre, 246 Clayton Road, Clayton, Australia, 3168

Although 1-4% of human oocytes fertilized in vitro are tripronuclear (contain 3 pronuclei) there is very little information about the subsequent development and chromosomal composition of preimplantation embryos which derive from these oocytes, and the data that does exist suggests a complex picture. In this study we investigate the pattern of the first cleavage division of tripronuclear human oocytes and the chromosomal constitution of these embryos before the second cleavage division, in order to determine both the proportion of tripronuclear human oocytes that develop into triploid embryos and to gain insight into the mechanisms by which these tripronculear human oocytes fail (if at all) to develop into the triploid embryos.

Human oocytes with three pronuclei were obtained from the Monash/Epworth in vitro fertilization programme. These oocytes were monitored constantly for dissolution of pronculei and cleavage. Six to eight hours after the first cleavage division, embryos were incubated in colcemid (0.1μg/ml) for a further 6h and the chromosomal constitution of these embryos evaluated as described by us (1).

Most (18 or 29) of the tripronuclear oocytes cleaved directly from 1 to 3-cells at the end of the first cleavage division. These embryos have a severely abnormal (but not triploid) chromosomal complement. Furthermore, some (4 of 29) tripronuclear human oocytes cleave to 2-cells plus an extrusion and these embryos are diploids, while only (7 of 29) cleave to 2-cells and these embryos are triploid after the first cleavage division.

These findings demonstrate that most tripronuclear human oocytes have an altered cleavage pattern at the first cleavage division, that most tripronuclear human oocytes (76% in this study) do not develop into triploid embryos and that a correlation exists between the pattern of the first cleavage division and the subsequent karyotype of these embryos. This study also gives insight into the mechanisms by which these tripronuclear oocytes fail to develop into triploid embryos.

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RESTRICTION ENDONUCLEASE VARIATION IN THE REGION OF THE ALCOHOL DEHYDROGENASE GENE: A COMPARISON OF NULL AND NORMAL ALLELES FROM NATURAL POPULATIONS OF *Drosophila melanogaster*

Chengshan Jiang, John B. Gibson, Ann V. Wilks and Allan L. Freeth

Population Genetics Group
Research School of Biological Sciences
Australian National University
Canberra, Australia

The restriction endonuclease variation in the 12 kb region surrounding twelve *Adh* null alleles extracted from three Tasmanian populations has been compared with normal alleles from the same populations. Each of the null alleles had the same haplotype as revealed by digestions with eight hexanucleotide restriction enzymes. This haplotype also occurred in four of the forty six chromosomes bearing normal alleles which were tested; these four chromosomes with the null allele haplotype carried the Adh^S allele. The data suggest that the *Adh* null alleles from geographically separate populations share a common ancestry and are probably derived from the same mutation in an Adh^S allele.

MOLECULAR ANALYSIS OF ALCOHOL DEHYDROGENASE NULL ALLELES FROM NATURAL POPULATIONS OF *Drosophila melanogaster*

Allan L. Freeth, John B. Gibson and Ann V. Wilks

Population Genetics Group
Research School of Biological Sciences
Australian National University
Canberra, Australia

A series of *Adh* null activity alleles isolated from Tasmanian populations of *D. melanogaster* do not produce any ADH CRM despite the gene being present (see Jiang *et al.*, this meeting). Northern blot analyses of the null alleles have shown that they all produce a transcript about 100 bases longer than that produced by the normal allele and they accumulate a precursor of 1800 bases. The amount of the major transcript produced by the null alleles is about 10% of that produced by normal alleles. S1 mapping experiments have indicated a defect in the splicing of intron 2 with several 5′ donor and 3′ acceptor sites. It is interesting that the nulls provide a further example of splice site mutations apparently maintained at higher than expected levels in natural populations.

GENETIC VARIATION OF FOUR POPULATIONS OF THE LITTLE BLUE PENGUIN, EUDYPTULA MINOR.

M.A.M. Meredith & F.Y.T. Sin

Department of Zoology,
University of Canterbury,
Christchurch, New Zealand.

The Little Blue Penguin, Eudyptula minor, is found only in Australasia. In New Zealand, E. minor is widely distributed along the coast and around the offshore islands. In this study we determined the morphological and biochemical variations of four geographical populations from (1) Poor Knights Islands (35°30'S, 174°45'E), (2) Maud Island (41°01'S, 173°52'E), (3) Motunau Island (43°08'S, 173°10'E), and (4) Onawe Peninsula (43°46'S, 172°55'E).

Six morphological characteristics including head length, bill length, bill depth, flipper length, tarsal diagonal length and toe length were examined. Statistically significant (ANOVA) differences were observed between the northern and southern populations in all these characteristics. Principle component analysis revealed extensive overlaps in all the four populations.

Biochemical variations* were detected amongst the four populations of E. minor. Of the twenty two loci examined, seven were polymorphic. A south-north cline was detected in the allelic frequencies of all the polymorphic loci. Population specific loci were also detected. The Yellow-eyed Penguin, Megadyptes antipodes offered a comparison between genera. The two genera shared the same alleles at seventeen of twenty loci, and loci specific for this genus were also detected.

Thus, both morphometrical and biochemical analysis suggest a clinal pattern of divergence with the Onawe Peninsula and Motunau Island populations at one end of the cline and the Poor Knights population at the other.

* M.A.M. Meredith & F.Y.T. Sin (1987) Genetic variation of four populations of the Little Blue Penguin, Eudyptula minor. Heredity, in press.

ANOTHER TEST OF THE ROLE OF TRANSPOSABLE ELEMENTS IN GENERATING QUANTITATIVE GENETIC VARIATION, USING THE P ELEMENT SYSTEM OF *DROSOPHILA*

A. Torkamanzehi, C. Moran and F. W. Nicholas
Department of Animal Husbandry, University of Sydney,
N.S.W. 2006

P-elements are transposable elements randomly located in the genome of *Drosophila melanogaster*. An extraordinary feature of these elements is that they are able to induce a whole range of unusual traits, termed hybrid dysgenesis. For example, insertion mutations occur at a very high rate in the germline of hybrids from crosses between M strain females and P strain males, but usualy at a much lower rate in the hybrids from reciprocal crosses.

P-element induced mutations which affect quantitative characters have been studied by a number of workers during the past few years. However, results of these experiments are not concordant. The most intriguing results were reported by Mackay (1984, 1985), who found a substantially higher response to selection in the lines derived from dysgenic crosses compared to those from non-dysgenic (reciprocal) crosses. However, in an attempt to repeat her experiment, we failed to detect any extra response to selection in the dysgenic lines, and more surprisingly we had an enhanced response to selection in one of our non-dysgenic downlines (results presented at the 33th Annual Conference of GSA, 1986). Furthermore, there is evidence that the enhanced response in our non-dysgenic downline is the result of P element transposition.

This paper reports results of a second experiment involving 10 generations of selection for abdominal bristle number, wthin dysgenic and non-dysgenic lines derived from crosses between Para Wirra (P) and Canton S (M) strains. Cytotype of flies was also determined throughout the course of selection, using ovarian dysgenesis and sn^{w} tests.

Response to selection for this character and change of cytotype in the dysgenic and non-dysgenic lines are discussed.

THE DISTRIBUTION OF *DROSOPHILA* A VIRUS IN AUSTRALIAN POPULATIONS OF *DROSOPHILIA MELANOGASTER* AND *D. SIMULANS*

P.D. Christian,
Population Genetics Group,
Resesarch School of Biological Sciences,
Australian National University.

Individual *D. melanogaster* and *D. simulans* from 34 Australian populations spanning 26.5° of latitude were surveyed for the presence of *Drosophila* A virus (DAV) using a cDNA hybridization assay. Infected flies were found in only 8 of these 34 populations, with infection frequencies ranging from 0.6% to 12.9% Within infected populations no difference was found in the frequency of DAV detection between *D. melanogaster* and *D. simulans*. For both species there was homogeneity in virus distribution between sexes.

Other species of *Drosophila* were found in association with *D. melanogaster/ simulans* at all localities, thereby forming communities. On the basis of the species composition of these communities, two major types of *Drosophila* fauna could be identified; a northern, tropical fauna and a southern, temperate fauna. No difference in the frequency of virus was found between *D. melanogaster/ simulans* populations arising from these faunas. Considerable heterogeneity however, was found in the infection frequency within infected populations. This was due to the high frequency found in the two populations collected in 1984 and 1985 from Coffs Harbour. Reasons for the abnormally high frequency of virus at this locality will be discussed.

Genetic characterization of a new homeobox gene in Drosophila melanogaster.

Wayne Knibb, Phillip England and Robert Saint

CSIRO Division of Entomology, GPO Box 1700, Canberra, ACT 2601.

The 20 or so homeobox genes that have been identified to date in Drosophila melanogaster appear to have two major features in common. Firstly, each contains a highly conserved 180bp DNA sequence (the homeobox sequence) that has been postulated to encode a sequence specific DNA binding protein. Secondly, they are all vital loci, essential for early development. Mutations at these loci cause disruption to normal pattern formation during development.

Hybridization screens for further members of the homeobox class of genes have identified a novel homeobox containing gene at cytological location 97D2-6 (see Saint et al. this volume). To elucidate the role of this new homeobox gene we have commenced genetic analysis of the chromosomal region in which this gene lies. The smallest deficiency covering this gene, Df(3R)ro^{XB3}, spans eight polytene bands and has been shown to delete the homeobox sequence. We identified vital loci within this region by testing whether EMS mutagenized chromosomes are lethal when heterozygous with Df(3R)ro^{XB3}. Six lethal complementation groups and two semi-lethal complementation groups have been identified to date. One of the semi-lethal complementation groups is the developmental gene Toll, while the other appears allelic to the rough gene, a gene implicated in the development of the eye imaginal disc. The discovery of a semi-viable rough 'allele' suggested that the rough gene may be involved more generally in Drosophila development, prompting us to explore the possibility that the homeobox gene corresponded to the rough gene. Molecular analysis has provided strong evidence for this.

To further characterize this gene, DEB mutagenesis is being carried out to obtain mutants that will be identifiable at the molecular level. These mutants will be examined to determine whether rough has a role only in eye differentiation or, in keeping with other homeobox genes, also has a vital role in early development.

Molecular analysis of a homeobox-containing region of Drosophila

Robert Saint, Bill Kalionis, Abigail Elizur and John Davidson.
CSIRO Division of Entomology, GPO Box 1700, Canberra, A.C.T. 2601

We have initiated a molecular analysis of a new homeobox gene located at 97D in Drosophila melanogaster. Genetic analysis of this region, suggested that the gene may correspond to the rough gene of Drosophila (see Knibb et al. this volume). Analysis of the only spontaneous rough allele revealed insertion of a moderately repeated element immediately 5' to the homeobox sequence in this gene. We conclude that the gene corresponds to rough, a gene implicated in the development of the eye-imaginal disc.

The gene is expressed at very low levels, consistent with highly localized regions of expression. The low levels of expression have prevented successful identification of cDNA clones from a variety of libraries screened exhaustively. We are particularly interested in testing for any embryonic expression of this gene to determine whether it has an embryonic function. All other characterized homeobox genes play a role in early development, being expressed during oogenesis and stored as maternal mRNA in the egg or at formation of the blastoderm during embryogenesis. We do not find transcripts present in maternal RNA, but do find a very low level of transcripts at blastoderm and later in embryogenesis. The function of such transcripts, if any, in embryogenesis has not been determined.

We are also attempting to engineer the expression of this gene in order to raise antibodies to the native product. We have fused the gene to a glutathione-S-transferase gene from Schistosoma japonicum which allows simple one-step purification of the protein product on a glutathione agarose affinity column. Initial attempts have been however hampered by lethality in the bacterial cells in which the fusion proteins are expressed.

THE TOPAZ AND WHITE GENES OF LUCILIA CUPRINA: STRUCTURE, FUNCTION AND ANALYSIS OF MUTANTS

A. ELIZUR, B. WICKSTEED, C. LANDSBERG AND A.J. HOWELLS

Department of Biochemistry, Faculty of Science, Australian National University.

The topaz and white eye colour genes of L. cuprina were isolated on the basis of homology with their already cloned D. melanogaster counterparts, scarlet and white respectively. The structures of the homologous genes from the two species have been examined by nucleotide sequencing.

The topaz and scarlet genes have virtually identical exon-intron structures. The exons are identical in size, are interrupted by introns at the same places and are 80-90% homologous at the predicted amino acid level. The introns show virtually no sequence homology and are generally longer in topaz. In addition three of the topaz introns contain moderately repeated DNA sequences, whereas no repeated DNA is found within scarlet.

Although the sequence data for the white gene of L. cuprina is incomplete, some interesting differences between the white genes of the two species have been found. In some regions the structures of the genes have been strongly conserved whereas in others they have diverged considerably. Some of the changes may be due to the presence of additional introns within the L. cuprina gene, while others suggest that there are marked differences in the amino acid sequences of the proteins.

In relation to functions, the evidence is mounting that the topaz and white proteins are both involved in the transport of pigment precursors across cell membranes. Both proteins contain several highly hydrophobic, potential membrane-spanning domains. In addition, both contain regions of homology to the putative ATP-binding domain found in a number of E. coli metabolite permeases.

Two spontaneous mutant alleles ($topaz^1$ and $white^1$) have been compared at the sequence level with their wild type counterparts. The most likely explanation for the mutant phenotype of $topaz^1$ is a deletion from an exon of 18 nucleotides; in the case of $white^1$, an inversion involving 7 kb of DNA sequence has been found. Thus, neither seems to have been caused by the insertion into the gene of a transposable element; this is different from the situation in D. melanogaster in which most spontaneous mutations are due to such insertions. Other mutant alleles are currently being examined.

THE CLONING AND PRELIMINARY CHARACTERIZATION OF THE CINNABAR LOCUS OF DROSOPHILA MELANOGASTER

W.D. Warren and A.J. Howells
The Department of Biochemistry, Faculty of Science
The Australian National University

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

The cinnabar locus has been isolated following a short chromosome walk within polytene region 43E. Southern blot analysis of genomic DNA has shown that at least five cinnabar alleles have molecular changes that fall within a region of approximately three kilobases. This region is also absent in deletions that cover the cinnabar locus. The exact nature of these molecular alterations are currently being investigated.

A mutant strain carrying a cinnabar allele (cn^{rbr}) has been reported to produce wild-type revertants at this locus. Destabilization of both the cn^{1} and cn^{2} alleles has also been observed in individuals heterozygous with cn^{rbr}. Attempts are being made to reproduce these observations, and if successful, the nature of these changes at the molecular level will be investigated.

CHARACTERISATION OF THE *gatA* GENE OF *ASPERGILLUS NIDULANS*.

I.B. RICHARDSON, M.J. HYNES and M. KATZ

Department of Genetics, University of Melbourne

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

Functionally, *gatA* has been localised to a 4kb fragment. Within this the approximate extent of a 1.8kb message has been determined, as well as the direction of transcription. 1.5kb (including approximately 350bp of non-transcribed 5' region) has been sequenced. An ATG start codon leading into a possible long open reading frame has been found at position +394 of the sequence.

Multiple copies of the 5' region of *gatA* have been found to be capable of titrating amdR protein *in vivo*. This titrating capacity has been localised to a 450bp 5' fragment. Comparison of sequence between this 450bp region and a 100bp 5' fragment of *amdS*, also known to titrate, has revealed a possible *amdR* binding site.

DNA MEDIATED TRANSFORMATION OF *MUCOR CIRCINELLOIDES*

R. van Heeswyck* and M.I.G. Roncero

Department of Physiology, Carlsberg Laboratory,
Gamle Carlsberg Vej 10, DK-2500 Copenhagen, Germany

*Presently at

Department of Genetics,
University of Melbourne, Parkville, 3052

The filamentous fungi belonging to genus *Mucor* are of biochemical and industrial interest because of the production of a number of extracellular enzymes, and because of the dimorphic nature of some species providing useful system for the study of morphogenesis. Genetic transformation of *Mucor circinelloides* has been achieved by incubating protoplasts from a leucine requiring strain with recombinant plasmid DNA in the presence of polyethylene glycol and $CaCl_2$. A plasmid was isolated from a genomic library of *M. circinelloides* DNA constructed in YRp17 by direct complementation of the *Leu*$^-$ auxotroph. Subclones containing a 4.4kb *Pst I* fragment of the pasmid complement the *Leu*$^-$ mutation and exist as extra chromosomal elements within *Mucor* transformants indicating that both a *Leu*$^+$ gene and a *Mucor ars* (autonomous replication sequence) are located therein.

INTERGENIC SEQUENCES ARE INVOLVED IN TRANSMISSION OF THE YEAST MITOCHONDRIAL GENOME

Jure Piškur

Molecular Genetics Group,
Research School of Biological Sciences
The Australian National University, Canberra, Australia

Yeast strains having deletions in their mitochondrial DNA (mtDNA) have been isolated (Clark-Walker *et al.* 1985). The mtDNA of these strains exhibits a normal gene order but various noncoding segments of the *wild type* mtDNA, encompassing various *ori* sequences, are missing. The loss of those segments does not affect mitochondrial function.

Haploids containing various intergenic deletions from two loci (between *pro tRNA* and *15S rRNA* and between *oli2* and *glu tRNA*) have been crossed to the *wild type* parental strains and between themselves. In all cases the *wild type* genome is preferentially transmitted to the diploid progeny. In crosses between different mutants transmission is not a simple function of deletion size at the same locus. Surprisingly, deletion at different loci affect transmission capacity very differently.

The results imply that transmission is influenced by particular intergenic sequence elements. A candidate is the *ori* sequence which is also involved in the enhanced transmission of highly suppressive petite mutants.

Clark-Walker G.D., Evans R.J., Hoeben P. and McArthur C.R. (1985) In: Quagliariello E. *et al.* (Eds.) Achievements and Perspectives of Mitochondrial Research. Elsevier Science Publishers, Amsterdam, Vol II, pp 71-78.

ELECTROPHORETIC VARIATION AND PLASMID DIVERSITY IN THE ENTERIC BACTERIA OF *Caledia captiva* AND OTHER GRASSHOPPERS

COLGAN, D. J. and D. A. WILLCOCKS, Population Genetics, R.S.B.S., A.N.U.

Three main conclusions are drawn from surveys of electrophoretic variation and plasmid diversity in the Enterobacteria of acridid grasshoppers.

1. There is extreme diversity in the nominal bacterial species *E. cloacae*, both in isolates from the same host species and in those taken from a variety of grashoppers. This diversity is consistent with a prediction of the neutral theory of molecular evolution but appears too high to be explained by the differential fitness of electromorphs.

2. Even, within nominal bacterial species, clones taken from one grasshopper species are more similar to each other than they are to isolates from other grasshoppers collected from the same 20x25 m^2 area. Dendrograms of bacterial relatedness are not, however, closely correlated with those of the insects, implying a complex pattern of coevolution between the hosts and their associates.

3. 52 different plasmids were revealed by restriction endonuclease profiles in 110 isolates of *E. cloacae* from *C. captiva*. Southern blots were separately challenged with 5 of the plasmids. At most, only one other plasmid was homologous to any of the probes. This sequence diversity is contrasted with the uniformity of plasmid size. The most probable explanation of the results is that whilst plasmids may serve *E. cloacae* in many functions, there are selectively important physical constraints on these cellular elements.

ASYMMETRICAL INTROGRESSION OF rDNA BETWEEN SUBSPECIES: EVIDENCE FOR THE INVOLVEMENT OF BIASED GENE CONVERSION

M.L. Arnold, N. Contreras and D. Shaw

Population Genetics Group,
Research School of Biological Sciences
Australian National University,
Canberra, ACT.

Ribosomal DNA (rDNA) restriction fragment length polymorphisms (RFLPs) have been identified for two parapatrically distributed grasshopper subspecies. We have also discovered that the rDNA loci are positioned in different regions of chromosomes 10 and 11 in individuals from these two subspecies. These two subspecies, which belong to the species *Caledia captiva*, meet and form a narrow hybrid zone in south-east Queensland. The zone is characterized by a major change-over in the frequencies of chromosomal markers that occurs over a distance of only 500 metres. In contrast, the rDNA from the Moreton subspecies has been detected in the Torresian form up to 450 km from the present-day hybrid zone.

There are several findings that suggest the involvement of a biased gene conversion-like process in the maintenance and spread of the Moreton rDNA markers in the Torresian individuals: 1) There is a marked discordance between the RFLP data from hybrid individuals and chromosomal, *in situ* hybridization data from the same individuals; 2) In addition, previous meiotic analyses have demonstrated that chiasmata do not occur within the regions of chromosomes 10 and 11 that possess the rDNA repeats; 3) RFLP data derived from using two restriction enzymes (Bam HI and Cla I) have identified a number of individuals that have conflicting patterns of RFLP markers. This finding suggests that only a portion of the "foreign" rDNA is being incorporated into the recipient subspecies; 4) Introgressed populations that are the farthest from the present-day hybrid zone, and are thus postulated to have possessed the "foreign" Moreton rDNA for the longest time, also demonstrate the highest Moreton rDNA frequencies.

SESSION 4C

COMPARISON OF ALLOZYME, MITOCHONDRIAL AND RIBOSOMAL DNA VARIATION, ACROSS A CHROMOSOMAL TENSION ZONE

A.D. Marchant, M.L. Arnold, P. Wilkinson, D.D. Shaw and D.M. Rowell

Population Genetics Group
Research School of Biological Sciences
Australian National University

The "Torresian" and "Moreton" subspecies of *Caledia captiva* (Orthoptera: Acridinae) are parapatric and form a narrow hybrid zone which is maintained by hybrid breakdown mediated by the chromosomal differences between the two taxa.

Studies of allozyme, mtDNA and rDNA markers indicate that the Moreton forms of these not-chromosome elements have introgressed into the Torresian chromosomal background. In one area, Moreton allozymes and rDNA restriction-fragment markers penetrate 300-400 km, while Moreton mtDNA is fixed within an area opproximately 200km long and 50 km wide, in the Torresian taxon. We propose that these Moreton markers have been left behind by movement of the chromosomal hybrid zone, which has itself remained a sharp transition of karyotpic markers, because of the role of chromosome structure in maintaining physical relationships among regulatory genes.

A transect across another part of the contact line between the two taxa also indicates asymmetrical introgression of non-chromosome markers, but on a much smaller scale. We have data on all markers used for the same individuals within this transect, which allows us to analyse within-individual correlations. With these data, the process of zone movement and the uncoupling of certain markers from their original karyotypic backgrounds can be studied, and we can investigate the functioning of a chromosomal tension zone as an obstruction to gene flow between speciating taxa.

EXTENT OF CLONAL DIVERSITY OF THE PARTHENOGENETIC GECKO *HETERONOTIA BINOEI*

C. MORITZ*, S. DONNELLAN+, M. ADAMS+ and P. BAVERSTOCK+

*Museum of Zoology and Department of Biology, University of Michigan, Ann Arbor, MI48109, USA.

+Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA 5002, Australia.

Electrophoretic variation at 18 allozyme loci was assayed in representatives of the geographically widespread, triploid parthenogenetic form of the gekkonid lizard *Heteronotia binoei*. A minimum of 58 different genotypes were observed among 146 individuals. Virtually all localities sampled were polyclonal. This represents unusually high genotypic diversity for a unisexual vertebrate. Heterozygosity in the triploids was higher than in diploid bisexual populations of *H. binoei*. Comparison with the occurrence of alleles in the diploid bisexuals confirms that the parthenogens are hybrids and suggests that most of the genotypic diversity stems from repetitive hybrid origins. However, some "orphan" alleles suggest that mutation adds to the genetic diversity of the parthenogens. The genetic structure of this geographically widespread parthenogen is consistent with the view that the persistence and spread of clonally reproducing organisms is facilitated by genotypic diversity.

Cytogenetic studies in the Mediterranean fruit fly, *Ceratitis capitata*

D. Bedo, Division of Entomology, CSIRO

The mitotic chromosome complement, in particular the sex chromosomes, of *C. capitata* have been studied in detail using fluorescent staining techniques. Triple staining with Chromomycin, Distamycin and DAPI allow the same chromosome to be examined for fluorescence of AT and GC rich regions. In the autosomes and the Y chromosome mutually exclusive patterns of AT and GC rich regions were found. However in the X chromosome both DNA types were seen in the same location. This may reflect an unusual chromosome structure with small alternating segments of AT and GC rich DNA.

Staining with DAPI/Actinomycin D produces the same fluorescence as quinacrine but with increased contrast. During spermatogenesis fluorescence of the X chromosome can be followed throughout but becomes less intense and more diffuse in the meiotic divisions. The X and Y chromosomes can still be distinguished in tightly paired metaphase I bivalents. Very bright fluorescence is restored during sperm maturation so that X-bearing sperm can be readily identified.

NUCLEOTIDE AND AMINO ACID POLYMORPHISMS WITHIN AND BETWEEN ESTERASE-6 ELECTROMORPHS OF *DROSOPHILA MELANOGASTER*

Peter Cooke[1&2] and John Oakeshott[1]
1: CSIRO, Division of Entomology, Canberra.
2: RSBS, ANU, Canberra.

Ten electrophoretic classes for *Est6* were detected in a sample of 157 individuals from a single population of *Drosophila melanogaster* using high resolution cellulose acetate electrophoresis. The ten classes clustered into four distinct groups; a Slow group with five classes, a Fast group with three classes, and two well separated faster groups with one class each.

Following the electrophoretic survey *Est6* alleles were cloned from representatives of the ten electrophoretic classes. Nucleotide sequences were obtained and analysed for eight of the ten electrophoretic classes. Thirty-two nucleotide differences occurred within the eight sequences; nineteen were silent substitutions in exons, one was within the small intron and twelve resulted in amino acid replacements.

As there were twelve amino acid replacements for eight electrophoretic classes, it is clear that electrophoresis has not detected all the amino acid variation. However, of the twelve amino acid replacements, eight were in hydrophilic regions likely to be on the surface of the EST6 protein molecule. Furthermore, these eight amino acid replacements were of two types. The first type of replacement results in charge changes that are associated with large electrophoretic mobility changes. The second type of replacement is charge conservative but non-conservative with respect to hydropathy (effects protein solubility) and results in small changes to electrophoretic mobility.

The remaining four amino acid replacements not detected by electrophoresis were in hydrophobic regions likely to be in the interior of the EST6 protein and were conservative changes with respect to charge, hydropathy and molecular volume. A previous study revealed four thermostability alleles within the Fast electrophoretic group and three within Slow. When representative lines from this study were electrophoresed on our high resolution cellulose acetate system no correlation was found between electrophoretic class and thermostability class, which implied that the two types of variation were independent. This suggests that the four conservative amino acid replacements in hydrophobic regions are in fact those affecting EST6 thermostability.

Previous electrophoretic studies, which classified *Est6* variation as Fast and Slow only, revealed a consistent latitudinal cline in Fast/Slow allele frequencies. As this cline for *Est6* occurred on three continents it has been suggested that forces of natural selection rather than those of chance are responsible for the cline. Five of the eight physicochemically non-conservative amino acid replacements differentiate the most frequent Fast and Slow alleles. Three of these cause changes to regions of likely functional significance; a putative glycosylation site, the signal peptide, and a putative dimerization site. Thus, any of the five, individually or in combination may be responsible for the clinal variation, and so be maintained by natural selection.

ESTERASE 6 ACTIVITY VARIATION IN *Drosophila melanogaster*: ASSOCIATIONS WITH RESTRICTION SITE POLYMORPHISM.

Anne Y. Game and John Oakeshott.

Research School of Biological Sciences, ANU. and
CSIRO Division of Entomology, Canberra.

Forty-two third chromosome lines of *Drosophila melanogaster* were isolated from the Coffs Harbour population and four day old virgins of each sex assayed for Esterase 6 (EST6) activity.

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

Some association was found between EST6 electromorphs and activity in males but not in females.

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

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

With an aim to discover if nucleotide polymorphism in the 5′ flanking region of the gene correlates with the activity levels, genomic Southern blot hybridisation analyses have been used to screen for restriction fragment length polymorphisms (RFLPs) around the *Est-6* gene using 9 six-base cutters and 6 four-base cutters.

In the 1.15kb region 5′ of the structural sequence 7 polymorphisms were found among 16 sites scored, giving a heterozygosity value of 0.0018. However in the coding region of the gene, only 1 polymorphism was found among 24 sites scored giving a heterozygosity value of effectively zero, and indicating sequences upstream of the gene to be much more variable than those within it.

Associations were evident between some of the 5′ RFLPs and both activity and electrophoretic phenotypes. It is suggested that those RFLPs define variable sequences involved in the differential regulation of the *Est-6* gene. The previously observed association of activity and electromorphs would be due to gametic disequilibrium between these 5′ regulatory sequences and sites within the coding region underlying the electrophoretic variation.

MOLECULAR BASIS OF RAPID EVOLUTIONARY CHANGE IN THE STRUCTURE AND FUNCTION OF *Drosophila* ESTERASE 6

Lis van Papenrecht[1] and John Oakeshott[2]

1.Department of Zoology, ANU and CSIRO, Division of Entomology
2.CSIRO, Division of Entomology

The Esterase 6 gene-enzyme system in the sibling species *Drosophila melanogaster, D.yakuba* and *D.erecta* has been used to investigate change in the structure and expression of a protein associated with evolutionary change in its physiological role.

In *D.melanogaster* EST6 is a monomeric protein produced mainly in the sperm ejaculatory duct of the adult male. In *D.yakuba* EST 6 is a dimer for which major sites of synthesis are the testes and accesory glands of the adult male. In *D.erecta* EST 6 is a dimer produced mainly in the thoraces of adults of both sexes. The 5' halves of the EST 6 coding regions of *D.yakuba* and *D.erecta* have been sequenced and compared to each other and to *D.melanogaster*. (Putative regulatory regions and the remaining parts of the coding region are currently being sequenced.)

In all three pairwise comparisons among the species, the percentages of amino acid replacement sites which were variable was found to be significantly less, at about one third, than the percentages of silent sites which were variable. This indicates some selective constraint against amino acid replacement in EST6 protein among the three species, despite their differences in EST6 structure and expression. However, the level of constraint was relatively low, as the ratio of replacement to silent site variation (0.34) was higher than has been calculated for other *Drosophila* genes studied (for example, approximately 0.1 for *Adh*).

The ratio of 0.34 for *Est6* among *D.melanogaster, D.yakuba* and *D.erecta* was also significantly higher than the corresponding figure of 0.22 for *Est6* among *D.melanogaster, D.simulans* and *D.mauritiana* (see Poster 13). In all three of the latter species EST6 is a monomer produced in the ejaculatory duct. This suggests that some of the extra amino acid variation among *D.melanogaster, D.yakuba* and *D.erecta* is due to divergent selection pressures associated with these species differences in the structure and expression of the enzyme.

Two further lines of evidence support this contention. First, the proportion of amino acid replacements in EST6 which are physicochemically non-conservative is much higher among *D.melanogaster, D.yakuba* and *D.erecta* (0.64) than among the three species with the same structure and expression of EST6 (0.28). Second, the amino acid differences among *D.melanogaster, D.yakuba* and *D.erecta* are clustered in regions of the EST6 protein likely to have specific functional significance. These regions include the signal peptide, a potential glycosylation site and a region tentatively identified as the dimerisation site for the enzyme in *D.yakuba* and *D.erecta*.

THE BOVINE β-CASEIN GENE 5' FLANK : CONSERVED ELEMENTS IN RELATED GENES.

John Bonsing, A. Francis Stewart and Antony G. Mackinlay.

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

The caseins are the major lactoproteins produced and secreted by the mammary gland during lactation in response to peptide and steroid hormones and supply the newly born infant with a source of amino acids and calcium phosphate, which is transported by aggregates of the caseins termed casein micelles. There are four casein species, α_{s1}, α_{s2}, β and κ, the first three of which have been shown (by cDNA analysis) to be evolutionarily related (1). Of the four, β and κ have been found in all eutherian milks examined, wheras the α caseins appear to be supplementary, being absent from some species, such as man.

We have recently determined the sequence of the bovine β-casein gene's 5' flanking sequences. The 5' flanking sequences of the bovine α_{s1}-casein gene have also been determined by us (2). Those of the rat α, β and γ casein genes have been determined elsewhere (2,3).

When these five sequences are compared, it is found that within the first 200 bp upstream from their transcriptional start sites occur several conserved elements. Some of these elements are recognisable motifs also found in other gene systems, such as the TATA, octamer and SV40-type core enhancer sequences, found within the first 70 bp upstream of the transcriptional start site. Also found are two sequences previously suggested as being putative progesterone receptor binding sites for the α_{s1}- and α-caseins. The β and γ casein sequences do not support this suggestion. An 'Eco RI palindrome' (a pair of Eco RI recognition sites or very similar sequences separated by three bp) and a large inverted repeat are also found. The latter is associated with a sequence similar to and in the same relative position as one found in the mouse whey acidic protein gene that is a known factor binding site (4).

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(3) Jones et al. J. Biol. Chem. 260 : 7042-7050 (1985)
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The Inheritance of Supernodulation in Soybeans

Angela Delves, Bernard Carroll and Peter M. Gresshoff
Department of Botany/Genetics, Australian National University

Leguminous plants, by mean of their ability to fix atmospheric nitrogen, can produce high protein seeds without the addition of expensive external fertiliser. This makes legumes particularly valuable as a crop and pasture plant. The nitrogen fixing symbiosis between legumes and soil bacteria of the genus Rhizobium is a closely regulated phenomenon with nodule number, development and activity governed by both external and internal factors.

In wild type soybeans such as cultivar Bragg and other legumes, external nitrogen sources such as soil nitrate inhibit nodule formation. In addition the plant possesses its own internal "Autoregulation" system which controls the amount of nodules allowed to develop on the root.

A number of soybean mutants have recently been isolated (see Carroll et al 1985) that produce nodule numbers far in excess of wild type cultivars and that continue to nodulate even in the presence of an external nitrogen sources. These mutants have been termed supernodulating and nitrate tolerant symbiosis or nts mutants and appear to have an altered autoregulatory system, governed by shoot factors (Delves et al 1986).

From the initial selection procedures and patterns of segregation displayed by the various nts lines, it became clear that the supernodulating character was inherited as a simple Mendelian recessive in most of the lines which showed increased nodulation (Delves et al 1987).

A hybridisation programme was done between a number of the nts lines (each nts line was originally isolated as a separate mutational event) to determine how many genes were involved. Complementation analysis showed that, with the exception of one line, nts1116, all the remaining lines tested were altered in the same complementation group. Crosses of these lines with wild type soybean, and subsequent analysis of F_2 and F_3 data, confirmed that they were all single recessive genes. The mutant nts1116 acted as a dominant when crossed with other nts lines, but as a recessive when crossed with normal wild type soybeans. This line may be a leaky mutant which shows partial autoregulation and nitrate tolerance.

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HOST GENETICS OF NON-NODULATION MUTANTS OF SOYBEAN

Anne Mathews, Bernard J. Carroll and Peter M. Gresshoff
Botany Department, The Australian National University, GPO Box 4,
Canberra, A.C.T. 2601 AUSTRALIA

The genetic contribution of the legume in the establishment of nitrogen-fixing nodules can be determined by the isolation and characterization of symbiotically altered plant mutants. Three stable non-nodulation mutants (nod49, nod139 and nod772) were isolated by induced mutagenesis of the soybean cv. Bragg (Carroll et al. 1986). These mutants, as well as, the naturally-occurring non-nodulation mutation rj_1 (Williams and Lynch, 1954) and the supernodulation mutant nts382 (Carroll et al. 1985) have been characterized.

Complementation tests indicate that nod139 is not allelic to the other non-nodulation mutants (nod49 and nod772), and the naturally-occurring non-nodulation mutation rj_1. Mutants nod49, nod139 and nod772 are inherited as Mendelian monogenic recessives.

The epistatic suppression of supernodulation by the non-nodulation mutants was observed by crossing the supernodulation mutant, nts382 with the non-nodulation mutants (nod49, nod139 and nod772). The F_2 plants segregated into three phenotypic classes in the ratio of 9 wild-type:3 supernodulators:4 non-nodulators. True double recessive mutants were detected by shoot grafts onto wild-type roots. nod49 and nts382 are unlinked and segregate independently.

Mutants nod49, nod139 and nod772 have been characterized and several attempts have been made to circumvent non-nodulation (Mathews et al. 1987). The stage(s) of blockage of nodulation in these mutants have been determined by studying the initial stages of nodulation.

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BACTERIAL GENES ESSENTIAL FOR SYSTEMIC PATHOGENESIS IN PLANTS

N.M.UPADHYAYA, K.F.SCOTT AND P.J.DART
Plant Molecular Biology, Research School of Biological Sciences,
The Australian National University, A.C.T. 2601, Australia.

Under field-conditions plant-bacterial interactions play an important role in plant nutrition *eg Rhizobium* nitrogen fixation. Many other associations are pathogenic such as the crown galls formed by the related *Agrobacterium*. *Rhizobium* infection of roots usually causes developmental changes at the cellular level such as formation of infection threads, and changes of structure coincident with release of bacteria into the cell, and at the organ level the development of the unique and complex nodule structure. Infection by *Agrobacterium* modulates tissue development resulting in the production of non-differentiated tumour tissue. We are studying another interaction which modulates shoot development as a result of nodulation. When the fast-growing, cowpea group *Rhizobium* strain IC3342 nodulates the tropical legumes pigeonpea, siratro and *Vigna spp*, a systemic response is produced which results in leaf-curling (hypernasty), suppression of apical dominance, sprouting of lateral buds and stunted growth. Grafting and sap feeding studies (Kumar Rao *et al* 1984; Upadhyaya *et al* 1985) indicated that the curl-inducing principle is produced in the roots and/or nodules and is translocated to the leaves to produce the symptoms, and that a continuous supply is essential for the manifestation of the disease. Adding combined nitrogen to roots suppressed nodule development and leaf-curling. A heat-cured, non-nodulating derivative did not induce curling, and curling was only induced in hosts which fix nitrogen suggesting that nodulation and fixation are essential for symptom development.

By Tn5 mutagenesis, we have identified 5 unlinked loci involved in the curling response. Two of the loci have a pleiotropic effect on nitrogen fixation. Hybridization studies show that 3 of the mutants have the Tn5 inserted on one of the 3 mega-plasmids present in the strain which also carries nodulation and nitrogen fixation genes (Upadhyaya *et al* 1987). These mutant fragments were cloned and wild-type sequences were isolated from a genomic cosmid library constructed from ~20 kb fragments of DNA from the strain IC3342. Cosmids containing homologous sequences, when transferred to the respective mutant strains, complemented the mutation restoring the curl-inducing ability in the mutants. Overlapping cosmids containing sequences homologous to one of the loci conferred curling ability to a normal, non-curling stain, ANU240. Hybridization studies indicated the presence of structural homologues to all except this locus in strain ANU240. We designated this gene as *lcrA*. Structural analysis of *lcrA* indicated a fairly strong homology to the *ompR* gene of *E.coli*. The gene *ompR* is a regulatory gene whose product controls the transcription of outer membrane porin proteins in *E.coli* and has homology with other regulatory genes from different systems namely, *virG, ntrC, cheY, spo0F* and *tppB* (Tracy Nixon *et al* 1986; Gibson *et al* 1987). The gene, *ompR* also has pleiotropic, regulatory effects on at least 4 different genes in *Salmonella typhimurium* (Gibson *et al* 1987).

By analogy to *ompR*, the gene *lcrA* is probably a regulatory gene which modulates the expression of many genes involved in the curling response, and these genes are probably common to several *Rhizobium* strains including strain ANU240.

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REGULATION OF *AGROBACTERIUM* Ti PLASMID VIRULENCE GENES.

Timothy J. CLOSE

CSIRO Division of Plant Industry, G.P.O. Box 1600, Canberra, A.C.T. 2601.

Crown gall, a tumor disease of plants caused by *Agrobacterium tumefaciens*, affects a wide range of gymnosperms and dicotyledonous angiosperms. There is mounting evidence that a number of monocots can also be infected, though the production of tumorous growths is not generally characteristic of infection in monocots. The genetic determinants of pathogenicity are carried on a large (200kb) autonomously replicating element called the Ti plasmid. The infection process involves attachment of the bacterium to the plant cell and subsequent transfer of a segment of DNA (T-DNA) from the Ti plasmid to the plant genome. The Ti plasmid also contains a cluster of genes whose functions are to aid and abet the process of infection. This segment of the Ti plasmid is called the virulence (*vir*) region. The *vir* region of pTiC58 contains six complementation groups: *virA*, *virB*, *virC*, *virD*, *virE*, and *virG*. Only the *virA* and *virG* genes are normally expressed at substantial levels in bacteria that have not been exposed to compounds present in plant wound sites. The remaining *vir* genes are quiescent until the infection process begins.

Genetic evidence indicates that a least two regulatory controls modulate the expression of the *virB*, *virC*, *virD* and *virE* genes. A two component sensory system is encoded by *virA* and *virG*, which mediate the induction of all of the other *vir* genes in response to phenolic compounds such as acetosyringone. Another control involves the *ros* locus, which is in the bacterial chromosome and affects only *virC* and *virD*. Expression of the *vir* genes was measured in each case using reporter genes encoding chloramphenicol acetyl transferase (*cat*) or bioluminescence (*lux*). Antibodies to VirC1 protein and T-DNA border cleavage were also utilized as more direct assays for the *vir* gene products.

HOW COMPLEX IS THE *LOZENGE* LOCUS OF *DROSOPHILA MELANOGASTER*?

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

Department of Genetics,
University of Melbourne, Parkville, 3052

The *lozenge* (*lz*) locus was identified as a complex locus by the classical studies of M.M. Green. Nineteen recessive alleles defined four discrete sites across a locus spanning 0.14 map units (X chromosome, position 27.7). In cis-trans tests most pairs of alleles failed to complement, however exceptions to this rule prevented a simple complementation map from being drawn. This genetic complexity extends to the phenotypic level ; *lz* mutants are typically pleiotropic displaying the following traits

1) Abnormal eye structure and pigmentation
2) Antennal basiconica reduced in number or absent
3) Absence of spermathecae and degeneration of ovaries
4) Tarsal claws reduced in size and depigmented, or absent
5) Some *lz* mutants lack the activity of an intracellular phenol oxidase enzyme (PHOX) ; structural locus - chromosome 2:80.6
6) Some *lz* mutations suppress the phenotypic expression of the Black cells mutation (chromosome 2:80.6)

In the context of an ongoing molecular study of the lz locus nine recently isolated *lz* alleles (along with eight previously characterized alleles) have been subjected to complementation and phenotypic analysis. Three key observations were made in these experiments

1) All mutations affecting the eyes and tarsal claws also result in the absence of spermathecae

2) Three mutations (lz^{49h}, lz^{50e} and lz^{MRI}) affect only eye phenotype. These mutations map to the spectacle sub-locus

3) Non complementation was observed in virtually all tests, except those involving lz^{49h}, lz^{50e}, lz^{MRI}. These alleles fail to complement all other mutations mapping to the *lz* locus (other than a putative deletion, lz^{XRI}).

It is therefore concluded that these mutations define two cistrons which can be aligned with the genetic map. Cistron A, defined by the mutations lz^{49h}, lz^{50e} and lz^{MRI}, which is apparently confined to the DNA sequences corresponding to the spectacle sub-locus. This cistron affects *lz* eye function. Cistron B spans the entire locus and affects eyes, tarsal claws and spermathecae. At the molecular level these cistrons could produce distinct polypeptides or represent different functional domains of a single polypeptide.

ISOLATION AND CHARACTERISATION OF *DROSOPHILA* GENES WITH HOMOLOGY TO A FELINE GLUTAMATE DECARBOXYLASE cDNA

M. PHILLIPS, L. SALKOFF* and L. KELLY

Department of Genetics, University of Melbourne, Parkville, Victoria 3052 Australia
* Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Glutamate decarboxylase, GAD, is the rate-lratting enzyme in the biosynthetic pathway producing the major inhibitory neurotransmitter δ-aminobutyric acid, GABA.

We have used a feline cDNA to glutamate decarboxylase, isolated by Dr Alan Robin, U.C.L.A., California, U.S.A., to screen a *Drosophila* whole genomic library.

Four clones with homology to the mammalian gene were isolated. These clones are being restriction mapped. At least two genes with homology to the feline gene are transcribed in *Drosophila*. On the basis of hybridization affinity one of these genes, from genomic clone G-2, is a likely candidate for the GAD gene.

The hybridisation indicates sequence homology in these two genes between *Drosophila* species separated by 60 million years of evolution, and the mammalian gene. The genes will be sequenced to determine the extent of this homology and the likely function of the conserved region.

The clones are being mapped *in situ* to polytene chromosomes to allow identification, or generation, of mutants of interest for genetic studies. G-2 maps to Chromosome 3 in a region genetically well characterised and for which deletion mutants are available.

A cDNA to G-2 has been obtained in an expression library and this is currently being used to isolate funtan proteins for antibody production.

Whole animal *in situs* to determine the spatial and temporal limits of gene exrmassion are planned.

OPERANT CONDITIONING IN *DROSOPHILA MELANOGASTER* WILD-TYPE AND MUTANTS WITH DEFECTS IN NEUROTRANSMITTER SYNTHESIS AND IN THE CYCLIC AMP METABOLISM.

H.A. MARIATH

Department of Neurobiology, Research School of Biological Science, Australian National University, Canberra , ACT, Australia.

Learning is usually classified into two categories: non-associative (comprising habituation and sensitisation) and associative learning, which includes higher types such as classical and operant conditioning. The similarity of learning phenomena across species suggests some simplicity and universality for the underlying mechanism. One way of attempting to understand this mechanism is through a neurogenetic approach, by characterizing single-gene mutations that code for the biochemical and structural machinery subserving learning. Wild-type *Drosophila* and the mutants *tan*, *ebony*, *Dopa-decarboxylase* type (affecting synthesis of 5-HT and DA), *Choline acetyltransferase* and *acetylcholine esterase* (affecting the metabolism of ACH), *dunce*, *amnesiac* and *rutabaga* (affecting different enzymes of cAMP metabolism) were tested using a new operant conditioning paradigm for single flies. All strains were able to learn but memory was impaired in some of the mutants. The relationship between memory and cAMP levels will be discussed in the light of a model for the cellular mechanism underlying learning in *Drosophila*.

CROSSING OVER IN MALE *LUCILIA CUPRINA*

G.G. FOSTER, G.M. CLARKE and G. WELLER

CSIRO, Division of Entomology, G.P.O. Box 1700, Canberra, A.C.T. 2601 Australia

Field-female killing systems incorporating conditional lethal mutations and Y-autosome translocations have been tested in several field trials of genetic control of the sheep blowfly *Lucilia cuprina*. Experience has shown that such systems are subject to genetic deterioration caused mainly by genetic recombination in translocation-bearing males. In a previous study it was implicitly assumed that enhanced crossing over in translocation bearing males was caused by breakage of the Y chromosome. The present investigations show that the stability of female-killing systems in *L. cuprina* is not related to structural alteration of the Y chromosome, but depends largely on which autosomes are involved in the Y-autosome rearrangement.

The frequency of spontaneous crossing over in chromosomally normal *Lucilia cuprina* males is approximately 0.14% in third and fourth chromosome regions which in females span 82 and 98 map units respectively. Crossover frequency was enhanced 10-fold in males heterozygous for 3;5, 4;5 or Y;5 translocations, but was suppressed several-fold compared to controls in males heterozygous for 3;4 and Y;3 translocations. Breakage of the Y chromosome thus appears to have no measurable effect on crossing over.

These data suggest that female-killing systems based on Y;3 or Y;3;4 translocations will be considerably more stable than the Y;3;5 systems which have been tested so far.

Evolutionary Change in Species from the Triticeae.

C.L. McIntyre
Evolutionary Biology Group
Research School of Biological Sciences
Australian National University

The grasses of the tribe Triticeae form a complex taxonomic group of more than 300 species. Approximately 75% of the species are perennial and ploidy levels vary from the diploid (2x=2n=14 chromosomes) to the dodecaploid (12x=2n=84 chromosomes). Over 20 haploid chromsome sets or genomes are recognised in the tribe, which are thought to have evolved from a common ancestor. Many genomes have remained diploid and restricted in geographical distribution. Some have combined to form an autoploid series while others have united in complex alloploid arrays producing a series of species that occupy vast areas on all continents. The classification of this large complex continues to be a matter of dispute, especially because a certain degree of crossability occurs between species with different genomes.

The tribe is best known for the three economically important cereals, wheat, rye and barley. However, many of the other Triticeae species are useful both in their own right as forage crops and as a source of agronomically desirable traits, such as disease resistance, salt and drought tolerance, for the improvement of the cereals.

The evolutionary relationships between a number of annual and perennial species representing 12 different genomes (A, B, D, S, E, J, R, V, N, P, H and I) have been examined utilising information obtained from 1) the distribution and variation in copy number of repeated sequence families, 2) variation at isozyme loci, and 3) DNA sequence analyses of regions of the rDNA and 5S DNA spacers. Both phenetic and cladistic analyses have been attempted for these data sets. These genomic relationships can then be compared with relationships derived from other criteria such as morphology and chromosome pairing.

The different parameters studied did not produce entirely concordant results. The data suggested that the A, B, D, E and J genome species were very closely related while the I and H genome species of the genus *Hordeum* (sensu lato) were the most divergent of the species examined. The S and R genome species clustered consistently with the A, B, D, E and J genome group. However the relationships between the V, N and P genome species relative to the clusters mentioned above varied between analyses and depended on the parameter studied. Introgession of a chromosome or chromosome segment from one species to another and variable rates of protein evolution in different lineages may explain the inconsistencies between phylogenies based on different parameters.

Species	Genome
Triticum monococcom	A
T. speltoides	B
T. tauschii	D
Pseudoroegneria spicata	S
Thinopyrum elongatum	E
Th. junceiforme	J_1J_2
Secale cereale	R
Dasypyrum villosum	V
Psathyrostachys juncea	N
Agropyron cristatum	P
Hordeum vulgare	I
Critesion bogdanii	H

Evolutionary change at the *Adh* locus in wheat and its relatives.

L. Mitchell, CSIRO Division of Plant Industry, G.P.O. Box 1600, Canberra, A.C.T. 2601

We have cloned and determined the nucleotide sequence of an alcohol dehydrogenase (*Adh*) gene from wheat. The cloned gene has been mapped to the long arm of chromosome 1A and hybridizes to an anaerobically induced RNA. Sequences important for eucaryotic gene expression such as the TATA box, polyadenylation signal and intron splice sites are found in the expected positions.

Comparison of the nucleotide sequence of the cloned wheat *Adh*-1A gene and a related gene from barley (*Adh3*) reveals extensive homology in both the coding and non coding regions of these genes. The homology is however, discontinuous due to a 1.8 kb insertion (TLM) in the wheat *Adh*-1A gene. This insertion is associated with a direct duplication of 8 bp of flanking genomic DNA, contains several large open reading frames, and the termini of the insertion have an imperfect 14 bp inverted repeat. These structural features are similar to those of eucaryotic transposable elements. Evolutionary studies of ancestral wheats suggest that the insertion is not active as a transposable element. Our attempts to induce transposition of the element have been unsuccessful.

VARIATION AT THE DNA LEVEL AMONG *SECALE* SPECIES

PRADNYA REDDY
CSIRO Division of Plant Industry

Eleven species from the *Secale* genus were tested for variation at the spacer region which separates the 18S and 26S rDNA gene subunits. Restriction fragment length polymorphism was detected between as well as within species, with fragments ranging from 1.5 kb to 4.0 kb in length when cut with Taq-1 enzyme. This length polymorphism is largely due to quantitative differences in number of 133 bp subrepeat units which are tandemly arranged in the spacer region and are bounded by Taq-1 sites on either side of the array. Annual ryes (*S. cereale* L. emend. Sencer) did not show marked differences from perennial ryes (*S. montanum* Guss. emend. Sencer), but *S. silvestre* possessed a 0.4 kb fragment shared by no other species. This short fragment could be the result of an extra Taq-1 site in the spacer region of *S. silvestre* or due to an extensive deletion in this region.

In contrast, relatively little length variation was found at the 5S DNA locus in the *Secale* genus. However, from agarose gel electrophoresis two major size classes of 5S DNA repeat units were found, the smaller is 420 bp long and the larger about 460-500 bp long. Sequence data revealed a 40 bp insertion in the larger spacer region which is not present in the smaller variant. This dual lineage pattern at the 5S DNA locus is found in some other Triticae species and it is speculated that the two variants in rye originate from two separate chromosomal loci.

HOMOLOGIES TO CHLOROPLAST DNA IN NUCLEAR DNA OF A NUMBER OF PLANT SPECIES.

M.A. Ayliffe[1], N.S. Scott[2] and J.N. Timmis[1].

[1] Department of Genetics, University of Adelaide, South Australia.
[2] CSIRO Division of Horticultural Research, Adelaide, South Australia.

Sequences homologous to chloroplast (ct)DNA have been found in nuclear DNA in 5 species of the Chenopodiaceae, extending the earlier observations of 'promiscuous' DNA in Spinacia oleracea (Timmis and Scott, Nature, 305: 65-67, 1983). Using the 7.7 kbp spinach ctDNA Pst I fragment as a hybridization probe, several separately located homologies to ctDNA were resolved in the nuclear DNA of Beta vulgaris, Chenopodium guinoa and Enchylaena tomentosa. In Chenopodium album and Atriplex cinerea the major region of homology was to a nuclear Eco RI fragment (6 kbp) indistinguishable in size from that in ctDNA. These homologies may therefore involve larger tracts of ctDNA because the same restriction sites are apparently retained in the nucleus. This suggests that in these latter two species there is a contrasting more homogeneous arrangement of ctDNA transpositions in the nucleus

DNA METHYLATION AND ITS POSSIBLE ROLE IN GENE REGULATION

Peter L. Molloy, Fuji Watt and David Tremethick
CSIRO Division of Molecular Biology, P.O.Box 184, North Ryde, NSW, 2113.

Cytosine methylation within CpG dinucleotides is the major post-synthetic modification of DNA within vertebrate genomes. Methylation at specific sites, and thus overall methylation patterns, are stably inherited via the post-replication action of a "hemi-methylase" enzyme. Developmental alterations in methylation patterns do occur and for many genes differences in methylation of specific CpG sites are seen when comparing DNA isolated from different tissues. For many genes a correlation has been observed between low levels of DNA methylation and active gene expression. Experiments involving either demethylation by treatment with 5-azacytidine or introduction into cells of methylated DNAs have demonstrated that DNA methylation can be causative in repression of gene expression (for Reviews see 1,2). These experiments do not, however, define what role, if any, of DNA methylation has in gene regulation during normal development.

To approach this issue three major areas are currently being pursued:

(1) What are the mechanisms by which DNA methylation inhibits transcription? Evidence from our laboratory and others suggests that there are at least two ways in which methylation can inhibit transcription - either indirectly, probably by favouring packaging into inactive chromatin (3,4) or directly by blocking binding of specific transcription factors, MLTF or E2F(5).

(2) How are patterns of DNA methylation established and altered? *De novo* methylation of DNA has been shown to principally occur in pre-implantation embryos of mice and may act to switch off all tissue-specific genes not then being expressed. Recently two systems have been found in which promoter-specific demethylation of transfected DNA is seen, for a skeletal α-actin gene in myoblasts (6) and for a metallothionein gene in mouse L cells. These systems offer the prospect of unravelling events leading to specific demethylation.

(3) What is the relative timing of gene activation and demethylation (or the reverse). The timing of DNA de-methylation in relation to gene activation has been best studied in the case of the chick vitellogenin gene, for which hemi-methylated DNA has been shown to be actively transcribed but it is not yet clear whether the fully methylated DNA is active (7). In the case of the X-chromosome it has been demonstrated that, although methylation can "silence" X-linked genes, X-inactivation precedes methylation of the inactive X-chromosome (8).

These recent findings suggest an interplay between DNA methylation and specific protein factors, in some cases active transcription factors, in the regulation of gene expression.

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METHYLATION IN CpG ISLANDS

M. Frommer, M. Gardiner-Garden, L. MacDonald and C. Paul

The Kanematsu Laboratories, Royal Prince Alfred Hospital,
Missenden Road, Camperdown, N.S.W. 2050

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

Although vertebrate genomes are depleted in the dinucleotide CpG, many vertebrate genes contain regions of clustered CpG dinucleotides called CpG islands or HTF islands. CpG islands are composed of unique sequence DNA with a very high G+C content and a frequency of CpG close to the expected value for the base composition (Bird, 1986). We have identified CpG islands at the 5' ends of all widely-expressed and many tissue-specific genes (5' CpG islands), and at the 3' ends of some tissue-specific genes (3' CpG islands). CpG islands are generally over 500bp in length, with an average length of about 1kb. Most 5' CpG islands extend through 5' flanking DNA plus adjacent exons and introns, whereas 3' CpG islands appear to be associated with exons (Gardiner-Garden & Frommer, 1987).

The CpG depletion of vertebrate genomes is believed to result from a high level of cytosine methylation at CpG dinucleotides, since 5-methylcytosine has a tendency to mutate by deamination to thymine. The best evidence currently available indicates that in CpG islands levels of CpG are not depleted and levels of (TpG + CpA) are not elevated because these regions are largely or completely unmethylated in all tissues, including germline. However, CpG islands are not intrinsically unmethylatable, and methylation within 5' CpG islands of housekeeping genes blocks transcription of the associated genes. It is not known whether this effect is mediated by gross structural changes, brought about by extensive methylation of CpG-rich DNA, or by changes in binding of protein factors, resulting from methylation of a limited number of key CpG dinucleotides. It is also not known if methylation within 5' CpG islands of tissue-specific genes will block transcription in cells that would otherwise be capable of expressing the genes. There is no data at present on levels of methylation of 3' CpG islands or on the effect of methylation of these islands on transcription of the associated genes.

Accurate methylation studies of CpG islands are difficult due to the high frequency of CpG dinucleotides, but it is clearly important to carry out such studies on CpG islands of tissue-specific genes, where differential methylation of a few CpG dinucleotides could have profound effects on rates of transcription. In our laboratory, we are studying the effect of methylation on two tissue-specific genes—c-*myb* which contains a well-defined CpG island at the 5' end of the gene, and proopiomelanocortin (POMC) which contains a 5' and a 3' CpG island separated by about 5kb of CpG-depleted DNA.

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DIFFERENTIAL RESPONSE OF MOUSE KALLIKREIN GENES TO HORMONAL INDUCTION

Barbara H. van Leeuwen*, Jennifer D. Penschow, John P. Coghlan and Rob I. Richards.
Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Vic. 3052 and *Medical Molecular Biology Unit, John Curtin School of Medical Research, A.N.U. A.C.T. 2601.

Glandular kallikreins are encoded in the mouse by a multigene family of 25 members which are closely linked on chromosome 7. Synthetic gene-specific oligodeoxyribonucleotides were used to investigate the hormonal regulation of expression of these genes. RNA dot blot analysis demonstrated that the expression of the kallikrein genes mGK-3, 4 and 5 in the salivary gland is sexually dimorphic and inducible in females by administration of testosterone or thyroxine. However mGK-6, which exhibits a nucleotide sequence homology of 80-85% with these genes and is contiguous on the chromosome with mGK-3, 4 and 5, is expressed at apparently constant total levels in male and female and is not induced by these hormones.

In situ hybridization histochemistry showed that all of these kallikrein genes including mGK-6, are expressed in the same cell type in the salivary gland: the granular convoluted tubule (GCT) cells in the male and the striated duct (SD) cells in the female. Testosterone or thyroxine treatment of females induces the differentiation of SD cells to GCT cells. The morphological changes take 3 to 7 days, and Northern analysis showed that the induction of expression of responsive genes accompanies the change in cell type. This slow time course, the generality of the response to different hormones and the accompanying differentiation of the responsive cells suggests that the induction is probably not a primary transcriptional response.

In situ hybridization histochemistry also showed that all kallikrein genes analysed exhibit uniform cellular distribution of expression in the SD cells of the female submandibular gland. The expression of mGK-3, 4 and 5 in the induced cells is also uniform. However the pattern of expression of mGK-6 in induced cells is non-uniform, suggesting that there are two distinct populations of kallikrein-expressing cells. The first express mGK-6 constitutively, and the second are the hormonally responsive cells which turn off expression of mGK-6 at a time when the expression of other kallikrein genes is induced. The latter cell type is only found in the submandibular gland, while the former is also found in the parotid and sublingual glands. From these results a complex distribution of cellular phenotypes has been identified and is a major contributor to the differential response of mouse kallikrein genes to hormonal induction.

CHARACTERIZATION OF *facB*, A GENE IN *ASPERGILLUS NIDULANS* WITH PROPERTIES OF A STRUCTURAL AND REGULATORY GENE

M.E. KATZ and M.J. HYNES

Department of Genetics, University of Melbourne

In *Aspergillus nidulans* acetate is metabolized via the glyoxylate pathway. It is known that the *facB* gene plays a critical role in acetate utilization in *Aspergillus*. The nature of that role, however, is obscured by the many different phenotypes observed in the *facB* mutants which have been isolated. Several mutations which abolish induction by acetate of the three glyoxylate pathway enzymes, acetyl CoA synthase, isocitrate lyase and malate synthase, have been characterized. The induction of acetamidase and isocitrate dehydrogenase is also affected. A mutation of *facB* (*facB*88) leads to increased levels of acetamidase and unaltered induction of glyoxylate pathway enzymes, but reduces growth on acetate. The *facB*88 mutation has not been separated from a translocation. Temperature sensitive *facB* mutants have been isolated. Some of these mutations result in the thermolability of glyoxylate pathway enzymes. Induction by acetate is not temperature sensitive in these mutants. The latter observations have lead to the proposal that *facB* plays both a structural and regulatory role in acetate metabolism (Kelly and Hynes, unpublished observations).

A molecular analysis of *facB* is being undertaken in order to determine the function(s) of *facB* in acetate utilization. *facB* has been cloned by complementation using a cosmid gene bank. A plasmid carrying the *facB* gene was recovered from the *Aspergillus* transformant by partial restriction endonuclease digestion, ligation and transformation of *E. coli*. A transcriptional analysis of *facB* is in progress.

Subclones of the original plasmid have been used to transform both *facB*$^-$ and *facB*88 translocation mutants. The stronger growth of *fac*88 translocation mutants on acetamide is unaltered in the transformants but some show increased growth on acetate. The breakpoint in the *fac*88 translocation has been determined. We are trying to isolate a plasmid carrying the *fac*88 mutation.

"Origins and speciation of viruses"

Adrian Gibbs
Research School of Biological Sciences,
Australian National University, Canberra.

Our recently acquired ability to determine the nucleotide sequences of genes is revolutionizing the study of virus evolution; not only providing the information from which past evolutionary events may be deduced, but also insights into the genetic forces moulding extant virus populations

Comparisons of the nucleotide sequences of viral genomes mostly confirm traditional taxonomic groupings of viruses. They also reveal unexpected homologies between virus groups previously thought to be unrelated, and show homologies between some viral and non-viral genes, indicating a common ancestry. Some of the sequence differences have been used to contruct phylogenies, however there is evidence that the 'molecular clock' in some of these does not always function at a consistent rate.

The observed nucleotide differences between the genomes of related viruses, or different isolates from a single viral population, seem to result from phenotypically neutral evolutionary 'noise', that is under various selective constraints. Phenotypic differences, important in the speciation and ecology of viruses, may result from a very small proportion of the observed differences.

SESSION 7A

VERTEBRATE β GLOBIN GENE EVOLUTION AND THE MOLECULAR CLOCK

SIMON EASTEAL

ABSTRACT.

The hypothesis of rate constancy in nucleotide evolutionary change is tested by multiple gene comparison involving a total of fifty genes of the beta globin gene family from five mammalian orders. The tests are designed to avoid any dependence on fossil record-derived estimates of species divergence times, as these may be unreliable. The results provide conclusive evidence that the rate of nucleotide change is the same among genes and taxa. There is no evidence for the proposed "hominoid slowdown". The analyses also provide novel insights into the evolutionary history of the genes and taxa.

X-Y EXCHANGE AND THE COEVOLUTION OF THE X AND Y rDNA ARRAYS IN *DROSOPHILA MELANOGASTER*

II. CLUSTERING OF rDNA CONTAINING TYPE 1 INSERTION SEQUENCES IN THE DISTAL NUCLEOLUS ORGANISER

P.R. ENGLAND[1], H.W. STOKES and R. FRANKHAM

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

The ribosomal RNAs produced by the multigene families on the X and Y chromosomes of *Drosophila melanogaster* are very similar despite the apparent evolutionary isolation of the X and Y chromosomal rDNA. X-Y exchange through the rDNA is one mechanism that may promote coevolution of the two gene clusters by transferring Y rDNA copies to the X chromosome. This hypothesis predicts that the proximal rDNA of X chromosomes will be Y-like. Consequently, rDNA variants found only on the X chromosome (such as those interrupted by Type 1 insertions) should be significantly clustered in the distal X nucleolus organiser. Proximal and distal portions of the X chromosome nucleolus organiser were separated by recombination between the inverted chromosomes $In(1)sc^{V2}$ (breakpoint in the centre of the rDNA) and $In(1)sc^{4L}sc^{8R}$ (no rDNA). Molecular analyses of the resulting stocks demonstrated that rRNA genes containing Type 1 insertions were predominantly located on the chromosome carrying the distal portion of the X rDNA, thus confirming a prediction of the X-Y exchange hypothesis for the coevolution of X and Y chromosomal rDNA. Distal clustering is not predicted by the alternative hypotheses of selection or gene conversion.

RAPID SEQUENCING OF SMALL SUBUNIT RIBOSOMAL RNA FOR PHYLOGENETIC ANALYSIS

A. JOHNSON* AND P.R. BAVERSTOCK+

* Flinders Medical Centre
Bedford Park, South Australia

\+ Evolutionary Biology Unit, South Australian Museum,
North Tce, Adelaide 5002.

ABSTRACT: A technique is described that allows rapid nucleotide sequencing of about 700 bases of the small subunit (16S - 18S) ribosomal RNA. Earlier studies using recombinant DNA technology have shown that this gene is sufficiently conserved in parts to allow phylogenetic inferences across both eukaryotes and prokaryotes. This rapid technique will therefore prove valuable for elucidating higher order relationships among taxa. The technique is illustrated with data from Toxoplasma, which show that it is not especially closely related to any other organism for which sequence data are available, including Plasmodium. The data available suggest that this gene behaves in a clock-like manner and can therefore be used as a molecular clock to date evolutionary events back to the divergence of eukaryotes and prokaryotes.

REGULATION OF THE *aro* F *tyr*A OPERON OF *ESCHERICHIA COLI*

C.S. Cobbett

**Department of Genetics,
University of Melbourne, Parkville, 3052**

Recent studies in prokaryotes have identified genes which are regulated by multiple operator sites, sometimes separated by hundreds of nucleotide pairs. A feature of these systems is that the upstream operator can exert its regulatory effect at various positions with respect to the promoter providing that the two sites are separated by an integral number of turns of the DNA helix. These observations have led to a model in which repressor protein bound at distant sites interacts via the formation of a DNA loop.

The *aro* F *tyr* A operon, codes for enzymes involved in the biosynthesis of tyrosine. Regulation of this operon by the *tyr*R$^+$ gene product requires the presence of three operator sites or TYR R boxes. TYR R boxes 1 and 2 lie adjacent to each other and overlap the promoter while the third TYR R box is upstream of the promoter. In this study the distance between TYR R boxes 2 and 3 has been increased by various amounts and the effect on repression by *tyr*R$^+$ in vivo has been measured.

TYR R box 3 contributes to the regulation of the *aro* F promoter at distances up to about 1,300 bp. However the repression exerted by TYR R box 3 is not dependent on its separation from box 2 by a integral number of turns of the DNA helix.

ANAEROBIC REGULATION OF ALCOHOL DEHYDROGENASE FROM *PISUM SATIVUM*

D. LLEWELLYN, J. TOKUHISA, B. TAYLOR, E.S. DENNIS and W.J. PEACOCK

CSIRO, Division of Plant Industry, G.P.O. Box 1600, Canberra, A.C.T. 2601.

The sessile nature of plants has resulted in the evolution of some unique adaptive responses which ensure their survival in an ever changing environment. Fluctuations in environmental parameters such as temperature, light and water availability induce specific physiological and biochemical reactions that are often termed stress responses. We have been concerned primarily with the response of plants to anaerobic stress caused in the field of flooding.

The anaerobic response has been well characterised in maize, where reduced oxygen causes the repression of normal protein synthesis and the inducion of a new set of about twenty anaerobic polypeptides (ANPs). A number of the ANPs have been identified as enzymes involved in anaerobic glycolysis and hence are critical for the maintenance of ATP supplies in the absence of respiration. Anaerobically induced alcohol dehydrogenase (Adh) is an important terminal step in this process and has been extensively studied in maize, but little is known about the regulation of *Adh* genes in dicot plants.

We have isolated and sequenced an anaerobically regulated *Adh* from the common garden pea, *Pisum sativum* and begun to analyse its expression using plant transformation techniques. A chimeric gene was constructed containing 350bp of the pea promoter linked to a CAT reporter gene and introduced into protoplasts of *Nicotiana plumbaginifolia* by electroporation. The gene was expressed and correctly regulated in tobacco. Deletion studies and hybrid promoter constructions has identified an 80bp region essential for anaerobic expression of the pea gene. This region contains sequence homology with the regulatory elements we have identified as functionally important in the maize *Adh1* gene. It seems that there is a control sequence common to many of the anaerobically regulated genes from different species and that the anaerobic response is an ancient one in the plant kingdom.

ALCOHOL DEHYDROGENASE GENE PROMOTER USAGE DURING *DROSOPHILA MELANOGASTER* DEVELOPMENT

T. Lockett[1] and M. Ashburner[2]

[1] CSIRO Division of Molecular Biology, North Ryde, 2113
[2] Dept. of Genetics, Cambridge University, Downing St., Cambridge, CB23EH, U.K.

The alcohol dehydrogenase (Adh) gene of *Drosophila melanogaster* is unusual in that its expression is regulated from two promoters. To a first approximation the proximal promoter is responsible for Adh expression during larval development while the distal promoter is active in adults. Detailed analysis of Adh transcripts during development by northern analysis using probes specific for transcripts initiated at either promoter, however, reveals that there are three stages in development at which transcripts from both promoters are present in the same organism. Early (0-2 h) embryos, mid (9-14 h) embryos and mid third instar larvae all carry transcripts made from both promoters. We have carried out tissue *in situ* hybridization experiments using probes (a) common to both transcripts, (b) specific for distally promoted transcripts and (c) specific for proximally promoted transcripts on embryos and larvae at and around these three developmental stages to determine whether transcripts from both promoters occur simultaneously in the same or different cells or tissues. Our results suggest that (a) transcripts from both promoters are randomly distributed in the early embryo, (b) at the onset of zygotic Adh transcription in the mid embryo, transcripts from both promoters first appear in the same tissue at the same time, and (c) in mid third instar larvae, distal promoter usage seems to be limited to fat body while gut Adh transcripts are presumably total produced from the proximal promoter.

MOLECULAR CHARACTERISATION OF TWO NATURALLY OCCURRING REGULATORY VARIANTS OF Adh IN *DROSOPHILA MELANOGASTER*.

P. EAST*, K. PAIGEN and D. SCHOTT

Department of Genetics, University of California, Berkeley.
*Department of Animal Science, University of New England, Armidale.

Several Drosophila stocks, made isogenic at the *Adh* locus, were characterised with respect to their ADH activity and *Adh* mRNA levels. Two of these stocks, both belonging to the Adh^S electromorph class, were shown to possess only half the ADH activity typical of Adh^S alleles. Furthermore, this difference in activity was manifest primarily in adults, larval activity being similar to that in other Adh^S stocks. *Adh* mRNA levels were assayed by quantitative dot blot hybridisation, and the two low activity stocks had only half the normal levels of *Adh* message. Cis/trans tests of these apparent regulatory variants were done for both ADH activity and *Adh* mRNA. In one instance the low activity/mRNA was shown to be cis acting and largely specific to the adult stage. Genomic Southern analysis of DNA from this stock revealed the presence of a 4.5kb insert approximately 3kb 5' to the distal promoter. This insertion may be responsible for the mutant regulatory phenotype of this stock, although the developmental specificity implies an effect which is more subtle than a gross interference with transcription.

The second regulatory variant appeared to be trans acting at both the protein and mRNA levels, and additive in its effect. Use of promoter specific probes revealed that reduced mRNA levels were only found for transcripts arising from the distal promoter, the observed developmental specificity of the phenotype resulting from differential promoter utilisation. Preliminary genetic analyses indicated that the locus, or loci, responsible for this promoter specific trans regulatory effect maps to the second chromosome.

ABNORMAL EXPRESSION OF INTERLEUKIN-3 AND LEUKAEMIA

I. G. Young
Medical Molecular Biology Unit
John Curtin School of Medical Research
Australian National University.

The haematopoietic system contains a variety of different functional cells derived from a common ancestor, thus potentially providing a model for studying the regulation of gene expression in both cell division and differentiation. A better understanding of this system could lead not only to improved therapies for leukaemias, anaemias, thrombocytopenias and immune disorders, but also to a better grasp of the molecular interactions that regulate cell growth and differentiation.

A number of glycoprotein growth factors have been identified which appear to play a central role in regulating blood cell development via interaction with specific cell surface receptors. Over the past few years rapid progress has been made in the molecular cloning and characterization of the genes encoding the various known haemopoietic growth regulators. Our own studies have concentrated on the multi-lineage regulator interleukin-3 (IL-3) and more recently on the eosinophil growth regulator IL-5.

The isolation of cDNA clones for murine IL-3 provided us with a valuable entry point for molecular studies of IL-3 and has allowed expression of IL-3 for biological studies and the characterization of the IL-3 gene for studies of gene regulation. The murine myelomonocytic leukaemia line WEHI-3B has been shown to possess a rearranged IL-3 gene resulting from insertion of an endogenous retroviral element near the IL-3 gene promoter. This rearrangement which results in the constitutive expression of IL-3 is postulated to have been an important step in the development of this leukaemia. A more direct investigation of the role of autostimulatory loops in leukemogenesis has been carried out by constructing a retroviral expression vector carrying the IL-3 gene and introducing it into the factor-dependent myelomonocytic cell line FDC-P1. The resulting autocrine-stimulated cell line has been shown to be leukemogenic, confirming the oncogenic potential of abnormal expression of IL-3 by haemopoietic cells.

A further area of interest concerns the structure and role of IL-3 in other mammals. The recent characterization of the rat and human IL-3 genes indicates a rapid divergence between the coding regions of the different mammalian IL-3 genes. This is in contrast to our recent findings with the mouse and human IL-5 genes. The availability of the human genes for IL-3 and IL-5 should facilitate molecular analysis of disorders of blood cell development in which the abnormal expression of these genes could be involved.

GENETICS AND MULTIFACTORIAL DISEASE

John Shine

Garvan Institute of Medical Research, St Vincent's Hospital, Sydney

Chronic, degenerative diseases such as cardiovascular disease, cancer, diabetes, osteoporosis and senile dementias represent the major challenge to present day medical research and clinical practice. Such diseases are slow in onset, complex in their presentation and multifactorial in origin. Both environmental factors (eg. smoking and obesity in heart disease) and genetic factors (a family history of heart attack) play a role in their genesis. Because there are so many factors involved in these types of diseases, studies of environmental factors have yielded complex and often conflicting results. Similarly, studies of familial inheritance by classic genetic epidemiology have failed to identify recognisable patterns of inheritance. At present, the application of molecular genetics to the study of such multifactorial diseases, through the use of DNA probes and restriction fragment length polymorphisms (RFLPs), constitutes an exciting approach to unravel the complexity of these disorders. Major advances have already been made in the ability to predict an individual's susceptibility to cardiovascular disease. Furthermore, identification of RFLPs which correlate with increased incidence of disease often provides important clues to some of the molecular lesions involved and suggests new therapeutic approaches.

The New Fungal Genetics

Michael Hynes

Department of Genetics, University of Melbourne

Many filamentous fungal systems have been well developed for genetic analysis and have made great contributions to the development of classical genetical theory. The availability of cloned genes has led to the development of efficient gene transfer systems for fungi which now enable many of these old problems to be addressed at the molecular level. The nature of fungal transformation and its application to problems of development, gene regulation, fungal sex, heterologous gene expression, plant pathogenicity and industrial microbiology will be discussed.

POSTER 1

GENETIC AND BIOCHEMICAL ANALYSIS OF COPPER TRANSPORT IN *ESCHERICHIA COLI*

D. ROUCH, J. CAMAKARIS, G. ADCOCK and B.T.O. LEE

Department of Genetics, University of Melbourne

Escherichia coli is an organism in which it should prove relatively easy to use mutants to identify all the steps of copper transport and mechanisms of copper tolerance. Although some steps may be different in mammalian systems, the findings in *E. coli* will provide leads for research in mammalian cells.

Plasmid-determined copper resistance has been studied in depth. The resistance is inducible and its basis is a reduced accumulation of copper. The major copper-resistance determinant on the plasmid has been cloned and contains at least four genes, one of which codes for an inducible 26 kd protein.

A number of copper-sensitive mutants have been isolated, some of which are also copper-dependent. Studies of these mutants has allowed partial characterisation of uptake, efflux and storage components of normal copper transport.

On the basis of these studies a general model for copper metabolism in *E. coli* has been formulated which is capable of further testing at the genetic and biochemical levels.

POSTER 2

TRANSLATIONAL COUPLING IN THE *aroFtyrA* OPERON OF *ESCHERICHIA COLI*

L. FARMER and C.S. COBBETT

Department of Genetics, The University of Melbourne

In *Escherichia coli* the *aroFtyrA* operon encodes two enzymes involved in the biosynthesis of tyrosine. These genes are coordinately regulated at the level of transcription. This poster presents evidence which suggests that they are also coordinately expressed at the level of translation.

An *aroFtyrA-lacZYA* gene fusion has been constructed and used to demonstrate the existence of translational coupling between *aroF* and *tyrA*. A mutation in the 5' untranslated region of *aroF* which reduces expression of *aroF* at the level of translation also causes a 4-fold decrease in the expression of the *tyrA-lacZ* protein fusion. Expression of *lacA*, which in this fusion is regulated only at the level of transcription, is not altered. Thus the effect of the *aroF* mutation on the expression of the *tyrA-lacZ* hybrid protein product is also exerted at the level of translation. These results suggest that efficient translation of *tyrA* requires the previous translation of *aroF* indicating that the two genes are translationally coupled.

POSTER 3

ANALYSIS OF THE POSITIVELY-ACTING REGULATORY GENE *amdR* OF *ASPERGILLUS NIDULANS*

A. ANDRIANOPOULOS and M.J. HYNES

Department of Genetics, University of Melbourne

The positively-acting regulatory gene *amdR* of *Aspergillus nidulans* co-ordinately controls the expression of four unlinked structural genes involved in the utilization of certain amides, omega amino acids and cyclic amides (lactams). These structural genes regulated by *amdR* are *amdS* (acetamidase), *gatA* (GABA transaminase), *gabA* (GABA permease) and *lamA* (lactamase/lactam permease). Induction of *amdR*-mediated expression of these genes requires the presence of omega amino acids as coinducers.

The *amdR* regulatory gene was cloned by complementation in *A. nidulans* using a cosmid based genomic library. Characterization of *amdR* subclones has indicated that the gene resides within a 4.2 kb Cla I-Eco RI fragment, as this is the smallest complementing fragment obtained. Transcriptional analysis has revealed the presence of two *amdR* transcripts of 2.7 kb and 1.8 kb, which are both synthesized constitutively at a very low level under induced and uninduced conditions. Given this very low level of transcription, we have initiated a study on the dosage effects of *amdR* on the expression of the activities under its regulation, using DNA-mediated transformation of *A. nidulans*.

In summary, the results indicate that elevated levels of the *amdR* gene product in *A. nidulans*, produced by either multiple copies of the *amdR* gene or by an *in vitro* manipulated *amdR* gene, lead to partial relief for the requirement of coinducer in activation of gene expression. In addition, high levels of *amdR* gene product result in an altered colony morphology with respect to conidiation, under induced conditions but not uninduced conditions, the basis of which is not clearly understood.

EXPRESSION OF THE HETEROLOGOUS REGULATORY GENE, NIT-2 OF NEUROSPORA CRASSA, IN ASPERGILLUS NIDULANS

M.A. Davis and M.J. Hynes

Department of Genetics,
University of Melbourne, Parkville, 3052

Aspergillus nidulans is a metabolically diverse fungus, capable of utilizing a wide range of nitrogen sources. Synthesis of the appropriate catabolic enzymes and permeases is controlled by nitrogen metabolite repression. Nitrogen control of gene expression is thought to be mediated by the positively acting regulatory gene, *are* A.

A new approach to the study of nitrogen control in *A. nidulans* was made possible by the availability of a clone of the *nit* -2 gene of *Neurospora crassa* (provided by George Marzluf). The *nit* -2 gene is thought to perform an analogous role to *are* A in the determination of the response to nitrogen status in *N. crassa*. The plasmid pNIT-2 carrying the *nit*-2 gene was transformed into *are* A^- mutants of *A. nidulans*. Loss of function *are* A^- mutants are unable to use nitrogen sources other than ammonium.

The *nit* -2 gene was found to complement several different *are* A alleles of *Aspergillus*, restoring the range of useable nitrogen sources to that of the wild-type organism. Although the *nit* -2 gene product restored activator function, studies of nitrogen control in the transformants indicated that enzyme synthesis was partially derepressed. Clearly there are differences in the expression and/or function of the *are* A and *nit* -2 gene products in *A. nidulans*.

Genetic mapping showed that the pNIT-2 transformants arose from non-homologous integration events. Attempts are underway to direct integration of the pNIT-2 plasmid to the *are* A gene with a view to producing hybrid *are* A-*nit* -2 proteins for further study of nitrogen metabolite repression in *A. nidulans*.

POSTER 5

ACTIVATION SEQUENCES OF THE *amdS* GENE IN *ASPERGILLUS NIDULANS*

T.G. LITTLEJOHN and M.J. HYNES

Department of Genetics, University of Melbourne

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

The *amdS* (acetamidase) gene of *Aspergillus nidulans* has been analysed in the above way. Transformants carrying multiple copies of the *amdS* controlling region (*amd* 1) can readily be generated, including those carrying many copies of mutant 5' regions. Through their ability to titrate regulatory gene products, these transformants have allowed identification of a 64 bp sequence that titrates the *amdR* gene product and a 38 bp sequence that titrates the *facB* gene product, as well as determining the relative affinities of wildtype and mutant 5'DNA sequences for regulatory gene products.

Titration analysis has identified DNA that binds most of the regulatory gene products known to control *amdS* expression (viz. *amdR*, *facB*, *amdA* and *areA*). Further characterisation of the regulatory elements of *amdS* has been made using transformants carrying single copies of *in vitro* mutated *amdS-lacZ* gene fusions. Together, these approaches have allowed identification of the sequences important for regulation and for regulatory gene product recognition and binding, providing a precise method for dissection of the mechanisms of gene regulation.

POSTER 6

INVESTIGATION OF *ACIA* IN *ASPERGILLUS NIDULANS*

J.A. SALEEBA and M.J. HYNES

University of Melbourne

Two approaches have been taken to study *aciA*, a gene of unknown function in *Aspergillus nidulans*.

Firstly, the function of the gene has been studied by inactivating *aciA*. The gene was manipulated *in vitro* and transformed back into the organism. Transformation occurs in *A. nidulans* by integration of sequences into the genome, here used to replace a wild-type copy of *aciA* with an inactivated copy. Plate tests have been carried out with this mutant to define a phenotype for *aciA*.

The second approach to the study of *aciA* has been to use the regulators of *aciA* to focus in on important regulatory regions in the gene. A series of subclones were made to represent 1.5 kb around the transcription start point of *aciA*. These have been individually transformed into *A. nidulans*. *amdS*, a gene coregulated with *aciA* via *amdA7*, is responsible for growth of *A. nidulans* on acetamide. When the *amdA7* product was present in limiting quantities growth on acetamide was reduced. Limiting quantities of available regulator were present in transformants in which multiple binding sites for the regulator were included in transformed sequences. These isolates showed the predicted reduced growth on acetamide. This study will be extended.

THE REGULATION OF ACETATE UTILISATION IN *ASPERGILLUS NIDULANS*.

R.A. Sandeman and M.J. Hynes
Genetics Department
University of Melbourne

Acetate utilisation in *Aspergillus nidulans* is under the control of two regulatory systems. One of these is a positive control system mediated by the regulatory product of the gene *facB*. Four genes have been found to be coordinately regulated by the *facB* gene product. They are

amdS encoding acetamidase,
facA encoding acetyl CoA synthase,
acuD encoding isocitrate lyase,
acuE encoding malate synthase.

The *amdS* gene product, acetamidase, converts acetamide into acetate and ammonia. The *facA*, *acuD* and *acuE* gene products are directly involved in the utilisation of acetate as a carbon source.

Two of these genes, *facA* and *acuE*, have been cloned and sequenced. Their regulation has been studied at the transcriptional level by Northern analysis. A comparison of the 5' control regions of these two genes and also of the *amdS* and *acuD* genes is underway to determine if any sequence homologies between them can identify the *facB* gene product binding site.

The *facA* gene has also been used to clone the homologous gene from *Neurospora crassa*. There is substantial homology at both the DNA and amino acid level between these two genes.

A SITE-SPECIFIC RECOMBINATION THROUGH SHORT DIRECT REPEATS IS INVOLVED In THE GENERATION OF SMALLER *RHO+* YEAST MITOCHONDRIAL GENOMES.

J. Piškur, E. Wimmer and G.D. Clark-Walker.

Molecular Genetics Group,
Research School of Biological Sciences
The Australian National University, Canberra, Australia

We have determined the nucleotide sequence of a 0.54 kb HhaI mitochondrial DNA (mtDNA) fragment from *Saccharomyces cerevisiae* strain R 0.54. The 0.54 kb fragment was derived after recombination-deletion processes from the original *wild type* 5.5 kb HhaI fragment which contains two short segments of coding regions for *pro tRNA* and *15S rRNA* genes at both end of the fragment and the *ori1* sequence in the middle. A recombination event involved in the generation of the novel mtDNA might have proceeded through short direct repeats (5' AC$^{C}_{T}$CCTTCGGG 3').

In R 0.54 mtDNA the distance separating the above genes is reduced to only 0.38 kb and the mtDNA does not contain the *ori1* sequence.

Although the strain contains a deleted mtDNA, it exhibits the respiratory (*rho*$^{+}$) phenotype in a non-fermentable medium. This implies that the intergenic region between *pro tRNA* and *15S rRNA* is dispensable for expression of the respiratory phenotype.

CLONING CALMODULIN BINDING PROTEINS FROM DROSOPHILA

A. Bull and L. Kelly
Department of Genetics
University of Melbourne

Calmodulin is a small highly conserved calcium binding protein present at high levels in the nervous system. It is thought to act as a modulator of the calcium signal in the nervous system through activation of various enzymes in response to calcium. Calmodulin binds to a wide range of enzymes, many of which are known to be important in nerve function.

To detect calmodulin binding proteins in Drosophila, head extracts were run on SDS gels which were subsequently western blotted and probed with I^{125}-labelled calmodulin. This technique detected approximately sixteen bands which bound calmodulin in the presence of calcium.

Screening of a Drosophila head cDNA library in λgt11 has been carried out using I-125-labelled calmodulin as a probe. A number of clones have been isolated one of which has been shown to produce a fusion protein with β-galactosidase which also binds calmodulin. This clone contains a 1.1 kilobase insert and it is currently being further characterised.

Isolation and characterization of lethal mutations at the *shibire* locus in *Drosophila melanogaster.*

M. Morgan and L. Kelly
Department of Genetics, University of Melbourne.

Shibire (*shi*) is an X-linked locus in *Drosophila melanogaster* which maps to position 52.2 (13F-14A cytologically). All alleles which have previously been isolated at the *shi* locus are conditional (temperature sensitive (ts)). These mutations cause adult paralysis at 30°C, which is reversible at 22°C. Further analysis of *shibire*^{ts}(*shi*^{ts}) has shown that some alleles are ts maternal effect, embryonic and pupal lethal.

Screens for new unconditional mutations at the *shi* locus have been carried out using several mutagens (X-rays, ^{60}Co, P-transposon and EMS). Some of the mutagens chosen are likely to cause chromosomal rearrangements, in order to facilitate the cloning and molecular characterization of the *shi* locus. It was predicted from studies of the ts alleles that such unconditional mutations at *shi* should be lethal. A number of new mutations have been isolated. All are unconditional, and lethal as predicted. Four were induced using X-rays and have been characterized cytologically. Three show no visible rearrangement at the level of polytene banding, while one shows a small rearrangement which appears to involve 13DE-13F. This may be a small inversion and is presently being characterized genetically. Several ^{60}Co induced and one P-transposon induced mutation are also being characterized.

CHARACTERIZATION OF THE SUPPRESSOR OF STONED MUTANT.

T. Petrovich and L.E. Kelly.

Department of Genetics, University of Melbourne.

The suppressor of the stoned allele, stn^{ts2}, has been isolated and characterized in Drosophila melanogaster. Genetic analysis of this mutant has revealed a number of interesting phenomena. It has been shown that the suppressor allele, su(stn), is co-dominant with the $su(stn)^{+}$, wildtype allele. The suppressor appears to suppress both the behavioural component of the stn phenotype and the reduced viability of the stn alleles. The suppressor gene maps to the X chromosome at approximately map position 22.0 near the mutant marker ocelliless. Cytological examination of this region in the su(stn)mutant has shown that there exists a chromosomal rearrangement. It is believed that a band duplication or insertion has occurred in region 7F of the su(stn) X chromosome.Molecular studies of this region are underway with the aim of cloning the su(stn) locus. Preliminary southern analysis has revealed some unusual results.

THE PHENOTYPE OF A PRESUMPTIVE HOMEOBOX GENE IN *DROSOPHILA*

By N. Brink[1] and R. Saint[2]

[1] School of Biological Sciences, Flinders University
[2] Division of Entomology, C.S.I.R.O. Canberra

A study has been made of the function in development of a presumptive homeobox containing gene located at $97D^{2-3}$ on the cytological map. The phenotype produced by loss of this gene was initially studied using deletions which removed the gene. More recently the phenotype has been assayed using zygotic lethals which map into this chromosome region. In contrast to other homeobox containing genes functioning early in development (e.g. *fushi tarazu* and *engrailed*) which alter segment number or polarity, the gene located at $97D^{2-3}$ does not alter segmentation but appears to interfere with gastrulation and head involution. The effects produced by loss of the gene are first evident around cellular blastoderm formation and show up later in development as defective gastrulation, failure of germ band shortening and abnormal head involution. On the basis of its phenotypic effects this gene may belong to another class of homeobox genes in *Drosophila*.

CONSERVATION AND CHANGE IN ESTERASE 6 NUCLEOTIDE SEQUENCES AMONG SIBLING *Drosophila* SPECIES.

Jill Karotam[1] and John Oakeshott[2].

1. Dept. of Botany, ANU and CSIRO Division of Entomology.
2. CSIRO Division of Entomology.

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

The results suggest that stabilising selection has operated to retain a similar structure for the EST6 protein among species in which the physiological role of the enzyme has been conserved.

The overall percentage of nucleotide differences between each pair of species was 4% between both *D.melanogaster* and *D.simulans* and between *D.melanogaster* and *D.mauritiana* and 2% between *D.simulans* and *D.mauritiana*. Of the total number of nucleotide differences between each pair of species, fewer occurred at amino acid replacement sites (22%) than in the intron or silent sites in exons (78%). This indicates a level of selective constraint against amino acid substitutions which may alter protein structure or function.

Detailed examination of each amino acid change provided further evidence for such selective constraint. It was found that 72% of amino acid differences between species were physicochemically conservative (with respect to hydropathy index, molecular volume and charge state of the amino acid residues involved), so likely to have little or no effect on the structure or function of the protein as a whole. In addition, changes were not found in regions believed to have particular structural or functional significance.

Nevertheless, comparison of the ratio of replacement to silent site variation (R/S) in *Est-6* with those for *Drosophila* genes for which analogous data are available suggest that the intensity of the stabilising selection is relatively weak for *Est-6*. Thus R/S=0.22 for *Est-6* but only 0.13 for alcohol dehydrogenase and for heat shock protein 82 and heat shock cognate genes, R/S=0, because no replacement changes at all have been found for these two genes among the *Drosophila* species studied.

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

P. Matthew and S. W. McKechnie

Genetics Department, Monash University

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

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

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

To test this possible role of transcription, tests will be carried out using P element transformation. To do this I intend to subclone fragments of sequenced Adh genes (Kreitman, 1983) and so produce constructs which only differ by the presence or absence of V2. A large number of transformants will be used and these lines will be tested for increased ADH, induction and other aspects which may be influenced by V2.

POSTER 15

Glycerol-3-phosphate Oxidase: a gene-enzyme system in Drosophila melanogaster

J. Ross and S.W. McKechnie

Genetics Department, Monash University

Glycerol-3-phosphate oxidase (GPO) is essential for flight in Drosophila. The structural locus maps to the 52D5 region of chromosome 2 and to date little is known about the molecular genetics of the GPO locus; the gene has only recently been isolated by Davis and MacIntyre at Cornell University. A study by McKechnie and Geer (1986) showed GPO enzyme activity is modulated by dietary factors in larvae. Enzyme activity increases of 5-7 fold occur when larvae are reared on a dietary combination of high ethanol and sucrose. The enzyme functions in larvae as part of the glycerolphosphate shuttle, regenerating NAD necessary for continued speedy glycolysis and oxidation of ethanol.

GPO mRNA levels were determined using dot blots quantified by scanning and using actin RNA levels as control. Ethanol had a significant effect on the elevation of mRNA levels.

Recently McKechnie and Hoffman (unpublished data) showed that larvae derived from adults collected inside the cellar at Tahbilk winery had significantly lower induced enzyme levels than larvae from flies collected from nearby orchards. This observation was confirmed for 33 isogenic substitution lines collected from these sites four years previously. Our objective now is to provide a GPO restriction map of these 33 lines and to determine whether there is an association between restriction haplotype and enzyme activity levels among the lines.

POSTER 16

CLONING OF A SEX DETERMINING GENE IN *CHIRONOMUS*

J. Martin and B.T.O. Lee

Department of Genetics,
University of Melbourne, Parkville, 3052

The region containing the male determining (MD) gene near the distal end of arm G of *Chironomus oppositus* form *tyleri* was microdissected by Prof. J.E. Edström. From the lambda clones so obtained, approximately 7, all about 3.9kb in length, showed some of the characteristics expected of an MD. One of these clones, E2.1, has been studied in more detail. This clone in-situ hybridizes back to the distal end of arm G and also to a number of other locations in the genome. The locations all have one thing in common - they are all sites at which the MD is known to occur in the various *Chironomus* species of south-eastern Australia. The same in-situ pattern was obtained on the chromosomes of both female and male larvae.

We therefore believe that E2.1 contains recognition sites for the MD region when it changes location. It may contain part of the actual MD itself, but probably only a small region.

POSTER 17

A SEX-INFLUENCED PROTEIN IN CHIRONOMUS - PROPERTIES AND CLONING STRATEGY

S.A. FABB, B.T.O. LEE AND J. MARTIN

DEPARTMENT OF GENETICS
UNIVERSITY OF MELBOURNE

A sex-influenced protein detected in the haeolymph and yolky oocytes of early-mid 4th instar larvae of several species of Chironomus was used to raise a polyclonal antibody.

The antibody, which binds only to proteins from female haeolymph, was used to screen a λgt11 expression library of total Chironomus tentans genomic DNA. Two positive clones were isolated and found to contain inserts, one of approximately 2000bp and the other of 400bp. Only the smaller of the two positives produces a large β-galactosidase - fusion protein, the other appears to be unstable. However, both react with the polyclonal antibody in native but not SDS-urea Western blots.

In order to isolate the entire gene, fragments from the larger of the two positives were oligo-labelled and used to probe a C. oppositus library constructed in λL47. A 5.6 kb sequence of DNA was isolated and is being further characterized.

COMPARISON OF PROTEASES PRODUCED BY LARVAE OF LUCILIA CUPRINA AND OTHER BLOWFLY SPECIES.

S.A. Constable

Genetics Department
Melbourne University

Blowfly larvae produce a number of proteases, including those which help them to invade flesh. Protease activity in blowfly larvae was compared using thin layer isoelectric focusing in agarose gels. Samples of Lucilia cuprina (7 laboratory and 5 field strains) and field isolates of L. sericata, Calliphora augur and C. stygia were used. Using gelatine, casein and collagen as protein substrates, a number of different proteases could be detected.

For each blowfly species a distinct zymogram phenotype was observed for a particular protein substrate. Zymogram phenotypes were similar for the two Lucilia species; patterns seen with the Calliphorids were more diverse. Overall, very little variation was observed within species. No difference in zymogram phenotype was observed between strains of L. cuprina, from either the laboratory or field, or between field strains of L. sericata. However, some minor variations were observed for each of the two Calliphora species.

POSTER 19

FIELD TRIALS OF GENETIC CONTROL OF *LUCILIA CURPINA*

G.G. FOSTER and R.J. MAHON

CSIRO Division of Entomology, G.P.O. Box 1700, Canberra, A.C.T. 2601.

During the summer of 1984-85, males of a sex-linked translocation strain (T(Y;5;3)23-3/w ru ar;to) were released in the Shoalhaven Valley (area 20,000 ha), south of Braidwood, N.S.W. The level of matings of native female flies by released males averaged 56% and remained reasonably constant throughout the season. This generated a constant genetic death rate of approximately 50%, which was sufficient to suppress the natural blowfly population to a level which reduced strike frequency and severity. However, the degree of population suppression and the genetic death rate were both substantially less than predicted from computer simulation studies (assuming no immigration into the test area). With the frequencies of mating achieved by released males, the death rate should have risen to approach the theoretical maximum of 93% for this strain, and the population should have been suppressed 10-fold more than was observed. Analysis of the genetic data using simulation methods suggested that both the lower death rate and the reduced suppression were probably caused by an influx of fertile wild flies to the valley.

In order to provide more substantial data concerning the effects of immigration, a similar trial was conducted on an island remote from potential sources of immigrants. Flies were released during 1985-86 season on Flinders Island (area 4000 ha containing 6000 merinos), 32 km off the Eyre Peninsula in South Australia. The proportion of matings by translocation males (both released males and their field-reared descendants) rose from approximately 50% during the spring months to 100% during March 1986. The rate of genetic death reached 88% towards the end of the summer. Estimates of population density derived from the weekly trappings showed that the mid-spring (October 1985) fly population on Flinders Island was unexpectedly high (some 10 times higher than the maximum density observed in the Shoalhaven region). After October, the population began to decline. This decline is attributable to a combination of management practices, climatic conditions, and the high rate of genetic death imposed by the released strain. The greater genetic loads imposed later in the season increasingly suppressed the native population until it was barely detectable at the end of the season.

The Flinders Island trial is probably the most successful demonstration of the potential of genetic methods to control a pest species to date. It was noteworthy that the genetic death rate approached the theoretical maximum for the system used, demonstrating the biological and technical feasibility of using genetically altered strains for the control of *Lucilia cuprina*.

Polytene chromosome map of the Mediterranean fruit fly, *Ceratitis capitata*

D. Bedo, Division of Entomology, CSIRO

Using trichogen cells from male pupal orbital bristles, a standard polytene chromosome map for the autosomes of *C. capitata* has been prepared. Mappping of a Y-autosome translocation allowed the correlation of the polytene element to its homologue in the mitotic karyotype. This translocation also confirmed earlier conclusions on the structure of the sex chromosomes in trichogen cells. Only one other polytene-mitotic chromosome correlation has been made.

Measurements of mitotic chromosomes show that two of the six pairs have indistinguishable lengths and arm ratios. In the absence of other recognition criteria the application of a systematic nomenclature system and full correlation of mitotic and polytene complements is not possible.

The polytene chromosomes from larval salivary glands have been compared to the trichogen chromosome map showing there is no similarity in banding patterns. This could reflect very different developmental gene expression in the two tissues. Distinct differences in the structure of the sex chromosomes support this contention.

FITNESS OF HALF-CHROMOSOME HOMOZYGOTES OF *DROSOPHILA MELANOGASTER*.

A. N. Wilton, J. A. Sved, K. Hu and F. J. Ayala.

Genetics Dept, Adelaide Univ.; Sch. Biol. Sci., Sydney Univ.; Entomology Dept., Hunian Univ., China and Genetics Dept., U.C. Davis, USA.

The fitness of whole-chromosome homozygotes when tested against balancer heterozygotes in population cages is drastically reduced to 20% of wild-type. From this it is difficult to infer anything about the effect on fitness of small regions of homozygosity since the consequences of assuming different selective interactions become more important as overall inbreeding depression becomes higher. The fitness of half-chromosome homozygotes was studied because it can provide information on a) selective interactions, the limited knowledge of which makes it difficult to construct realistic multiple locus models, and on b) the selective values and distribution of individual loci or small chromosome regions.

Using the multiply marked stock all (al dp b pr cn c px sp), lines homozygous for a wild-type left arm or right arm were constructed from 10 viable 2nd chromosome lines. Homozygotes for all combinations (10x10) of wild-type left arm with right arm were constructed. Lines homozygous for only half of the 2nd chromosome were made by combining all of the lines with a particular chromosome arm in common. Besides testing 2nd chromosome isozymes by electrophoresis to check for contamination, each half chromosome was tested 4 times, using non-overlapping subsets of lines on two occasions, and the results were examined for inconsistencies. In most cages the balancer, SM5, was eliminated so relative fitness had to be estimated from the rate of elimination. The low fitness (13%) of the SM5/+ heterozygote relative to wild-type was a major reason that stable equilibria were not reached in more cages. The average fitness of quasinormal right arm homozygotes (n=9) was 43% of wild-type and of left arm homozygotes (n=8) was 35%. The average fitness of half-chromosome homozygotes is 0.39.

Extrapolating to the fitness of whole-chromosome homozygotes under an additive model gives an unreal expected fitness of -0.22. Under a multiplicative model the expected fitness is 0.15 which is very close to the observed of approx. 0.2. Although the multiplicative model gave reasonable fitness estimates for most reconstructed whole-chromosome homozygotes, for the few with high fitnesses it gave estimates that were much lower than observed. This could be due to synergistic interactions between genes on the left and right chromosome arms or to contamination of these lines. Contamination is unlikely since duplicate lines give similar results.

POSTER 22

GENETIC VARIATION IN THREATENED AND SECURE BANDICOOT POPULATIONS

W.B. SHERWIN[1], N.D. MURRAY and J.A.M. GRAVES[2]

[1] Department of Genetics, Adelaide University.
[2] Department of Genetics & Human Variation, La Trobe University.

Recent formulations of conservation genetics theory have relied heavily on the hypothesis that a small, isolated population will lose genetic variation at a rate determined from the effective population size, using neutral theory.

The Eastern Barred Bandicoot, *Perameles gunnii*, has two populations, one in Tasmania that is dense, widespread, and secure, and a threatened remnant at Hamilton, Victoria. The Hamilton population appears to have an effective size of less than 100, and it has been isolated from other populations for up to 50 years.

On the assumption that the Tasmanian and Victorian populations originally had the same average heterozygosity (H), a comparison of $\overline{H}$ in the two populations constitutes a test of the hypothesis stated above. It was estimated that the predicted drop in $\overline{H}$ could be detected in a moderate-sized study, if the Tasmanian population showed the "average" mammalian $\overline{H}$ value of 0.051.

Twenty-six protein-coding loci were studied by electrophoresis of red cell extracts and plasma. Although crosses could not be performed, variants were investigated biochemically. Most variants appeared to be secondary and presumably non-Mendelian.

Genetic variation was minimal in both populations, so there was little power in the test of the hypothesis that average heterozygosity has dropped in the Hamilton isolate since the crash of the Victorian population. Further investigation of this hypothesis must await methods that allow the study of more loci and/or loci that are more variable.

POSTER 23

THE POSSIBLE INFLUENCE OF MAJOR GENE HETEROZYGOSITY ON VARIATION OF QUANTITATIVE TRAITS

G.R. BISHOP[a], O. MAYO[a] and L. BECKMAN[b]

[a] Biometry Section, Waite Agricultural Research Institute, The University of Adelaide.

[b] Department of Medical Genetics, University of Umeå, Sweden.

The possible explanations for heterosis and heterozygous advantage have included the hypothesis that the metabolic versatility of heterozygotes for functional alleles of structural genes would enhance resistance to environmental insult, i.e. would result in enhanced developmental homeostasis. Evidence on this hypothesis is conflicting.

The poster presents additional evidence, based on four human polymorphisms and nine quantitative traits in a sample of mother-offspring data from Sweden. These data do not support the hypothesis of interest. Reasons for the conflicting results are discussed.

CYTOGENETIC COMPARISONS BETWEEN THE AMERICAN MARSUPIAL MONODELPHIS DOMESTICA AND AUSTRALIAN MARSUPIALS.

D.L. Hayman[1], H.D.M. Moore[2] and E.P. Evans[3].

[1] Genetics Department, University of Adelaide, South Australia 5001.
[2] Institute of Zoology, Regent's Park, London, England.
[3] Sir William Dunn School of Pathology, Oxford University, England.

G banding comparisons are shown between chromosomes of the American laboratory marsupial Monodelphis domestica 2n=18(XX/XY) and chromosomes of an Australian and an American marsupial species with the conserved 2n=14(XX/XY) complement. These show that the two extra chromosomes of Monodelphis are derived by "centric fission" from the conserved complement. All other autosomal chromosomes are G band identical. Thus genetic data from this species and from the Australian laboratory marsupial Sminthopsis crassicaudata are likely to show similar linkage patterns.

One complete cell at prophase of meiosis obtained from a female Monodelphis shows markedly terminal localisation of chiasmata of all autosomal chromosomes. Preparation of meiosis from male Monodelphis show that interstitial chiasmata are found. These observations suggest that, like Sminthopsis crassicaudata, recombination may be more frequent in male Monodelphis than in female. The considerable evolutionary distance between these two species suggests that this sex difference may well be common to marsupials. This work is still in progress.

THE TAMMAR WALLABY MAMMARY GLAND; A POTENTIAL MODEL SYSTEM TO STUDY PROLACTIN DEPENDENT CONTROL OF GENE EXPRESSION

K. R. Nicholas and C. Collet

CSIRO Division of Wildlife and Rangelands Research, P.O. Box 84, Lyneham, A.C.T. 2602

Abstract

Traditionally, the mammary gland has been recognised as a primary site of prolactin action. In eutherian mammals, lactation is initiated by the withdrawl of the negative influence of progesterone at parturition followed by a positive stimulation of the gland with a combination of insulin, glucocorticoids, thyroid hormone, estrogen and prolactin. However, given the complexity of this system, the intracellular action of prolactin, post receptor binding, has been difficult to elucidate.

In marsupials, stimulation of the mammary gland to initiate lactation appears to be a function of prolactin alone. The hormone-dependent induction of casein and alpha-lactalbumin synthesis has been measured in mammary gland explants from the late pregnant tammar wallaby (Macropus eugenii). The primary role of the pituitary (the site of prolactin production) can be demonstrated by the induction of alpha-lactalbumin synthesis in mammary explants co-cultured for 2 days with anterior pituitary explants. Mammary explants cultured for 4-6 days in a chemically defined medium with prolactin show a progressive increment in accumulated casein and alpha-lactalbumin and this induction was maximal at a prolactin concentration of 10-20ng/ml which is within physiological levels measured in late pregnancy and early lactation. No increment was observed when prolactin was omitted from the media. The addition of cortisol, insulin, estrogen and triiodothyronine had no effect on prolactin-stimulated synthesis of either alpha-lactalbumin or casein. Furthermore and in contrast to eutherians, the prolactin-induced synthesis of milk proteins was not inhibited by the addition of progesterone to the media.

The single hormone induction of milk proteins in the Tammar mammary gland is unique and provides an ideal model system to study prolactin action, particularly in relation to the mechanism by which information is transferred from the receptor on the plasma membrane to the nucleus. The concept that an intracellular messenger exists for prolactin is controversial but has remained untested largely due to the lack of an appropriate experimental system. To this end the prolactin receptor from the tammar mammary gland has been isolated and characterised and the casein genes are being cloned. A model is presented to examine for the presence of a putative second messenger for prolactin which is released from the receptor and is transmitted to the nucleus to induce expression of specific milk protein genes.

POSTER 26

THE ISOLATION AND CHARACTERISATION OF A β-GLOBIN GENE FROM SMINTHOPSIS CRASSICAUDATA

S.J.B. Cooper

Department of Genetics,
University of Adelaide.

In higher vertebrates, haemoglobin is composed of two α-like and two β-like globin polypeptides each encoded by a family of related genes, under differential but coordinate control during development. The β-like globin gene family has been extensively studied in a wide range of organisms, and comparisons of β-globin genes both within and between species have provided much information on the molecular processes responsible for gene evolution and the developmental regulation of these genes.

The availability of data on the molecular structure of β-globin genes in marsupials will facilitate comparisons of considerable evolutionary interest. For this reason a project has been undertaken, the preliminary results of which are presented in this poster, to study the β-globin gene family in the marsupial Sminthopsis crassicaudata.

A S. crassicaudata genomic DNA library has been constructed in the bacteriophage EMBL3 and one clone was isolated from the library showing hybridization to a marsupial Dasyurus viverrinus cDNA β-globin clone (PDG-5) (Wainwright and Hope, 1985). Southern blot analysis of this clone localized the hybridization to a 3.7 kb BamH I/Sal I fragment. This fragment was subcloned into pBR322, restriction mapped and partially sequenced using the dideoxy-chain termination procedure, and has been shown to contain a β-like globin gene. Sequence comparisons show conserved upstream sequences characteristic of regulatory regions, including CCAAT and TATA boxes and also reveal conservation of the intron/exon splice sequences.

Wainwright, B. and Hope, R. (1985) Proc. Natl. Acad. Sci. U.S.A. 82: 8105-8108.

Genome instability in interspecific cell hybrids I. Aminopterin resistance and gene amplification in lines arising from fusions of cells from divergent mammalian species.

J.M. Wrigley, L.M. McKay and J.A.M. Graves
Department of Genetics and Human Variation,
La Trobe University, Bundoora, Victoria,
3083, Australia.

In order to investigate instances of genetic instability in divergent cell hybrids, we studied several HAT-resistant colonies recovered from fusions between HPRT or TK-deficient rodent cells and marsupial or monotreme cells. Most of these colonies proved to lack HPRT or TK activity and to have survived by acquiring resistance to aminopterin; such aminopterin-resistant lines were never recovered from parent cells subjected to HAT selection. Two of the aminopterin-resistant hybrids over-produced DHFR, and possessed either double minutes or a homogeneoulsy staining region, the cytological manifestations of gene amplification. Selection in higher aminopterin concentrations yielded a highly resistant line with 100X wild type DHFR activity and a large HSR. We suggest that interspecific cell hybrids are predisposed to gene amplification, and may also show many other types of genetic and chromosomal instability. We propose that such genetic variability constitutes the *in vitro* equivalent of the plethora of "genomic shock" phenomena described for inter-strain or interspecies hybrids of plants or animals.

POSTER 28

METALLOTHIONEIN mRNA LEVELS IN MENKES' DISEASE LYMPHOCYTES

R.A. FARRELL[#*], J. CAMAKARIS[#], J.F.B. MERCER[*] and D.M. DANKS[*]

[#] Department of Genetics, University of Melbourne, Parkville, Victoria 3052.
[*] Murdoch Institute, Royal Childrens' Hospital, Parkville, Victoria 3052.

Menkes' Disease is a human sex-linked inherited disorder of copper metabolism which is characterized by abnormal distribution of body copper. Features include progressive cerebral degeneration, connective tissue, arterial and skeletal abnormalities. Death usually occurs by two years of age.

Continuous Lymphocyte Cells (CLC's) isolated from Menkes' patients accumulate more copper than normal cells in culture. Excess copper in Menkes' cells has been shown to be associated with a low molecular weight, cysteine rich, protein which has been identified as metallothionein (MT). The overproduction of MT may be due to a primary defect in regulation in Menkes' cells or as a result of a secondary induction by Cu accumulating due to a block in intracellular transport.

MT gene expression has been studied in response to extracellular copper in both normal and Menkes' CLC's. MT mRNA levels were measured by "northern transfer" and "dot hybridization' using human MT-$1l_A$ specific cDNA probes (Karin and Richards, 1982). At low extracellular copper levels Menkes' cells produce higher levels of MT-$1l_A$ mRNA than normal cells, however, at higher levels of extracellular copper normal cells have more MT-$1l_A$ mRNA than Menkes' cells. The latter result was unexpected but can be explained if pre-existing concentrations of MT in Menkes' cells bind Cu effectively preventing this Cu from inducing more MT synthesis. Overall these data do not support the notion that there is a primary defect in MT regulation in Menkes' cells.

Karin, M. and Richards, R.I. (1982) Human metallothionein genes: Molecular cloning and sequence analysis of the mRNA. *Nucl. Acids Res.* 10, 3165-3173.

POSTER 29

CHROMOSOMAL LOCALIZATION OF THE 70 KILODALTON HEAT SHOCK PROTEIN (HSP70) GENE IN HUMANS.

Anna Davey, and Ismail Kola.

Centre for Early Human Development, Monash University, Monash Medical Centre, 246 Clayton Road, Clayton, Australia, 3168.

The one cell mouse embryo is under maternal control and does not require the transcription of the embryonic genome. At the 2-cell-stage of mouse embryo development the embryonic genome is activated. The first major transcription product of the embryonic genome has been shown to be Hsp 70 (Bensaude et al, 1983). Our current studies are aimed at evaluating the role of Hsp 70 in the preimplantation mouse embyro. One aspect of these studies involves the chromosomal localization of these genes in the mouse and the human.
A 2.3 kb cDNA fragment cloned in pUC 8 was nick-translated with tritium. Probe DNA was hybridized with chromosomal DNA fixed onto glass slides. Unbound DNA was washed off after hybridization using varying washing stringencies. Hybridization to chromosomes was detected by autoradiography.
Fifteen percent of grains were found on chromosome 6, the observed number of grains was 3 times higher than the expected number of grains. The peak of these grains was found on the short arm of chromosome 6 and more specifically in region 6 p2.1. this gene was syntenic with the c-ki-ras oncogene.
This study has localized the chromosomal position of hsp 70 in humans. Further studies are underway to localize the gene position in the mouse and these studies should contribute to understanding of the function of these genes.

(i) Bensaude, O., Babinet, C., Morange, M. and Jacob F (1983) Nature **305**, 331-333

VITRIFICATION INHIBITS THE ABILITY OF MOUSE OOCYTES FERTILIZED IN VITRO TO FORM VIABLE FETUSES.

Carol Kirby, Ismail Kola, Jillian Shaw, Anna Davey, Stephanie Edmondson and Alan Trounson.

Centre for Early Human Development, Monash University, Monash Medical Centre, 246 Clayton Road, Clayton, Australia, 3168.

The ovulated oocyte is in metaphase of the second meiotic division with chromosomes are gathered on the spindle. Magistrini and Szollosi (1) have reported that cooling down of such oocytes causes depolymerization of the spindle. It is thus possible that oocytes frozen at this stage could result in chromosome scatter, and give rise to embryos which develop abnormally. This study evaluates the viability of embryos derived from the in vitro fertilization of vitrified oocytes.

Unfertilized mouse oocytes were vitrified. After thawing the oocytes were fertilized in vitro and cultured to the 2-cell stage before transfer to pseudopregnant (Day 1) recipients. On day 15 the mice were sacrificed, and the number of implantations, resorptions and morphologically normal fetuses evaluated.

Table 1 : Development of embryos derived from the in vitro fertilization of vitrified oocytes.

Group	No of oocytes	% fertilized	% implanted	% resorbed	% foetuses
Controls	766	87	$61^{a}(70)^{b}$	$14^{a}(22)^{c}$	$47^{a}(55)^{b}$
Vitrification (10 mins)	932	19	$12^{a}(63)^{b}$	$7^{a}(61)^{c}$	$5^{a}(26)^{b}$
Solutions (10 mins)	663	26	$16^{a}(63)^{b}$	$7^{a}(41)^{c}$	$10^{a}(37)^{b}$
Vitrification (5 mins)	767	19	$8^{a}(43)^{b}$	$5^{a}(57)^{c}$	$3^{a}(19)^{b}$
Solutions (5 mins)	705	37	$27^{a}(73)^{b}$	$6^{a}(23)^{c}$	$20^{a}(56)^{b}$
Slow-cooling (DMSO)	745	42	$23^{a}(55)^{b}$	$11^{a}(45)^{c}$	$13^{a}(49)^{b}$

a - as a percentage of total oocytes b - as a percentage of fertilized oocytes c - as a percentage of implanted embryos.

These results demonstrate that vitrification of oocytes has a significant effect on the number of oocytes that are fertilized, number of fertilized oocytes that implant and number of embryos that get resorbed. Furthermore the number of preimplantation embryos that were chromosomally aneuploid was also significantly increased in the vitrified groups.

We conclude that oocyte vitrification and freezing adversely affects the viability and developmental potential of embryos derived from these oocytes.

(1) Magistrini, M. and Szollosi, D. (1980) Eur. J. Cell. Biol. **22**, 699-707

CYANOGENESIS IN AUSTRALIAN BRACKEN (PTERIDIUM ESCULENTUM): FACTORS INFLUENCING THE INDUCTION OF ENZYME AND SUBSTRATE.

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

Cyanogenesis in bracken involves hydrolysis of the glycoside prunasin by the enzyme beta-glucosidase to liberate mandelonitrile and glucose. The mandelonitrile is then hydrolysed to produce hydrogen cyanide.

The cyanogenic potential of field-grown bracken from 30 localities throughout Tasmania and 79 throughout NSW was determined using the alkaline picrate method at pH 5.5. 41% of the Tasmanian fronds and 23% of the NSW fronds were cyanogenic.

A total of 178 clones of P. esculentum (151 from throughout Australia) were also tested for their cyanogenic phenotype. These plants were tub-grown in standardised soil mix at the same site in Sydney. Only a small percentage of fronds from Australian clones raised under these conditions were cyanogenic (0% from Tasmania and 2% from NSW). However, the majority of fronds produced cyanide when supplied with prunasin indicating that endogenous beta-glucosidase was present.

The effect of three physical (shading, crushing, wilting) and two anti-metabolite treatments (p-phenylfluoroalanine and sodium arsenite) on the cyanogenic potential of fronds from normally acyanogenic clones was examined. No treatment resulted in cyanogenesis without exogenous substrate. All treatments resulted in increased beta-glucosidase activity in some clones. A few clones remained totally acyanogenic, showing no induction or activation of beta-glucosidase activity as a result of any of the treatment regimes tested.

Complex genetic polymorphisms involving at least 2 genetic systems appear to underlie cyanogenesis in P. esculentum. We interpret the results as showing that Group I gene(s) controlling presumed enzyme(s) involved in the synthesis of the cyanogenic glycoside prunasin may be constitutive or inducible, according to genotype, but factors affecting induction (or activation) of these enzymes have not yet been identified. Group II gene(s) controlling beta-glucosidase(s) hydrolysing prunasin are constitutive, uninducible or inducible to a degree varying according to genotype.

POSTER 32

RIBOSOMAL DNA VARIATION IN TARO

P. MATTHEWS

Department of Prehistory, Research School of Pacific Studies & Population Genetics Group, Research School of Biological Sciences, ANU.

Colocasia esculenta (taro) is an ancient starchy root-crop of the Asian and Pacific region. Cloned ribosomal DNA from taro has been used for hybridisation-probe analysis of plants from both tropical and temperate areas. The rDNA patterns observed suggest a long history of stable maintenance within vegetative lineages, as well as segregation and reassortment during sexual reproduction. Within the species, variation in rDNA patterns is compounded because every ancestral genotype has potentially been able to mate with every descendent genotype. It is not yet apparent what kinds of *de novo* mutation can be attributed to assexual and/or sexual cell lineages.

ABSTRACT

INHERITANCE OF ADULT PLANT LEAF RUST RESISTANCE TO RACE 77A - IN CULTIVARS WG138 AND TEZNOS PRECOS PINTOS.

R.G. Saini*,G. Malhotra and A.K. Gupta
Department of Genetics
Punjab Agricultural University
Ludhiana - INDIA

Cultivar WG138 (*Lr1, Lr3, Lr13*) from India and Teznos Precos Pintos (*Lr3, Lr13, Lr14*) from Mexico have remained free from leaf rust in India for over 12 years in artificial epiphytotics of race 77 (biotype A) which is the most frequent and virulent race in India. Leaf rust resistance genes *Lr1, Lr3, Lr13* (adult plant resistance) and *Lr14* identified from cultivars WG138 and Teznos Precos Pintos are ineffective against race 77 both at seedling and adult plant stage (Gupta et al, 1984; Saini et al, 1986). Seedlings of cultivars WG138 and Teznos Precos Pintos are susceptible but the adult plants develop highly resistant infection types against race 77, suggesting the presence of additional adult plant leaf rust resistance in these two cultivars. The segregation pattern for susceptibility in the F2 and F3 generations obtained from the crosses of these two cultivars with a susceptible cultivar Agra Local suggests that adult plant resistance to race 77 is conditioned by a dominant gene in both the cultivars. Some wheats with the adult plant resistane gene *Lr13* are known to give different levels of resistance to leaf rust in different parts of the world. It appears that the additional adult plant resistance such as that seen in cultivars WG138 and Teznos Precos Pintos is causing this variation in the reaction of different *Lr 13* bearing wheats.

*Visiting Scientist at the Plant Breeding Institute, Castle Hill, University of Sydney, N.S.W. Australia under the ACIAR-ICAR Co-operative Wheat Rust Research Programme.

GROUP TRAVEL TO:

4th International Congress of Cell Biology - Montreal, 14-19 August, 1988

XVI International Congress of Genetics - Toronto, 20-27 August, 1988

Members of the ANZ Society for Cell Biology and the Genetics Society of Australia who are contemplating attending one or both of these congresses may be interested in reducing their travel costs by availing themselves of group travel discounts. As the congresses are still a year away it is difficult to obtain firm quotes on airfares that will be operating in August 1988. However, tentative quotes have been obtained from two travel agents, and details are given below. At this stage we would like to hear from anyone who is contemplating attending and might avail themself of this offer, so we can see if there is sufficient interest to warrant continuing the exercise. ANZSCB members should contact the Secretary with details of their requirements and preferences. GSA members should contact Chris Gillies at the AGM or by post.

1.World Travel Headquarters Pty Ltd (12 retail agencies round Australia)

Proposed Itinerary

13 Aug 1988: Sydney - Montreal (via Los Angeles) : Qantas and Air Canada

20 Aug 1988: Montreal - Toronto : Air Canada

28 Aug 1988: Toronto - Sydney (via San Francisco) : Air Canada and Qantas

Cost - ex Sydney (per person): $2140 (economy group airfare only)
: $2675 (economy group airfare + twin share accommodation - Le Sheraton, Montreal; Delta Chelsea Inn, Toronto)

Price includes airport transfers in Montreal and Toronto. You may break return journey in North America on condition you use Qantas on the West Coast - Sydney sector.
(Fares subject to changes due to currency changes, airline changes etc.)

2.Everything Travel (Sydney)

Proposed Itinerary

13 Aug 1988: Sydney - Montreal (via Vancouver) : CP Air

20 Aug 1988: Montreal - Toronto : CP Air
OR
19 Aug 1988: Sydney - Montreal (via Vancouver) : CP Air

28 Aug 1988: Toronto - Calgary : CP Air
? : Calgary - Vancouver : surface
? : Vancouver - Sydney : CP Air
OR
28 Aug 1988: Toronto - Sydney (via Vancouver) CP Air
(Stopover in Honolulu at no additional cost to airfare)

Cost - ex Sydney (per person): $1950 (excursion group economy fare)

Based on travel with CP Air and approximately 30 persons travelling as 1 or 2 groups on departure leg. This could be one group going to Montreal on 13 August, and a second group going direct to Toronto on 19 August, provided the total number was approximately 30. Individual return travel is acceptable, provided CP Air via Vancouver is used for the Canada - Sydney sector.
(Fare based on present fare projections, subject to changes or special offers.)

OVERVIEW OF FASTS ACTIVITIES

The Federation of Australian Scientific and Technological Societies (FASTS) is the "umbrella body" to which your professional society belongs. FASTS has over 60 Member Societies and through these bodies represents over 60,000 people working in science, technology and engineering through industry, medical research institutes, government laboratories, in government and in all levels of the education sector.

FASTS was officially established in November 1985 and has had a full-time Executive Director, Dr David Widdup since June 1986. It is governed by an Executive which currently meets every six weeks, a Board which meets twice a year and a Council which meets every second year.

FASTS Constitution (Section 3) states:-

> "The objectives of the Federation are to foster close relations between the scientific and technological societies in Australia and to take concerted action for promoting science and technology in Australia. These objectives include:
>
> (a) To facilitate discussions within the scientific and technological community concerning matters of common concern;
>
> (b) To enhance communication between the scientific and technological community and governments, industry and commerce; and
>
> (c) To promote understanding among the Australian public of the work done within the scientific and technological community of the nation."

In pursuit of these objectives, FASTS has initiated the following action over the last year.

1. To facilitate communication within the scientific and technological community

   * FASTS continues to be strongly involved in NSTAG (the National Science and Technology Budget Analysis Group), which in addition to FASTS comprizes the Institution of Engineers (Aust), the Academy of Sciences and the Academy of Technological Services. NSTAG advises the government on the S & T budget and on related matters. This year FASTS is co-ordinating the NSTAG Report and Forum.

   * An agreement has been concluded with Australian Airlines to enable FASTS Board members to meet more often.

* A monthly newsletter on FASTS activities is sent to all Member Societies, to FASTS Board Members and to other interested parties including journalists

* In addition there is a bi-monthly report on FASTS activities in the journal Search. FASTS members can subscribe to 6 issues of Search at the reduced rate of $25.

* There will be a full Council Meeting in 1987 at which each Member Society can be represented.

* The Executive Director has made contact with a number of scientific and technological societies overseas and has investigated ways in which conferences of FASTS Member Societies can be promoted overseas.

* There is continuing liaison and joint activity with CSIRO CSIROOA, FAUSA.

2. To enhance communication between the scientific and technological community and governments, industry and commerce

During its first year of existence FASTS has seen its major role to be the enhancement of communication between the scientific and technological community and government, industry and commerce. A number of meetings and many more written submissions have brought FASTS views to the attention of Ministers, Opposition Spokespeople, Government and Opposition Committees, Senior Public Servants and Industry group representatives.

Most recently in the run-up to the Federal election FASTS contacted politicians stressing the need for support for a broad spectrum of research and voicing the concerns that have been expressed by FASTS Board concerning the funding for the Australian Research Council.

Whilst FASTS representations have covered a whole range of concerns felt by the scientific and technological community areas where Government and Industry have been particularly receptive are:-

* the provision of sufficient trained personnel;

* the quality of training received;

* the barriers to the commercial realisation of scientific and technological ideas.

2a. With regard to the provision of sufficient trained personnel

* A submission "Industry-Education for Science and Technology" showing the shortfall of trained personnel in certain areas of science and technology was presented to Senator Button, Senator Ryan and Barry Jones and then widely circulated. Discussions on the issue were also held with Opposition Members.

* The provision of sufficient trained personnel will be a major issue in the NSTAG Forum on 5,6 November.

* Collection of appropriate statistics on graduation rates and labour demand where possible (eg, Physics) has been carried out and discussions were held with the Department of Employment and Industrial Relations to have this done on a more comprehensive basis.

* Input provided to the ASTEC Working Party on Capacity of Australian Education System.

* Input to the Victorian Inquiry on S & T Education was provided. This was requested by the Inquiry and increasingly FASTS is being consulted by Government bodies.

* An Analysis of the impact of overseas students on higher education research was carried out by

  . collection of statistics on overseas student enrolment and graduation at higher degree level;

  . collaboration with the Australian Vice-Chancellors Committee on assessing the impact of the Overseas Student Charge.

* Meetings involving representatives from industry, mathematics and science teachers associations and the Australian Council of State Schools Organisation have been held in Sydney and Melbourne to examine the important issue of expanding opportunities in S & T employment. More meetings will be held in other cities.

2b. With regard to the quality of training received

* A submission "Economic Development and Tertiary Education" was submitted to Commonwealth Tertiary Education Commission drawing attention to the run-down of equipment in tertiary education institutions which was hampering education and training. This was distributed to CTEC and to other Government bodies.

* Funding was obtained from the Committee to Review Australian Studies in Tertiary Education for projects aimed at improving teacher training in:

  . Chemistry (with Royal Australian Chemical Institute);

  . Mathematics (with Australian Association of Mathematics Teachers).

2c. With regard to the barriers to the commercial realisation of scientific and technological ideas

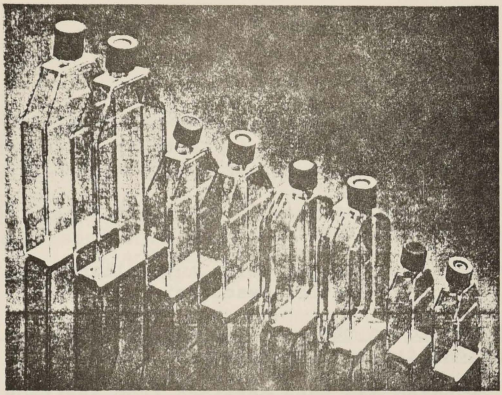
* Funding has been obtained from Austrade to conduct a study of the barriers to the commercial realisation of research ideas. A consultant has been employed to carry out the survey.

3. To promote understanding among the Australian Public of the work done within the scientific and technological community of the nation

* FASTS initiatives in this area are now getting started. FASTS hopes to provide an interface with the media for Societies to promote research in their areas. The Executive Director is working on a kit for politicians that would help them with, for example school speech day talks on advances in science and technology in Australia.

If you would like to become more involved in FASTS, nominate for representative to FASTS Council on 9 November. At this meeting FASTS Board members, the Executive and office bearers will be elected.

CORNING® DISPOSABLE TISSUE CULTURE FLASKS



The Corning® Disposable Tissue Culture Flasks are designed as culture vessels for the in vitro growth and study of cells and tissues. Flasks with orange plug seal caps are recommended for closed culture systems requiring gas tight seals; the black phenolic style caps are best for open culture systems requiring gas exchange with the environment.

MATERIALS

The Corning flasks are manufactured from virgin medical grade polystyrene carefully selected and tested for cell culture compatibility. All of the flasks are treated by a special process to modify the surface for better cell attachment and growth.

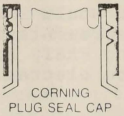
The linerless orange plug seal caps are high-density polyethylene. The black caps are manufactured from phenolic or polypropylene resins and contain a nontoxic liner.

PERFORMANCE

Corning Disposable Tissue Culture Flasks are tested for cell attachment and growth, sterility, toxicity and overall performance. In addition, every flask is individually leak tested to insure a quality product.

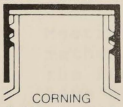
All three sizes (25cm², 75cm², and 150cm²) are now available with a choice of orange plug seal caps or black phenolic style caps with liner. The orange caps are designed to be used in closed culture systems requiring both a gas and liquid tight seal. The flexible plug allows the cap to mold itself to the flask on the inside of the neck as well as against the rim for double the protection from leaks.

SEALS INSIDE NECK AND RIM



CORNING
PLUG SEAL CAP

SEALS RIM AGAINST LINER



CORNING
PHENOLIC STYLE
CAP

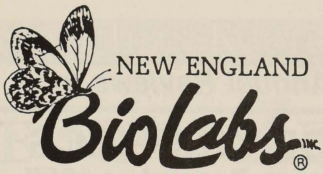
The black phenolic style caps are best for open culture systems requiring gas exchange with the environment. These use a traditional nontoxic liner for sealing the cap against the rim of the flask neck ensuring leak free use. With the black cap slightly loosened, gas exchange is permitted without the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

The 75cm² flasks are available in both the Corning canted neck design (Catalog No.'s 25110-75 and 25111-75) as well as the traditional straight neck design (Catalog No.'s 25115-75 and 25116-75). Although both designs have large openings, the canted neck offers better access to the growth surface and easier pouring.

CROWN CORNING

Science Products Division

Moorebank Avenue, Moorebank N.S.W.
Private Bag No. 4, Moorebank 2170, Australia
Telephone (02) 602 9800
Telex AA 21539



PRODUCTS FOR MOLECULAR BIOLOGY

New England Biolabs have been producing high quality products for researchers in molecular biology since 1975. Their continuing research on the discovery, characterization, purification and cloning of enzymes makes their products unsurpassed in range and purity, yet competitively priced. NEB scientists themselves are actively engaged in pure and applied research using their own products, ensuring that the highest quality is maintained.

The New England Biolabs product range now includes:

* *Over 100 different restriction endonucleases*
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* *DNA molecular weight standards*
* *pBR322 cloning vectors*
* *Cloning and sequencing kits and reagents*
* *Reagents for mRNA research*
* *DNA synthesis kits and reagents*

The NEB 1986/87 catalogue contains a full list of NEB products plus extensive technical and reference data of interest to all molecular biologists. For your free catalogue and Australian price list, write to:

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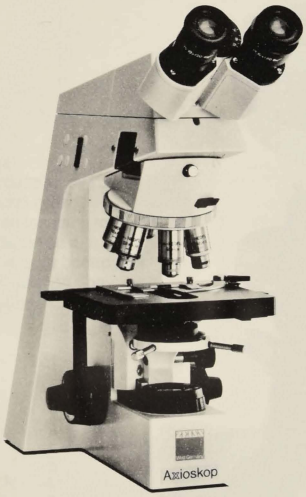
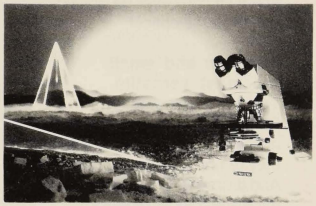
Genesearch is a biotechnology research and development company established in 1980 and wholly owned by scientists. Thus we understand the needs of researchers and are happy to assist our customers in any way we can.

THE MORE-THAN-ROUTINE MICROSCOPE: AXIOSKOP

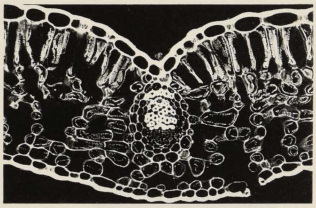
THE NEW ZEISS AGE IN MICROSCOPY CONTINUES.



Zeiss *Axioskop* with innovations to make microscopy more efficient: ICS-optics for high-contrast, bright, color-true images. SI-architecture for multi-purpose flexibility. Ergonomical design for fatigue-free hours of work.



In 1986 a new Zeiss age in microscopy dawned with the Pyramids of Zeiss – the *Axioplan*, *Axiophot*, and *Axiotron* microscopes. Now Carl Zeiss expands the series: The more-than-routine microscope: *Axioskop*.



Demanding routine becomes easy: examinations of stained or unstained cells and tissue, fluorescence-stained specimens, specimens with primary fluorescence.

Zeiss *Axioskop*
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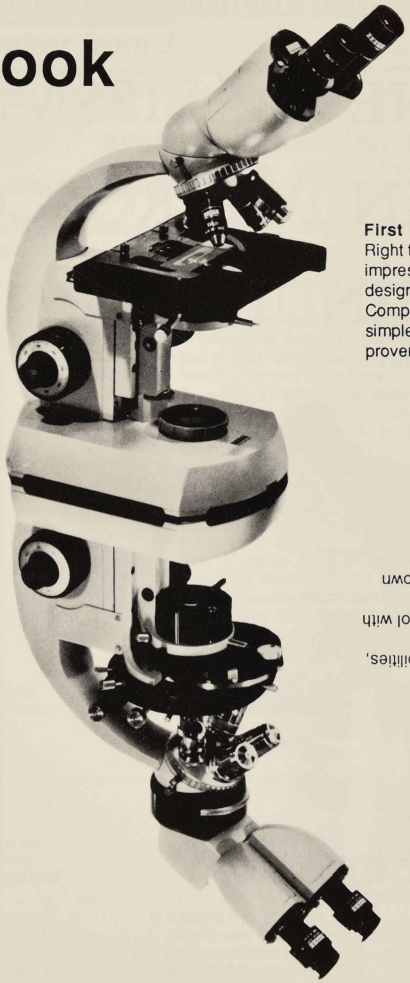

First look.
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