CHRIS MORAN

31st Annual Conference of the Genetics Society of Australia, Canberra 1984

M.J.D. WHITE

Born August 20, 1910, London, England

Early education in Italy (1914-1920) and France (1920-1927)
Entered University College, London, September 1927

Degrees
B.Sc in Zoology and Human Physiology (First Class Honours) [University of London] 1931
M.Sc [University of London] 1932
D.Sc [University of London] 1940
M.Sc [University of Melbourne] 1959
Dottore in Scienze Biologiche *honoris causa* [University of Siena] 1979

Positions held

19[illegible]1935	Assistant Lecturer in Zoology, University College, London
19[illegible]-1946	Lecturer in Zoology, University College, London
1937-1938	Rockefeller Research Fellow [Columbia University]
1940-1945	Wartime positions as Statistician and Entomologist in British Ministry of Food
Jan-Mar 1947	Reader in Zoology, University of London
Mar-Sept 1947	Guest Investigator, Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N.Y.
Sept 1947-June 1953	Professor of Zoology, University of Texas
July 1953-Dec 1956	Senior Research Fellow, Commonwealth Scientific Industrial Research Organization, Canberra, Australia
Jan 1957-June 1958	Professor of Zoology, University of Missouri
July 1958-July 1964	Professor of Zoology, University of Melbourne
Aug 1964-1975	Professor of Genetics, University of Melbourne
Jan-Mar 1968	Visiting Agassiz Professor, Harvard University
1976-	Visiting Fellow, Dep. Australian National

Membership of Academies etc.

Fellow, Australian Academy of Science (elected 1955) (Member of Council 1960-1962)

Fellow, Royal Society of London (elected 1961)

Honorary Foreign Member, American Academy of Arts and Sciences (elected 1963)

Fellow of University College, London (elected 1962)

Foreign Member, American Philosophical Society (elected 1978)

Socio Straniero, Accademia Nazionale dei Lincei (elected 1978)

Fellow of the Linnean Society of London *honoris causa* (elected 1979)

Foreign Associate, U.S. National Academy of Sciences (elected 1981)

Medals

Mueller Medal, Australian & New Zealand Association for the Advancement of Science 1965

Silver Medal for Research, Royal Society of Victoria 1979

Linnean Medal for Zoology, Linnean Society of London 1983

Minerva Medal of the University of Rome 1983

Membership in Professional Organizations

Society for the Study of Evolution 1946 to present (Vice President 1961 and 1967)

Genetics Society of America - 1947 to present

American Society of Naturalists - 1948 to present

Society of Sigma Xi - 1948-1972

Australian and New Zealand Association for the Advancement of Science [President of Section D 1962]

Genetics Society of Australia [President 1970-1972] Hon. Member 1980

Australian Entomological Society [President 1969-1971]

Royal Society of Victoria - 1958-1972

Association d'Acridologie

[illegible]na - socio onorario 1979

[illegible]liana di Entomologia - socio onorario 1

[illegible]delle Scienze 1982

1932 The chromosomes of the domestic chi[illegible]

1933 Tetraploid spermatocytes in a locus *Cytologia 5*: 135-139.

1934 The influence of temperature on chi[illegible] *29*: 203-215.

1935 Eine neue Form von Tetraploidie nac[illegible] *Naturwissenschaften 23*: 390-391.

[illegible]ism in natural populations of *Am. Phil. Soc. 1949*: 183-185.

[illegible]translation of *The Chromosomes* by C. [illegible] 144 pp.

[illegible]nd genes. Chapter 5 in *Cytology and* [illegible]don: Oxford University Press.

[illegible]ic mechanisms in animals. Chapter 16 [illegible]eth Century*.* Macmillan.

The effects of X-rays on mitosis in the spermatogonial divisions of *Locusta migratoria* L. *Proc. R. Soc. Lond. B 119*: 61-84.

1936 Chiasma localization in *Mecostethus grossus* L. and *Metrioptera brachyptera* L. *Z. Zellforsch. 24*: 128-135.

The chromosome cycle of *Ascaris megalocephala*. *Nature (Lond.) 137*: 783.

1937 The effect of X-rays on the first meiotic division in three species of Orthoptera. *Proc. R. Soc. Lond. B 124*: 183-196.

The Chromosomes. London: Methuen, viii + 128 pp.

1938 A new and anomalous type of meiosis in a mantid, *Callimantis antillarum* Saussure. *Proc. R. Soc. Lond. B 125*: 516-523.

1940 The heteropycnosis of sex chromosomes and its interpretation in terms of spiral structure. *J. Genet. 40*: 67-82.

The origin and evolution of multiple sex chromosome mechanisms. *J. Genet. 40*: 303-336.

A translocation in a wild population of grasshoppers. *J. Hered. 31*: 137-140.

Evidence for polyploidy in the hermaphrodite groups of animals. *Nature (Lond.) 146*: 132.

1941 Chromosomal evolution and the mechanisms of meiosis in praying mantids. *Proc. VII Int. Genet. Congr.*, p.313.

Cytological polymorphism and racial differentiation in grasshopper populations. *Yearb. Am. Phil. Soc. 1950*: 158-1[illegible]

A cytological survey of wild populations of *Trimerotropis* a[illegible] *Circotettix* (Orthoptera, Acrididae). II. Racial differenti[illegible] in *T. sparsa*. *Genetics 36*: 31-53.

Cytogenetics of orthopteroid insects. *Adv. Genet. 4* 267-33[illegible]

Structural heterozygosity in natural populations of the grasshopper *Trimerotropis sparsa*. *Evolution 5*: 376-394.

White, M.J.D. and Nickerson, N.H. Structural heterozygosit[illegible] a very rare species of grasshopper. *Am. Nat. 85*: 239-246.

Supernumerary chromosomes in the trimerotropine grasshopper (abstr.). *Rec. Genet. Soc. Am. 20*: 130-131.

Citologia Animal y Evolucion (Spanish translation of *Animal Cytology and Evolution* by F.A. Saez). Madrid: Espasa-Calpe 512pp.

1952 Review of H.F. Barnes: *Gall Midges of Economic Importance*. vol. 5. *Q. Rev. Biol. 27*: 219-220.

1953 Multiple sex chromosome mechanisms in the grasshopper genus *Paratylotropidia*. *Am. Nat. 87*: 237-244.

Review of J.A.G. Rehn: *The Grasshoppers and Locusts (Acridoidea) of Australia, vol. 1, Families Tetrigidae and Eumastacidae*. *Q. Rev. Biol. 28*:184-185.

31st ANNUAL CONFERENCE of the

GENETICS SOCIETY OF AUSTRALIA

Australian National University

Canberra

Friday 11th May - Monday 14th MAY, 1984

PROGRAMME & ABSTRACTS

GENERAL INFORMATION

Registration

Conference participants can register on Friday evening at Bruce Hall or on Saturday morning at 8.30 - 9.00 am in the Main Physics Lecture Theatre.

Papers

Contributed papers will be presented at concurrent sessions in Physics Lecture Rooms 6 and 8.

Guest lectures will be presented in the Main Physics Lecture Theatre.

Posters

Posters will be on display and attended by contributors in the Bruce Hall Common Room from 5.30 - 6.30 pm on Saturday and 4.10 - 6.00 pm on Sunday.

Refreshments

Morning Tea and Coffee will be served in the Common Room at Bruce Hall from 10.20 to 10.50 am and Afternoon Tea at 4.00 - 4.20 pm on Saturday and at 4.10 pm on Sunday.

Accommodation and Meals

Accommodation has been arranged at Bruce Hall. Fees (less deposit) should be paid upon arrival. Rooms must be vacated by 10.00 am on Monday, 14th May.

Lunch will be available at Bruce Hall on Saturday and Sunday at a cost of $3.50 per day. If you have not already booked for lunch but wish to do so please notify the organisers at Registration on Saturday morning at the latest.

Social Arrangements

A mixer will be held in the Bruce Hall Common Room from 6.30 - 10.30 pm on Friday, 11th May.

The Society Dinner will be held in the Bruce Hall Dining Room on Sunday, 13th May at 7.30 for 8.00 pm.

The Annual Business Meeting will be held at 5.30 pm in the Bruce Hall Common Room on Sunday 13th May.

No special arrangements have been made for Saturday evening but there are a variety of restaurants in Civic - about 15 minutes walk from Bruce Hall.

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Peter A. Parsons

Department of Genetics, La Trobe University

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- Integrating genetic change, natural selection, and the interaction of the species with its environment and other living systems therein, the colonizing species is transformed into a sophisticated and complex source of data for understanding evolutionary biology
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PROGRAMME

FRIDAY 11th MAY

6.30 - 10.30 pm MIXER and REGISTRATION - Bruce Hall

SATURDAY 12th MAY

8.30 - 9.00 am REGISTRATION - Physics Main Lecture Theatre

9.00 - 10.20 **SESSION 1A** (Physics Lecture Room 6)
Chairman: Dr. J.A. McKenzie

9.00 - 9.20 J.S.F. Barker, P.D. East & B.S. Weir (University of New England).
'Temporal and microgeographic variation in allozyme frequencies in a natural population of *Drosophila buzzatii*'.

9.20 - 9.40 D. Anderson (Australian National University)
'Alcohol dehydrogenase activity variation in a natural population of *D. melanogaster*'.

9.40 - 10.00 L.H. Schmitt (University of Western Australian), S.W. McKechnie (Monash University) & J.A. McKenzie (University of Melbourne)
'Quantity of alcohol dehydrogenase and alcohol tolerance in *Drosophila melanogaster*'.

10.00 - 10.20 S.W. McKechnie (Monash University)
'Genetic and biochemical correlates of alcohol tolerance in *Drosophila melanogaster*'.

9.00 - 10.20 am **SESSION 1B** (Physics Lecture Room 8)
Chairman: Dr. J. Langridge

9.00 - 9.20 C.H. Doy, J.A. Pateman, J. Olsen & H. Kane (Australian National University)
'Genomic cloning and molecular analysis of the region in *Aspergillus nidulans* containing the alcohol dehydrogenase structural gene (alcA) and the trans-acting regulatory gene alcR'.

9.20 - 9.40 D.E.A. Catcheside (Flinders University)
'A two strand break model for the initiation of recombination at the cog sites in *Neurospora*'.

9.40 - 10.00 C.M. Corrick & M.J. Hynes (University of Melbourne)
'The amdS gene of *Aspergillus nidulans*: the structure and DNA sequence of the wild type and its mutants'.

10.00 - 10.20 J.M. Kelly & M.J. Hynes (University of Melbourne)
'*Aspergillus nidulans* transformants'.

10.20 - 10.50 MORNING TEA & COFFEE - Bruce Hall

10.50 - 12.50 pm **SESSION 2A** (Physics Lecture Room 6)
Chairman: Dr D.L.Hayman

10.50 - 11.10 am D.J. Coates & D. Smith (Australian National University)
'The spatial distribution of chromosomes in the grasshopper *Caledia captiva*'.

11.10 - 11.30 D. Colgan (Australian National University)
Concerted developmental changes in the glucose metabolising enzymes of *Caledia Captiva*'.

11.30 - 11.50 D.D. Shaw, D.J. Coates, P. Wilkinson & M.L. Arnold (Australian National University)
'Temporal changes in the structure of a hybrid zone'.

11.50 - 12.10 pm M.L. Arnold, R. Appels & D.D. Shaw (CSIRO Division of Plant Industry & Australian National University)
'Sequence variation in a highly repeated DNA family from *Caledia captiva*'.

12.10 - 12.30 J.A. Marshall Graves (LaTrobe University)
'Gene amplification in an interspecific hybrid mouse embryo'.

12.30 - 12.50 I.S. Dundas, E.J. Britten & D.E. Byth (University of Queensland)
'A method of pachytene chromosome karyotyping for use in cytogenetic studies involving pigeon pea (*Cajanus cajan*) and *Atylosia* species

10.50 - 12.50 pm **SESSION 2B** (Physics Lecture Room 8)
Chairman: Professor M.J. Hynes

10.50 - 11.10 am J. Langridge (CSIOR Division of Plant Industry)
'Vectors for Plant Transformation'.

11.10 - 11.30 E.S. Dennis, W.L. Gerlach, M.M. Sachs & W.J. Peacock (CSIRO Division of Plant Industry)
'Expression of the alcohol dehydrogenase genes of Maize'.

11.30 - 11.50 W.R. Scowcroft & R.S. Brettel (CSIRO Division of Plant Industry)
'Inherited genetic instability in Tobacco somaclones'.

11.50 - 12.10 pm P. Schofield, M. Djordjevic, B. Rolfe & J. Watson (Australian National University)
'Genetic and molecular analysis of symbiotic nitrogen fixation in *Rhizobium trifolii*'.

12.10 - 12.30 M.A. Djordjevic, P. Schofield, J. Watson & B. Rolfe (Australian National University)
'Identification of host specificity determinants in *Rhizobium trifolii*'.

12.30 - 12.50 N. Morrison, B. Bassam & B. Rolfe (Australian National University)
'Identification of early nodulation genes in a broad host range *Rhizobium* species'.

Hogness

Developmental mutants

- enormous size.

~ 27 - 100 kb

- extension rates for messenger
  1kb per minute

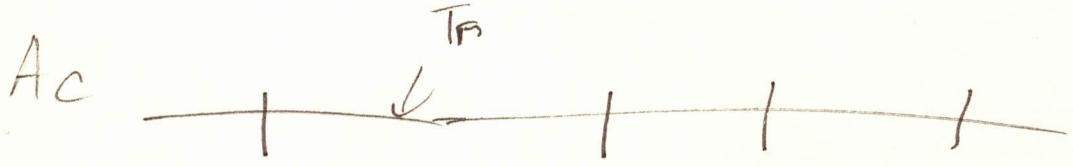
∴ possibility of developmental clock
inherent in size of developmental gene

Metallothioneins - 61 aa.

- 20 Cysteines

Metal induction
Glucocorticoid induction.

Specific DNA sequences
for metal + glucocorticoid induction
- identified by deletion experiments.



12.50 - 2.00 pm LUNCH BREAK - Bruce Hall

2.00 - 5.20 pm **GUEST LECTURES** in the Main Physics Lecture Theatre
Chairman: Professor J.A. Pateman

2.00 - 3.10 Professor D.S. Hogness (Department of Biochemistry, Stanford)
'Molecular analysis of genes controlling development in *Drosophila*.

3.10 - 3.35 W. Gerlach, E.S. Dennis & W.J. Peacock (CSIRO Division of Plant Industry)
'Molecular genetics of the Ac-Ds controlling element system of Maize'.

3.35 - 4.00 R. Richards, (Australian National University)
'Human metallothioneins: gene structure and regulation by steroid hormones and heavy metals'.

4.00 - 4.20 AFTERNOON TEA & COFFEE - Bruce Hall

4.20 - 5.30 Professor R. Williamson, (St. Mary's Hospital Medical School, London)
- Title to be announced - Progress towards a total human genome.

5.30 - 6.30 POSTER SESSION in Bruce Hall

SUNDAY 13th MAY

9.00 - 10.20 am **SESSION 3A** (Physics Lecture Room 6)
Chairman: Dr. D.D. Shaw

9.00 - 9.20 J.R. Ovenden, R.H. Crozier & A.G. MacKinley (University of New South Wales)
'Mitochondrial DNA and protein evolution within *Platycercus* (Aves)'.

9.20 - 9.40 L. Christidis (Australian National University)
'Evolutionary genetics of the family Estrildidae (Aves)'.

9.40 - 10.00 M.H. Thorne, R.K. Collins & B.L. Sheldon (CSIRO Genetics Research Laboratories)
'A study of the inheritance of triploidy in the Chicken'.

10.00 - 10.20 M.H. Thorne, R.K. Collins & B.L. Sheldon (CSIRO Genetic Research Laboratories)
'A report of live haploid-diploid and diploid-triploid chickens'.

9.00 - 10.20 am **SESSION 3B** (Physics Lecture Room 8)
Chairman: Dr. R. Richards

9.00 - 9.20 R.B. Saint, A.F. Cowman, D.J. Kemp, R.L. Coppel, K.R. Lingelbach, G.V. Brown & R.F. Anders (Walter & Eliza Hall Institute)
'A variable gene system in *Plasmodium falciparum*'.

Williamson –
First human gene cloned in 1976

Now 300 – mapped etc

Diagnostic probes.

Disease	Probe
α thal	α globin
β thal	β globin
emphysema	α-1 antitrypsin.
dwarfism	human growth hormone.
Lesch-Nyhan	HPRT
PKU	phenylalanine hydroxylase.
osteogenesis imperfecta	collagen
analbuminaemia.	albumin
Downs	chrom 21 specific probe.
Menkes disease	X chrom probe
HLA linked conditions	HLA genes
hyperlipidaemia	apolipoprotein genes
myasthenia gravis	acetylcholine receptor.
thyroid medullary carcinoma	calcitonin.
Haemophilia	Factor VIII C
Christmas disease	Factor IX

Restriction site mutations

Melting changes due to base substitutions

Duchenne muscular dystrophy. – X linked – Becker dystrophy allelic.

- used chromosome sorter to produce chromosome specific libraries
- 50,000 clones from X
- checked for X-chromosome specificity.
- location determined by 1) deleted X's in mouse-human hybrids.
  2) in situ hybridisation.

- probes RC8 & L128 bracket Duchenne musc dyst.
- allows detection of recomb, required for prediction.

9.20 - 9.40 M.J. Healy, R.J. Russell & G.L.G. Miklos (Australian National University)
'Molecular and genetic characterisation of the uncoordinated (unc) locus in D.melanogaster'.

9.40 - 10.00 A.R. Lohe (CSIRO Division of Entomology)
Multiplicity of satellite DNA sequences in D.melanogaster'.

10.00 - 10.20 L. vonKalm & D.R. Smyth (Monash University)
'Methylation patterns in ribosomal RNA genes of Lilium'.

10.20 - 10.50 MORNING TEA & COFFEE - Bruce Hall

10.50 - 12.50 pm **SESSION 4A** (Physics Lecture Room 6)
Chairman: Dr. D.W. Cooper

10.50 - 11.10 am K.M. Oakley (LaTrobe University)
'Mitochondrial genome of *Macropus giganteus*'.

11.10 - 11.30 P.G. Johnston & E.S. Robinson (Macquarie University)
'X-chromosome inactivation during marsupial embryogenesis'.

11.30 - 11.50 R. Davey & D.W. Cooper (Macquarie University)
'Active and inactive chromatin in Marsupials'.

11.50 - 12.10 pm A. vanDaal (Macquarie University)
'Use of a marsupial genomic library in the study of X inactivation'.

12.10 - 12.30 G.C. Webb (Royal Children's Hospital, Melbourne)
'Probable homogeneously staining regions in the chromosomes of normal individuals of *Homo sapiens* and *Chortoicetes terminifera*'.

12.30 - 12.50 R.M. Hope, S.J. Goss, P.N. Goodfellow & E. Solomon (Universities of Adelaide and Oxford)
'MIC-5 maps between HPRT and G6PD on the long arm of the human X chromosome'.

10.50 - 12.50 pm **SESSION 4B** (Physics Lecture Room 8)
Chairman: Professor J.S.F. Barker

10.50 - 11.10 am I.A. Boussy (Australian National University)
'Latitudinal variation of P-M hybrid dysgenesis in Australian *Drosophila melanogaster*'.

11.10 - 11.30 M. Yamanaha & L.E. Kelly (University of Melbourne)
'Electrophoretic variation in cyclic AMP phospho diesterase in *Drosophila*'.

11.30 - 11.50 S. Easteal (Australian National University)
'Allele frequency distribution at the Mpi locus in a native population of the Gian Toad, *Bufo marinus*'.

11.50 - 12.10 pm G.R. Singleton & T.D. Redhead (CSIRO Division of Wildlife and Rangelands Research)
'An ecological genetic study of house mice inhabiting a rice field'.

Max Whitten

Vetrazin - 1979 till present
- no resistance.

6-12 weeks to decay to discriminating dose.

Sublethal Level insecticide
- soft selection - no increase in mortality
- rather than random death due to competition, ++ genotypes are more stressed than resistant genotypes.
- hard selection models now considered inappropriate.

12.10 - 12.30	K.M. Nielsen (Monash University) 'The Tpi polymorphism of *D. melanogaster*: the meaning of environmental correlates?'
12.30 - 12.50	D. Ayre (Australian Institute of Marine Science) 'Phenotypic plasticity of genetic variation in Corals?'
12.50 - 2.00	LUNCH BREAK - Bruce Hall
	Chairman: Professor J.A. Thomson
2.00 - 3.00	Main Physics Lecture Theatre - Presidential address by Dr. Max Whitten, CSIRO Division of Entomology 'Ecological genetics of pesticide resistance using the Sheep Blowfly as a model system'.
3.10 - 4.10	**SESSION 5A** (Physics Lecture Room 6) Chairman: Professor W. Hayes
3.10 - 3.30	V. Krishnapillai & A. Puhler (Monash University) 'Cloning the promiscuous plasmid DNA primase gene in Tn5'.
3.30 - 3.50	V. Krishnapillai & J.H.E. Nash (Monash University) 'Role of the promiscuous primase gene in wide host range behaviour'.
3.50 - 4.10	D. Rouch, J. Camakaris & B. Lee (University of Melbourne) 'Copper resistance in *E. coli*'.
3.10 - 4.10	**SESSION 5B** (Physics Lecture Room 8) Chairman: Dr. G.C. Webb
3.10 - 3.30	D.W. Cooper (Macquarie University) 'Genetic control of pre-eclampsia'.
3.30 - 3.50	E.M. Deane & D.W. Cooper (Macquarie University) 'Immunogenetic markers in pre-eclampsia'.
3.50 - 4.10	T.J. Lockett & M. Sleigh (CSIRO Division of Molecular Biology) 'Developmental regulation of gene activity in mouse embryonal carcinoma cells'.
4.10 - 6.00	AFTERNOON TEA & COFFEE and POSTER SESSION - Bruce Hall
5.30	BUSINESS MEETING - ~~Bruce Hall Common Room~~ Main. L.T.
7.30 for 8.00	ANNUAL SOCIETY DINNER - Bruce Hall

MONDAY 14th MAY

9.00 - 10.20 am	**SESSION 6A** (Physics Lecture Room 6) Chairman: Professor B. John

9.00 - 9.20 J. Martin & B.T.O. Lee (University of Melbourne)
'Sex determination of some Australian Chironomus species'.

9.20 - 9.40 D. Bedo & G. Foster (CSIOR Division of Entomology)
'Cytogenetic mapping of the male determiner in *Lucilia cuprina*'.

9.40 - 10.00 M. King, G. Pasteur, C. Blanc & C. Moritz (Australian National University)
'Parthenogenesis in the Gekko *Lepidodactylus lugubris*'.

10.00 - 10.20 C. Moritz (Australian National University)
'A highly variable sex chromosome in *Gehyra purpurascens*'.

9.00 - 10.20 **SESSION 6B** (Physics Lecture Room 8)
Chairman: Dr. R. Brock

9.00 - 9.20 R.H. Crozier (University of New South Wales)
'On being the right size: Male contributions and multiple mating in social Hymenoptera'.

9.20 - 9.40 B. Oldroyd & C. Moran (University of Sydney)
'Analysis of continuous variation of humuli number in *Apis mellifera* by means of a diallel'.

9.40 - 10.00 F. Cockrem (Ruakura Research Centre)
'Physiological approach to animal genetics illustrated by research on the inheritance of susceptibility to bloat in cattle'

10.00 - 10.20 G.M. Clarke (University of Melbourne)
'Quantitative analysis of sheep and non-sheep populations of *Lucilia cuprina*'.

10.20 - 11.00 MORNING TEA & COFFEE - Bruce Hall

POSTERS

G.C. Webb, A. Maslin & J.M. Mercer (Royal Children's Hospital)
Localisation of the IgH locus to the distal region of chromosome 12 in the mouse using *in situ* hybridisation.

R. Koebner (Waite Agricultural Research Institute)
Use of biochemical marker genes to screen for rare recombinants between wheat and alien chromatin.

J. Tomasov (LaTrobe University)
Genetics of colour polymorphism in the St John's Wort beetle *Chrysolina quadrigemina*.

C. Shanahan (University of Adelaide)
Cytogenetics of Australian Scorpions.

D. Rowell (Australian National University)
Chromosomal variation in Huntsman spiders.

P. Zelesco & J.A.M. Graves (LaTrobe University)
Mitosis in mammalian cell hybrids - why are chromosomes segregated?

J.M. Wrigley & J.A.M. Graves (LaTrobe University)
Growth and DNA synthesis characteristics in Platypus cell lines.

N. Farrar (LaTrobe University)
The mitochondrial genome of the common Brushtail Possum *Trichosurus vulpecula*.

L. McKay (LaTrobe University)
Development of the thoracic sclerites of *Drosophila melanogaster*.

B. Evans & R. Richards (Australian National University)
The mouse glandular kallikrein gene family.

J. Plazinski, M.A. Djordjevic & B.G. Rolfe (Australian National University)
Transfer, characterization and expression of the *Rhizobium trifolii* infection genes in *Rhizobium* and non-*Rhizobium* backgrounds.

J. Watson, P. Schofield, C. Hayes & J. Shine (Australian National University)
Characterization of Sym plasmid-specific repeated DNA sequences in *Rhizobium trifolii*.

B. van Leeuwen, B. Evans & R. Richards (Australian National University)
Expression of glandular kallikrein in mouse kidney.

R.N. Oram (CSIRO Plant Industry)
The domestication of *Phalaris brachystachys* as a cereal crop.

J. Weinman, F. Fellows, P. Gresshoff, J. Shine & K. Scott (Australian National University)
Structure and organisation of nitrogenase genes in the *Parasponia Rhizobium* strain ANU289.

J. Michailidis, N. Murray & J.A. Marshall Graves (LaTrobe University)
The effects of development time and demethylating agents on variegated position effects in *Drosophila melanogaster*.

J.L. Harry (Macquarie University)
Multiple insemination in the Loggerhead turtle (*Caretta caretta*).

TEMPORAL AND MICROGEOGRAPHIC VARIATION OF ALLOZYME FREQUENCIES IN A NATURAL POPULATION OF *DROSOPHILA BUZZATII*

J.S.F. Barker[1], P.D. East[1] and B.S. Weir[2]

[1]Department of Animal Science, University of New England, Armidale N.S.W.

[2]Department of Statistics, North Carolina State University, Raleigh, North Carolina

Preliminary results for some aspects of this study were reported to the 1982 meeting of the Society, but complete analyses now have been done using new multiple-locus theory developed by Weir, Cockerham and colleagues.

Within one prickly-pear infestation, 10 sites were defined for collection, five in each of two approximately linear transects, which were 100-120 m apart, and differed in microenvironments. Within transects, sites were 60-70 m apart. Collections of *D. buzzatii* were made at each site, monthly over four years, and a total of 15,313 flies were assayed for up to six polymorphic enzyme loci.

Allele frequencies showed no obvious seasonal pattern, but every allele showed significant variation over time. In multivariate analyses, allele frequencies for *Est-1*, *Hex* and *Pgm* showed significant associations with variables relating to population size.

For the four highly polymorphic loci, the proportion of site x month samples showing significant deviations from Hardy-Weinberg equilibrium ranged from 0.115 to 0.267. For *Est-1*, *Est-2* and *Hex*, these significant deviations were largely due to observed homozygote excess, but for *Adh-1* all were due to observed heterozygote excess. Substantial two-locus disequilibria were found among *Est-2*, *Aldox* and *Hex*.

Estimates of the correlation between genes within individuals within each site x month sample (f) were used to analyse population structure in time and space. No spatial structure was detected, but there were significant differences between months within year 2 for all loci except *Adh-1*. Year 2 had the highest mean f and the smallest mean estimated population size of all years.

There was significant inbreeding in the population as a whole, but mean inbreeding differed among loci, from -0.082 for *Adh-1* to 0.169 for *Aldox*. Mates within sites are more related than random pairs of individuals, but the differences among loci imply differential effects of selection.

Variation in Alcohol Dehydrogenase Activity *In Vitro* in Flies from Natural Populations of *Drosophila melanogaster*

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Abstract

Alochol dehydrogenase (ADH) activity variation in single male fly assays from seven natural populations of *Drosophila melanogaster* is largely accounted for by segregation of alleles at the *Adh* structural gene locus. There was little overlap in the ADH activities of Adh^F and Adh^S homozygotes. Body weights varied only slightly between *Adh* genotypes and contributed little to ADH variation. Between and within population variation in ADH activity and ADH protein is mainly due to the relative frequencies of Adh^F and Adh^S.

THE QUANTITY OF ALCOHOL DEHYDROGENASE AND ALCOHOL TOLERANCE IN DROSOPHILA MELANOGASTER

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3. Department of Genetics, University of Melbourne.

Variation in Alcohol dehydrogenase and alcohol tolerance was investigated in 156 Adh^F iso-female lines of Drosophila melanogaster from the Tahbilk winery. Alcohol dehydrogenase variability was estimated from both the quantity of ADH protein (as measured by radial immunodiffusion) and ADH enzymic activity on two different substrates. Alcohol tolerance was estimated from the survival of larvae on ethanol impregnated media. There was a statistically significant negative association between larval alcohol tolerance and the quantity of ADH measured in these lines. Alcohol dehydrogenase quantity accounted for 4% of the variation in alcohol tolerance. There was no association between ADH activity and larval tolerance to ethanol. These results were similar in two distinct collections taken during successive vintages. In addition, the quantity of ADH present in 28 lines was negatively correlated with adult survival in a sucrose/ethanol ingestion test. No correlation was observed however when adults were acutely exposed to ethanol vapour.

GENETIC AND BIOCHEMICAL CORRELATES OF ALCOHOL TOLERANCE IN Drosophila melanogaster

Stephen W.McKechnie , Monash University.

To better understand the genetic basis of naturally occurring variation in ethanol tolerance, a set of 42 isofemale strains (isolated from a winery population) were tolerance tested and characterised for activity levels of three enzymes involved in ethanol metabolism, ADH , glycerol-3-phosphate dehydrogenase (GPDH) and the mitochondrial enzyme glycerol-3-phosphate oxidase (GPO). By inbreeding,the strains were made homozygous at the polymorphic Adh and Gpdh loci as follows: 11 strains Adh-FF/Gpdh-FF, 11 strains Adh-FF/Gpdh-SS, 10 strains Adh-SS/Gpdh-FF, and 10 strains Adh-SS/Gpdh-SS.

Larval tolerance was positively correlated (over the 42 strains) with both the development time and weight loss of the adults raised on ethanolic medium. A two-way factorial ANOVA on larval tolerance indicated no main effects of Adh or Gpdh genotype. However there was a significant interaction, suggesting epistasis. Thus, Adh-FF/Gpdh-FF and Adh-SS/Gpdh-SS were more tolerant than Adh-FF/Gpdh-SS and Adh-SS/Gpdh-FF.

A significant positive correlation (over all strains) occurred between larval tolerance and specific activity of GPO. No such association occurred for ADH of GPDH activities.

Genomic cloning and molecular analysis of the region in *Aspergillus nidulans* containing the alcohol dehydrogenase structural gene (*alcA*) and the trans-acting regulatory gene *alcR*.

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Problems associated with cloning DNA containing *alcA* and *alcR* (a trans-acting regulatory gene for at least *alcA* and *aldA*, the structural gene for aldehyde dehydrogenase) will be mentioned; for example it seemed necessary to use the EMBL3 vector rather than Charon30. A 3.85 kb BamHI fragment characteristic of wild-type and certain mutants, but not others, was cloned into pBR322 and used as a probe for the *alcA alcR* region. About 24 kb DNA has been cloned with *alcA* at the centre adjacent to *alcR*. The genes were located by Southern blot analysis of wild-type and mutant DNAs probed with various clones. Two *alcA* mutants (behaving as deletions in genetic tests) proved to contain insertions and of 4 *alcR* mutants tested, one contained an insert. These mutations could therefore be located within the restriction enzyme maps of the clones. All 5 *alcA alcR* mutants tested were deletions lacking all the DNA cloned (without affecting growth on minimal medium). Carbon catabolite repression is the over-riding control of transcription of the *alcA* region. The 3' end of *alcA* (and possibly *alcR*) is probably within a 1.9 kb SalI fragment contained within the original probe which contains *alcR* sequences at the opposite end and outside the 1.9 kb SalI fragment. The DNA involved in *alcA* probably contains introns; the mature m-RNA is about 1 kb (sufficient for coding ADH) yet a probe derived from m-RNA labels several kb. Yeast and maize ADH clones show weak and different patterns of homology to the *Aspergillus* gene. Another of our clones, which by inference could be *aldA* (which is unlinked to *alcA*) hybridises weakly to two regions 5' to *alcA* one of which may overlap the *alcA* region. We have commenced sequencing the DNA encompassing *alcA*, *alcR* and the flanking regions of obvious interest.

A TWO STRAND BREAK MODEL FOR THE INITIATION OF RECOMBINATION AT *COG* SITES IN *NEUROSPORA CRASSA*.

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A model for the initiation and control of recombination in Neurospora has been deduced from the properties of natural variants with altered local recombination frequencies (1). In this 'Repressor' model, it is envisaged that there are some 500 recognition sites scattered throughout the genome at which recombination can be initiated by a single strand nick. It is proposed that initiation is subject to control by repressors coded by about nine different recombination genes that each block the initiation of recombination from a subset of the *cog* sites by binding to a control site of the appropriate specificity situated adjacent to each of the affected *cog* sites. The processing of a nicked duplex and its interaction with an homologous duplex to yield recombinants has been described (2,3).

In its simplest form the model predicts that the nicked duplex is the donor of genetic information. However,the duplex that carries a recombinator is more often the recipient of information in conversion events. This difficulty can be overcome by modifying the model in various ways. The 'Restriction 'model proposed here offers a different explanation. The principal features of the model are that:i) *cog* sites are subject to epigenetic modification by *rec* gene products and that only unmodified sites are recombinators,ii) the endonuclease initiating recombination binds to the recombinator and translocates the DNA past the enzyme for a variable distance prior to making a double strand cut in the same duplex,iii) erosion of the ends to form a gap that is repaired using information from the homologous duplex.

The properties of crosses heterozygous for the translocation TM429 (2), indicate that although recombination is initiated at *cog*, the event leading to strand exchange occurs at a distance. D.G. Catcheside and Angel explained this by proposing the migration of a single strand nick rather than translocation of DNA as is proposed here. Enzyme with properties appropriate for the initiation of recombination in this manner are known in *E. coli* type I restriction and modification systems. Repair of double strand gaps has recently been implicated in recombination events in Yeast and a model for recombination involving gap repair that leads to the formation and resolution of two Holliday junctions has been proposed (4).

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The *amd*S gene of *Aspergillus nidulans* : the structure and DNA sequence of the wild type and some mutants

C.M. CORRICK and M.J. HYNES (University of Melbourne)

The acetamidase of *Aspergillus nidulans* enables the utilization of acetamide as a sole nitrogen or carbon source. The *amd*S gene is subject to complex multiple control mechanisms which are independent of one another and are not confined to the regulation of *amd*S alone. These regulatory gene products operate at a control region adjacent to the structural gene. The extent of this control region has been described by genetic and physical mapping of various strains containing control region mutations.

Transcriptional mapping has shown the *amd*S gene to have two small introns. Northern blot analysis shows an mRNA size of 1.6-1.7 kb.

To further understand the regulation of *amd*S the DNA sequence of the wild type gene has been determined including both the control and the structural regions. As yet only genomic clones have been sequenced, thus intron locations are tentative. The control regions of three *cis*-acting mutants are also being sequenced. The nature of these mutations will be described.

Aspergillus nidulans transformants.

J M Kelly and M J Hynes, University of Melbourne.

We have used a modification of the yeast DNA transformation system to transform the filamentous fungus, Aspergillus nidulans, and we are using this system to study the regulation of gene expression.

Using the cloned acetamidase structural gene, amdS, (see Corrick and Hynes, these abstracts), inserted into pBR322, we have obtained transformants that grow strongly on acetamide media. Analysis has shown that the transformants represent chromosomal integration events. We have analysed these for copy number, integration sites, and expression, and are presently looking at the regulation of the inserted copies.

Other proposed uses of transformation to study the regulation of acetamidase expression will be discussed.

THE SPATIAL DISTRIBUTION OF CHROMOSOMES IN METAPHASE NEUROBLAST CELLS FROM SUBSPECIFIC F_1 HYBRIDS OF THE GRASSHOPPER *CALEDA CAPTIVA*.

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ABSTRACT

The spatial distribution of chromosomes was analysed in radial metaphase neuroblast cells in F_1 hybrid embryos generated by crossing the Moreton (acrocentric X genome, MAX, and metacentric X genome, MMX) and Torresian (TT) chromosomal taxa of the grasshopper *Caledia captiva*. No significant associations were detected between any pair of homologous chromosomes in either male or female (MAX x TT) and (MMX x TT) F_1 hybrids. This result was supported by data which showed that the mean separation between homologues is greater, although not significantly so, than the mean separation between non-homologous chromosomes within the two Moreton genomes. Further, in a number of cases, genome separation was clearly observed in radial metaphase preparations from these F_1 hybrids. In comparison the analysis of pairwise associations between non-homologous chromosomes within the MMX and MAX Moreton genomes revealed a number of significant associations and dissociations which strongly suggest that chromosomes in these genomes are organised non-randomly at metaphase. Of particular interest was the highly significant X-5 association in the MMX genome since in a previous study X-5 rearrangements were found to occur repeatedly among different backcross progeny involving Moreton x Torresian F_1 hybrids. Additionally a comparison of the organisation of chromosomes in the MAX and MMX genomes, which differ primarily by the type of X chromosome, revealed that in a number of cases pairs of chromosomes are arranged very differently with respect to each other. The distribution of chromosomes on the hollow spindle was also analysed to investigate whether a specific spatial ordering of chromosomes exists within these Moreton genomes based on the association of pairs of short arms and pairs of long arms of most similar length (the Bennett model). In both genomes predictions based on the Bennett model place the twelve chromosomes in two sub-sets, one of nine chromosomes, the other of three chromosomes. An analysis of 48 cells in the MMX and 38 cells in the MAX genomes showed that the predicted order in the nine chromosome sub-set in each genome did not rank in the top 20% of the 20,160 possible orders. This suggests that although there is a good evidence that non-homologous chromosomes may be associated non-randomly at metaphase in these genomes, they do not appear to show a specific ordered arrangement as predicted by the Bennett model.

CONCERTED DEVELOPMENTAL CHANGES IN THE GLUCOSE METABOLIZING ENZYMES OF CALEDIA CAPTIVA

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I report the first example of a concerted developmental change in the isoenzymes controlling a biochemical pathway: more than half of the cytosolic glucose metabolizing enzymes of *Caledia captiva* differ in electrophoretic pattern between the embryonic and adult stages. The changes in pattern occur near the time of hatching from the egg. Most of the variable enzymes also show tissue specificity in the adult. In contrast, none of the four studied enzymes of the citric acid cycle change during development and only one has tissue specificity.

A few of the changes in the glucose metabolizing enzymes may be due to post-transcriptional modification, but the results of *in vitro* mixing experiments, breeding experiments and surveys of the developmental variation among the taxa of the species complex demonstrate that most changes are due to alteration in the regulation of individual members of gene families. There are a number of systems for which a given locus is active only in the embryos. This is surprising since, although there have been no systematic investigations in insects, such specificity is rare among enzymes in mammals.

The concerted isoenzymic changes during development in *Caledia* show that the hemimetabolous habit is a dynamic process which poses intriguing questions about the evolution of insect genomes. Is, for instance, a similar system found in species, such as *Locusta migratoria*, with lower cellular DNA contents? Will thoroughinvestigations reveal that holometabolous insects also possess such a system? And, most importantly, what is the evolutionary significance of the system in *Caledia*?

Temporal Variation in the Structure of a Chromosomal Hybrid Zone.

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The structure of the hybrid zone between the Moreton and Torresian taxa of the grasshopper Caledia captiva was first described by Moran (1979). The genomes of these two taxa show fixed differences in their patterns of chromosomal organisation which permits all members of the genome to be unambiguously identified. Moran showed that the zone was extremely narrow ($\lesssim$800m) with changes in chromosomal frequencies of more than 50% over only 200m. The zone was found to be asymmetrical with respect to the frequency of chromosomal introgression on either side of the zone. Furthermore, the patterns of gametic disequilibrium differed on the two sides of the zone providing evidence for asymmetrical hybrid breakdown.

A reanalysis of the zone six years later has shown that its chromosomal structure - in terms of the correlation between chromosomes (r), the direction of gametic disequilibria (Nr^2) and the directional changes in D' - has undergone a complete reversal during this time period. There now exist highly significant correlations between chromosomes in populations which only six years previously showed equilibrium frequencies. Those populations which now show significant chromosomal interactions are also characterised by significant reductions in embryonic viability. The changes in the pattern of disequilibrium have been shown to result from major reductions in the frequencies of chromosomal heterozygotes on the Moreton side of the zone but which is not occurring on the Torresian side. These dramatic changes in the structure of the hybrid zone correlate with equivalent changes in the pattern of rainfall between 1977 and 1983.

The data indicate that strong directional selection is operating within the zone and that the response to selection involves a major part of the genome. Moreover, the observed selective changes suggest that the chromosomal rearrangements per se may be directly involved. Significant phenotypic differences between the Moreton and Torresian taxa in some aspects of their developmental patterns across the zone provide a variable character which may allow the identification of the function of the chromosomal rearrangements in modifying the phenotype.

Sequence Variation in a Highly Repeated DNA Family from *Caledia captiva*

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Amount and pattern of sequence variation among members of a highly repeated DNA family was examined by cloning and sequencing over 50 copies of a "160 base pair" repeat. The isolated clones included multiple copies from individual grasshoppers belonging to the same natural population, as well as sequences cloned from pooled DNA samples from the various *C. captiva* taxa.

Sequence divergence among repeats from a single grasshopper can be as great as 10%, suggesting that a large amount of variation has been, and is being, generated in this family. In contrast, the base pair positions involved in the sequence variation do not appear to be randomly distributed. This pattern of evolution is consistent whether sequences from a single individual or divergent taxa are being compared. Two possible explanations for these findings seem apparent. Firstly, the conserved regions of each sequence may be of some functional importance and, therefore, are being maintained by natural selection. Alternatively, the present day distribution of base pair changes may be due to the presence of a mechanism which selects for the particular sequence types unrelated to any functional aspects. This mechanism would necessarily have been present in the common ancestor of the *C. captiva* taxa since each taxon studied shows similar patterns of change.

GENE AMPLIFICATION IN AN INTERSPECIFIC HYBRID MOUSE EMBRYO

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Double minutes were found to be present in six of seven clones derived from a 16-day female *Mus musculus* x *M. caroli* fetus. The DM-positive clones derived from three primary populations independently set up from the fetus, and included clones with an active *M. caroli* X chromosome as well as clones with an active *M. musculus* X. The simplest explanation of these findings is that DM were already present in cells of the *M. musculus* x *M. caroli* embryo at the time of X chromosome inactivation, and persisted during *in vivo* development and *in vitro* culture. This suggests that gene amplification occurred in the early embryo, or even the fertilized egg, perhaps because of interactions between components of germ cells contributed by the *M. musculus* and *M. caroli* parents. Alternatively, DM induction may have occurred independently in these lines, requiring that amplification is an unusually common occurrence in cells from interspecific hybrids.

DM - oncogenes.?

Not repet DNA.

Xinact - late blastocyst stage

Non random seg - no centromeres
- associate with normal chromosomes

A method of pachytene chromosome karyotyping for use in cytogenetical studies involving pigeonpea *(Cajanus cajan)* and *Atylosia* species

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Pachytene analysis provides an efficient option for karyotype determination in those plant species in which all somatic chromosomes are indistinguishable either by conventional or banding techniques. However, pachytene studies have been hindered by the tendency of chromosomes to tangle and the lack of any reliable quantitative method of characterizing the chromosomes.

A method has been developed in pigeonpea *(Cajanus cajan,* 2n = 22) which enables karyotype preparation from pachytene cells in which only one or a few chromosomes are separated from the nuclear cluster. It allows rapid identification of chromosomes based on small segments adjacent to the centromere.

Large chromomeres on either side of the centromere were the most reliable markers for chromosome identification since they remained relatively unaltered in position and structure from cell to cell. These large chromomeres were observed to be compound structures of smaller chromomeres and were termed singles, doubles, triples.... depending on their structure.

Karyotypic analysis proceeded in four steps: 1. description of chromomere pattern of 196 chromosomes from 79 cells. 2. grouping of chromosomes according to chromomere pattern. 3. testing of the validity and repeatability of these groups. 4. preparation of a key and idiogram to provide identification.

This karyotyping method was applied to accessions of a native Australian relative of pigeonpea *(Atylosia acutifolia,* 2n = 22). These chromomere patterns have been used to identify several chromosomes and their homeologues in *Cajanus* x *Atylosia* hybrids.

Vectors for Plant Transformation.

J. Langridge.

Separate molecular vectors have been constructed for the transformation of the nucleus of dicotyledonous plants, the nucleus of monocotyledonous plants, chloroplasts and Chlamydomonas. These are now being tested for their efficiency in effecting transformation of cells in culture.

Inherited Genetic Instability in Tobacco Somaclones

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The aurea mutant, sulphur (Su), has been used to monitor somaclonal variation in tobacco (Lörz and Scowcroft, 1983). This is a semi-dominant mutant which in sexually produced progeny segregates with precise Mendelian expectation. Both the homozygous and the heterozygous classes can be phenotypically distinguished with accuracy and the consequence of somatic genetic alterations can be observed in leaves as twin spots or single homozygous spots against a phenotypically heterozygous background.

Among plants regenerated from protoplasts of Su/su heterozygotes, a number of different somaclonal variants were found which affected vegetative and floral development as well as the pattern of leaf sectoring. One particular somaclonal mutant increases the frequency of twin spots on the leaf by approximately 500 fold. This mutant is inherited as a single dominant which appears to be lethal in the homozygous condition. The high spotting mutant is unstable and can be spontaneously "lost" during somatic cell division or gametogenesis.

Lörz, H., and Scowcroft, W.R., 1983. Variability among plants and their progeny regenerated from protoplasts of Su/su heterozygotes of Nicotiana tabacum. Theor. Appl. Genet. 66: 67-75.

Genetic and molecular analysis of symbiotic nitrogen fixation in *Rhizobium trifolii*

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Rhizobium trifolii interacts specifically with clover plants forming root nodules in which atmospheric nitrogen is fixed as ammonia. Genes essential for nodulation and nitrogen fixation are located on a large Sym (*symbiotic*) plasmid in this *Rhizobium* species.

The genetic regions encoding the subunits of the nitrogenase enzyme (*nifHDK*) and nodulation functions (*nod*) are located some 16 kb apart on the Sym plasmid (Schofield *et al.*, Mol. Gen. Genet. 192:459, 1983). A species-specific DNA sequence has been shown to be reiterated on the Sym plasmid of this species. One copy of the repeated sequence constitutes the promoter of the *nifHDK* operon (see Watson *et al.*, these proceedings).

A 14 kb HindIII fragment, spanning the nodulation gene region, was cloned into a broad-host-range vector to yield the recombinant plasmid pRt032. This plasmid was shown to restore clover nodulation ability to a Sym plasmid-cured, Nod^- mutant of *R.trifolii*. Transfer of pRt032 into *Agrobacterium tumefaciens* and into an unrelated "cowpea" *Rhizobium* conferred on these strains the ability to nodulate clover. Thus, the 14 kb fragment carries the genetic determinants for host specificity as well as nodule induction and development (Schofield *et al.*, Plant Mol. Biol., in press).

Nodulation-defective mutants have been generated by localized mutagenesis of the cloned 14 kb HindIII fragment using the transposon Tn5 (see also Djordjevic *et al.*, these proceedings). Four of the mutants were found to be defective in clover root hair curling, an early step in nodulation. The DNA region encompassing these four distinct mutations is currently being sequenced.

Further definition of the nodulation genes has been achieved by subcloning various regions of the 14 kb HindIII fragment. Complementation analyses of Tn5-induced Nod mutants, using these subcloned regions, have revealed that complementation of the Nod^- mutants can only be achieved when the corresponding wild-type fragments are present in low copy number (~5) and not when present in high copy number (~20). These observations suggest that the genes controlling early nodulation stages are coordinately regulated.

IDENTIFICATION OF HOST-SPECIFICITY DETERMINANTS IN *RHIZOBIUM TRIFOLII*

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Bacteria of the genus *Rhizobium* characteristically induce the formation of nitrogen-fixing root nodules on legume plants. *Rhizobium* recognises, attacks, distorts, and invades root hair cells of the host plant through a structure called an infection thread. These "early recognition" events largely govern the host-specific properties shown by *Rhizobium*. Rhizobia are, in fact classified according to their host specificity for example, *R.trifolii* nodulates *Trifolium* (clovers), *R.leguminosarum* nodulates *Pisum* (peas) and *R.japonicum* nodulates *Glycine* (soybeans).

Genes which are known to determine host range and nodulation ability occur closely linked on large, indigenous plasmids in many rhizobia (1). These plasmids also carry genes for nitrogen fixation (including the nitrogenase structural genes) and hence are called Sym (symbiosis) plasmids (2,3,4). It had been shown previously that a 14kb *HindIII* fragment from a *R.trifolii* Sym plasmid could restore nodulation ability to non-nodulating *R.trifolii* strains cured of their Sym plasmid, to *Agrobacterium tumefaciens* (a soil pathogen), and to other *Rhizobium* species (e.g. *R.leguminosarum*) which are normally unable to nodulate clovers (5). This verified that nodulation and host range genes occur on this 14kb *HindIII* fragment.

Extensive Tn5 mutagenesis of this 14kb nodulation fragment resulted in several distinct mutant phenotypes. Tn5 insertions into a central area (region 1) produced mutants unable to curl (attack) plant root hairs (Hac^-: hair curling). Insertions into flanking areas resulted in mutants which gave an exaggerated Hac response (Hac^{++}). Mutations in region 2 resulted in a non-nodulating (Nod^-) phenotype while mutations in region 3 produced strains able to nodulate some hosts but not others. The transfer of subcloned fragments containing region 3 (but not regions 2 or 1) to other *Rhizobium* species resulted in an extension of the host-range of these recipients to include clover plants. On this basis we conclude that region 3 is involved directly in determining the host range of *R.trifolii*.

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Identification of Early Nodulation Genes in a Broad Host Range *Rhizobium* Species

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The fast-growing *Rhizobium* strain NGR234 can successfully nodulate a broad range of tropical legumes as well as the non-legume *Parasponia* (Trinick and Galbraith, 1980). Symbiotic genes responsible for early nodulation functions in NGR234 exist on a heat-curable sym-plasmid (Morrison *et al.*, 1983) and have been identified by Tn5 mutagenesis, cross-hybridization and complementation studies using existing hair-curling mutants of *R.trifolii*. Site directed transposon mutagenesis is currently being used to precisely map genes essential for the early steps of nodule formation.

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Morrison, N.A., Cen, Y.H., Trinick, M.J., Shine, J., Rolfe, B.G. (1983). Heat curing of a Sym plasmid in a fast-growing *Rhizobium* sp that is able to nodulate legumes and the non-legume *Parasponia* sp. J. Bacteriol. 153: 527-531.

Trinick, M.J., and Galbraith, J. (1980). The *Rhizobium* requirements of the non-legume *Parasponia* in relationship to the cross-inoculation group concept of legumes. New. Phytol. 86: 17-26.

Human metallothioneins: gene structure and regulation by steroid hormones and heavy metals

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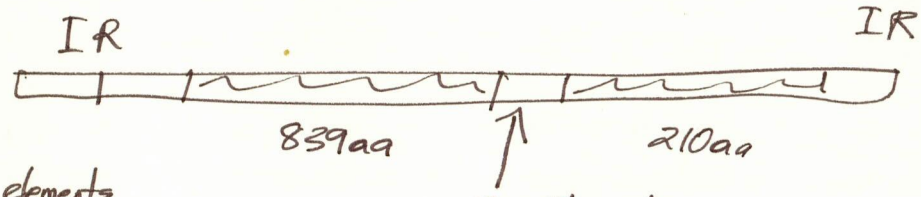
Metallothionein (MT) genes, encoding low molecular weight heavy-metal binding proteins, are a useful experimental system for studying transcriptional regulation of higher eukaryotes. In mammals, MT genes are induced in a variety of cell lines and tissues by administration of either heavy metal ions or glucocorticoid hormones. Gene transfer and *in vitro* mutagenesis techniques have been used in order to identify DNA sequence requirements of the induction responses. These experiments have defined two stretches of DNA (genetic elements), lying close to the promoter for the human gene for metallothionein-II, that separately mediate the induction of the gene by heavy metal ions, particularly cadmium, and by glucocorticoid hormones. The element responsible for induction by cadmium is duplicated, yet a single copy is fully functional; the element responsible for induction by glucocorticoid hormones is coincident with the DNA-binding site for the glucocorticoid hormone receptor.

One of the human MT-I genes (hMT-Ia), was also studied by gene fusion and transfer experiments. This gene was found to encode a functional protein, conferring heavy metal resistance to NIH 3T3 cells after transfer on a bovine papilloma virus derived vector. This gene is normally expressed in cultured human cell lines, although at a lower basal level than the hMT-IIa gene; and shows a different induction response, than the hMT-IIa gene, to heavy metals and glucocorticoids. This differential response is maintained when the respective 5' flanking regions are used in gene fusion/transfer experiments to drive expression of the thymidine kinase gene. Induction of the human MT multigene family therefore does not represent an equivalent elevation in the expression of individual genes, but the composite of the differential responses of the active members, as determined by their regulatory elements.

Ac element

- waxy M9 – Ac mutant.
- element ~~AE~~Ac9. 4.5 kb.



- two other elements similar in structure

∴ 2 active genes.

Ds element.

1) Ds 9.
   - waxy m 9 derivative mutant
   - element no longer autonomous
   - 4.3kb.
   - deletion from large open reading frame.

2) Shrunken (sh-m 6238, m
   - Ds sh
   - both 2kb.
   - deletion within large open reading frame.

Large open reading frame ⟶ trans acting "transposase"

3) Ds 1 element.
   - ~~about~~ 400 bp element
   - only 370 bp between terminal repeats.
   - several similar elements found with same structure. 101, 103, 105

Ds1 + Ds9 – only things in common are inverted repeats of 11 bp.

Abstract for Genetics Society of Australia Meeting, ANU.
11 May - 14 May

Molecular genetics of the Ac-Ds controlling element system of maize

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The molecular nature of Ac-Ds controlling elements of maize have been determined by cloning genes which are mutated by them and then characterizing the molecular changes. They are transposable DNA elements containing some features common to mobile sequences in other eukaryotes. Different families of Ds elements occur, with each family having similar sequence termini but little other sequence homology between the families. Upon entry into a chromosomal site, duplications of 6-bp or 8-bp are produced. The size of the duplication is related to the presence or absence of one base pair in the terminus of the Ds element. When Ds elements leave a chromosomal site they do so almost precisely or, sometimes, with deletions of adjacent DNA. In a Ds element mutation of the maize Adh1 gene there is a decreased level of mRNA transcript.

Insertion sites.
- no consensus sequence for site of integration.
Small duplications at target site

11bp inverted repeat

G G G A T

G G G A C T G A

8 bp flanking direct repeat.

Reversion to normal
- 8 bp flanking repeat +- 1-2 bases are left.

"Reversion" to null
- Ds excised & taken some Adh DNA with it
- deletion of N terminus of protein.

Co-ordinate transposition of Ds elements.

MITOCHONDRIAL DNA AND PROTEIN EVOLUTION WITHIN *PLATYCERCUS* (AVES).

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The Australian rosellas are an interesting assemblage of six, closely-related, morphologically similar species. This study tested hypotheses about their evolutionary history, their relationship to other parrot groups, the alleged slowdown of molecular evolution in birds and how the mitochondrial DNA (mtDNA) evolves in relation to the nuclear genome in naturally-occurring rosella populations.

Phylogenies were inferred from phenetic and maximum likelihood networks which were calculated from the liver enzyme gene frequencies of 88 individuals from 31 populations representing 26 parrot species. Gene frequencies were estimated after Cellogel electrophoresis of isozymes encoded by 21 loci. MtDNA was isolated from hearts and livers of 26 rosellas from the isozyme study using a technique especially devised for small amounts of avian tissue which had been frozen on liquid nitrogen for 1-25 months. MtDNA samples were digested with six four-base restriction enzymes; Dde 1, Hinf 1, Hpa II, Mbo I, Taq I and Zmo 37. The number of substitutions per base pair between each pair of mitochondrial genomes was calculated. From these values, a phylogeny for the 26 rosella mtDNA genomes was inferred.

The phylogeny proposed for rosellas from this genetical study corresponds well to the assumed environmental changes which occurred in Australia in the Quaternary. Rosella nuclear DNA which encodes liver proteins and rosella mtDNA are apparently evolving in a clock-like fashion, with mtDNA evolving more quickly than nuclear DNA. Comparisons with other studies suggest that rosella mtDNA may be evolving faster than mammalian mtDNA. Rosella molecular evolution was found to be just as rapid as for some mammal species studied, which refutes the alleged slowdown of molecular evolution at least in these bird species. To explain why genetic distances between bird species pairs are small if avian molecular evolution does occur rapidly, it is suggested that those species selected for genetic analysis could be younger than previously thought.

Evolutionary genetics of the family Estrildidae (Aves)

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An electrophoretic survey of 30 species of estrildid finches (family Estrildidae) has been conducted. A total of 25 enzymes coding for 39 presumptive loci were examined. The results were analysed by both quantitative phenetic and cladistic, and by qualitative Hennigian analyses. The data reveal the existence of three tribes within the Estrildidae, each largely confined to a specific geographical region. It is shown that the Australian grassfinches are a monophyletic group and that the morphological similarities between many African and Australian species are clearly due to convergences. These results endorse previous conclusions drawn from the chromosomal analyses.

Like many avian electrophoretic studies, the genetic distance between congeneric species was far lower than those observed in mammalian studies. This low amount of genetic divergence was apparent at all levels and so was not due to taxonomic oversplitting. Most species displayed levels of heterozygosity which were comparable to those seen in mammals and so it is unlikely that a reduced protein mutation rate in birds will explain the lower genetic distances. It seems that avian species may be a relatively young group compared to mammals and consequently speciation in birds may be rapid.

One interesting feature of this study was the lack of any correlation between levels of heterozygosity and genetic distance. Most loci which differed between species showed no intra-specific polymorphisms while some of the more polymorphic loci were highly conserved at the interspecific level. Thus, the low amount of genetic distance between many avian species may be due to a combined effect of recent origin and a low mutation rate.

ZWW - lethal

ZZW - intersex

ZZZ - male
- normal gonads + ducts
- sterile.

A STUDY OF THE INHERITANCE OF TRIPLOIDY IN THE CHICKEN (GALLUS DOMESTICUS)

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Triploidy has been reported frequently in the chicken in both embryonic and post-hatch stages. Triploids with karotypes 3A.ZWW are not viable, but some of those with 3A.ZZW and 3A.ZZZ karotypes do hatch (1). The 3A.ZZZ has a normal male phenotype, however, the 3A.ZZW adult has an intersex phenotype. Both are usually infertile.

In 1979, six closely related hens with intersex phenotypes were detected out of about 500 in one of the CSIRO layer strains (a synthetic Leghorn x Australorp crossbred line), and all were identified as 3A.ZZW triploids. Further examination of close relatives revealed a triploid male, 3A.ZZZ (2). The pedigree data supported an hypothesis that the triploidy was due to a single autosomal recessive gene, which, when homozygous, caused a high frequency of meiotic non-disjunction in at least the female, and possibly the male. The result is a diploid (2n) ova (or sperm) rather than the normal haploid (n). When this unites with a haploid gamete at fertilisation, a triploid results.

Selection has been applied to increase the frequency of triploidy in the subline. By the fourth generation of selection (S4), the frequency of matings producing triploid embryos was 50%. The frequency of hatched triploids has increased from 0.4% in the S1 to 2.5% in the S4. The data have remained consistent with the initial hypothesis and have clearly shown that the female produces diploid ova, but have not conclusively shown that the male produces diploid sperm. The results of each generation of selection will be discussed in detail and the direction of research with the subline will be outlined.

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A REPORT OF LIVE HAPLOID-DIPLOID AND DIPLOID-TRIPLOID CHICKENS (GALLUS DOMESTICUS)

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A total of 15 live mosaic chickens have been detected in a synthetic Leghorn x Australorp crossbred line of poultry. This line has been selected on its ability to produce a high frequency of triploid progeny. The mosaics consist of 11 haploid-diploids (4 1A.Z/2A.ZW; 7 1A.Z/2A.ZZ), 3 diploid-triploids (2 2A.ZW/3A.ZZW; 1 2A.ZZ/3A.ZZW) and 1 haploid-diploid-triploid (1A.Z/2A.ZW/3A.ZZW). Initially two of the diploid-triploid mosaics were detected in metaphase cells from feather pulp during routine screening of this line for triploids. Further screening of full-sibs and other birds of this line by DNA analysis of red blood cells (R.B.C.) on a cytofluorograph revealed the other mosaics. The mosaic ploidy levels have been confirmed in other tissues for each of the mosaics.

The percentage of 1N cells in the 11 haploid-diploids varies from 1-55% (R.B.C.) and the percentage of 3N cells in the diploid-triploids is approximately 5% (R.B.C.). All of the haploid-diploids and diploid-triploids have survived to maturity and are fertile. The 4 1A.Z/2A.ZW and 2 2A.ZW/3A.ZZW mosaics have a normal female phenotype and the 7 1A.Z/2A.ZZ and 1 2A.ZZ/3A.ZZW have a normal male phenotype. The haploid-diploid-triploid mosaic has an intersex phenotype and is not fertile. It has 20% 1N, 5% 2N and 75% 3N cells (R.B.C.).

The pedigree data has shown that the mosaics are all closely related, and test matings between mosaics and their full-sibs have been set up to examine the possibility of a genetic mode of inheritance. This will be discussed together with possible explanations for development and survival of the mosaics.

A VARIABLE GENE SYSTEM IN PLASMODIUM FALCIPARUM.

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The S antigen of the human malaria parasite Plasmodium falciparum is the dominant heat-stable antigen in the serum of infected individuals and in the supernatants of in vitro cultures of the parasite. The S antigens of different isolates of the parasite have been shown to be variable both antigenically and in size. We have studied the genetic basis of this variability by cloning DNA coding for antigenically different S antigens. We have shown that two such S antigens are encoded largely by a series of nearly exact tandem repeats. The repeat units of the different S antigen types, however, share no sequence homology and are different in length. Size variation in antigenically cross-reacting S antigens appears to be the result of expansion or contraction of repeat number encoded by the gene.

While the sequence of the coding regions of different S antigen genes share no homology, the sequence of adjacent non-coding DNA is highly conserved. In addition, the two different types of S antigen gene behave as if they are allelic, suggesting that this gene family is a series of at least two alleles. Such a family of alleles is presumably maintained in a polymorphic state by selection imposed on natural populations of the parasite by the human immune response to the type of S antigen expressed in a given infection. The pronounced conservation of degenerate bases in the codons of the tandem repeats suggests that a mechanism such as a specialized gene conversion mechanism is operating to maintain sequence homogeneity within the repeat portion of the coding region.

Molecular and Genetic Characterization of the *uncoordinated* (*unc*) locus in *D. melanogaster*

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A chromosomal walk was initiated in the proximal region of the X chromosome of *D. melanogaster* in order to a) isolate a number of loci of developmental and neurobiological signifiance, and b) study gene expression in a chromosomal region spanning the junction between euchromatin and heterochromatin.

The first cloned entry point into the region was isolated on the basis of RNAs enriched in the adult head. Southern blot hybridization analyses using DNA extracted from flies carrying deficiencies with genetically defined breakpoints localized the cloned DNA to the *uncoordinated* (*unc*) locus in polytene chromosome band 19E8. Mutations at the *unc* locus are semi-lethal. *Uncoordinated* flies lack coordination in leg movements and are unable to walk, suggesting a possible neurobiological function for the *unc* gene.

More than 40 kb of genomic DNA has been isolated extending in both directions from the initial entry point. Each EcoR1 fragment has been characterized with respect to representation in the genome.

Transcription products from this region of the genome were analysed by hybridizing each EcoR1 fragment to Northern blots containing both total cellular and total cellular polyA$^+$ RNA from 5 developmental stages. Three polyA$^+$ transcripts and one possible polyA$^-$ were identified, each of which is developmentally regulated. We have confirmed that the two adult transcripts originate from the *unc* locus by Northern blot hybridization using RNA extracted from flies homozygous deficient for the *unc* complementation group.

MULTIPLICITY OF SATELLITE DNA SEQUENCES IN D. MELANOGASTER

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Three D. melanogaster satellite DNAs (1.672, 1.686, 1.705 satellites) containing short tandem repeats were cloned into pBR322 as short fragments to maximize stability. Most clones appeared to delete spontaneously but 5% retained the insert undeleted and were subsequently stable. DNA sequencing revealed 11 simple repeat types with repeating sequences of either 5, 7 or 10 base pairs (bp). A tandem array of one sequence type usually extended the length of the insert. The repeating sequences are related and each 5bp repeat differs from another 5bp repeat by a single nucleotide. The expression $(Pu\ Pu\ N)_m\ (Pu\ N)_n$ with Pu = purine describes every sequence. Therefore if these repeat sequences have derived from a single ancestral repeat it has been in a non-random manner. Quantitation showed that one sequence class accounts for most of a given satellite DNA and that at least five repeat sequences are minor, amounting to 0.5% or less of the genome. Nevertheless these repeat sequences band as satellites with characteristic buoyant densities. Clones of the 1.688 satellite, comprised of more complex 359bp units in tandem, showed no evidence for spontaneous deletion. One clone contained a 353bp repeat 80% homologous to the 359bp repeat. Unlike the simple repeat types, constraints on sequence changes do not seem to apply to complex repeat types because comparison of the 353bp and 359bp sequences showed multiple changes scattered throughout the repeating units. Together the cloned satellite sequences amount to 18% of the genome, thereby accounting for most of the highly repeated DNA by amount but possibly only a fraction of the different sequence classes in this species.

Methylation Patterns in Ribosomal RNA Genes of *Lilium*

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In higher plants, many cytosine bases in the dinucleotide CG and in the CNG trinucleotides are methylated. Restriction enzymes can be used to assay the extent of methylation as most will not cut their recognition sequence if cytosines within it are methylated.

In the tandemly repeated rRNA genes of *Lilium henryi*, most enzymes affected by such methylation do not cut the repeat. However, single sites of digestion by *Hpa*II, *Eco*RII, *Hae*II and *Pst*I do occur. In each case the cut site is around 2 kbp upstream from the 5' end of the 18S rRNA gene, presumably near where transcription starts. All enzymes digest only 15-20% of rDNA from shoot meristem, and double digests suggest that the sites cut by the four enzymes occur within the same repeats.

It is possible that the minority of repeats undermethylated in this region are those being actively transcribed. Certainly the large number of rDNA genes per genome (around 5,000 to 10,000) implies excess transcriptional capacity. However, the extent of undermethylation in microsporocytes, cells at a stage when rRNA transcription is very low, is similar to that in actively growing shoot tissue.

THE MITOCHONDRIAL GENOME OF *MACROPUS GIGANTEUS*

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Mitochondrial DNA has been isolated from liver cells of the Eastern grey kangaroo *Macropus giganteus*. When subjected to restriction endonuclease analysis the genome was found to be 16.5kb and the restriction endonuclease patterns were consistent with the genome being circular. A large number of enzymes have been used to cleave the mitochondrial DNA and have also detected intraspecific variation.

Mt DNA in mammals ~16.5kb circular.

X CHROMOSOME INACTIVATION DURING MARSUPIAL EMBRYOGENESIS

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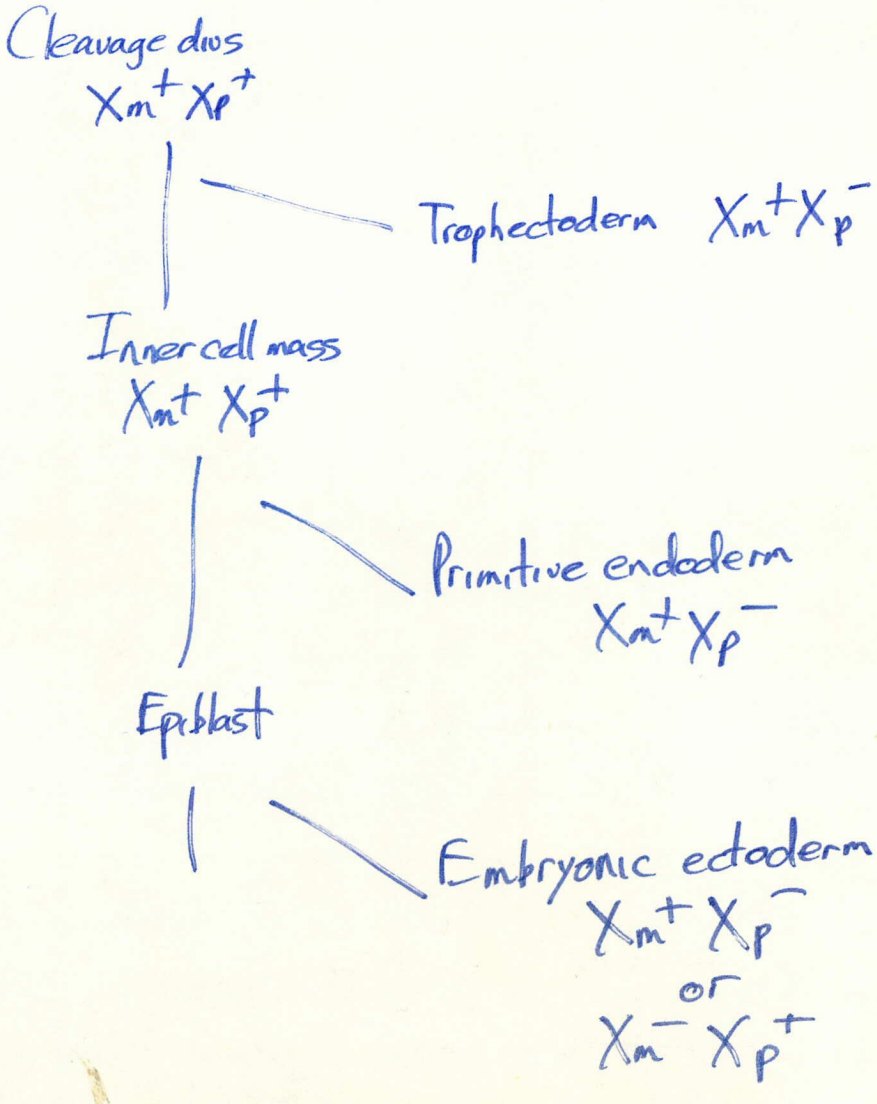
X chromosome inactivation during marsupial embryogenesis is being investigated by analysing X-linked enzyme expression and X chromosome replication patterns. We have found that in intra-uterine Macropus robustus females heterozygous for X linked G6PD, the tissues of the embryo proper, the amnion and the allantois show paternal X inactivation, whereas yolk cells express both maternal and paternal alleles and presumably have both X chromosomes active. By contrast, in eutherians such as mice, there is random X inactivation in the tissues of the embryo proper, amnion, allantois and mesodermal component of the yolk sac but paternal X inactivation in the yolk sac endoderm and the derivatives of the trophectoderm lineage. We are currently examining bilaminar kangaroo blastocysts to determine the state of X chromosome activity in the extra embryonic region (which forms the yolk sac) and the embryonic region.



ABSTRACT

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Active and Inactive Chromatin in Marsupials

High Mobility Group (HMG) proteins are non-histone chromosomal proteins which have been found associated with an active chromatin conformation in chicken red blood cells and trout testis. Proteins with the same electrophoretic mobility as calf HMG 1, 2, 14 and 17 have been isolated from several tammar wallaby tissues, and a number of tissue-specific differences have been observed. In particular, two testis-specific proteins (KT1 found in the HMG fraction, and a histone H1 variant found in both HMG and H1 fractions) have been discovered.

DNase I digestion of isolated nuclei from calf liver, tammar wallaby liver and red kangaroo testis has revealed that HMG 1 and 2 from all three tissues are preferentially released by this enzyme, which suggests these proteins are associated with active chromatin in calf and kangaroo tissues. In contrast, HMG 14 and 17 have not been preferentially released by DNase I treatment.

Treatment of DNase-digested nuclei with salt (0.35 M) results in the release of larger quantities of histone H1 than from undigested control nuclei in both calf and tammar liver. This may reflect a change in conformation of H1-containing nucleosomes after DNase I treatment. Interestingly, no histone H1 is salt-extracted from testis nuclei after DNase I treatment, which may indicate a different form of chromatin organization in this tissue.

Construction of a Marsupial Genomic Library and its use in the Study of X Inactivation

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In marsupials and in some of the earliest differentiating tissues of the extra-embryonic membranes of rodents, the paternal X chromosome is inactivated, whereas in other tissues of eutherians there is random X inactivation. This suggests that there is an intrinsic difference between the paternal and maternal X chromosomes. A considerable body of data implicates methylation of cytosine residues in the control of gene activity. In gene transfer experiments active X DNA is capable of transforming an $HPRT^-$ cell line, whereas inactive X DNA from somatic tissues is not. Inactive X DNA from the early differentiating extraembryonic membranes, on the other hand, is capable of transformation of an $HPRT^-$ cell line. To determine whether methylation is involved in marsupial X chromosome inactivation, a genomic library has been constructed. The approach is to use human X-linked cDNA clones to isolate the equivalent marsupial genes and to then use these as probes in MspI/HpaII digests of male and female DNA. The construction of the library and its potential uses in the study of X chromosome inactivation will be discussed.

PROBABLE HOMOGENEOUSLY STAINING REGIONS IN THE CHROMOSOMES OF NORMAL INDIVIDUALS OF **HOMO SAPIENS** AND **CHORTOICETES TERMINIFERA**

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In two human families, six normal individuals have been found who are heterozygous for similar variants which result in an increase in the length of the short arm of chromosome 9. In both families the variants appear to be segregating normally. In one family the additional segments vary in length and they are overall much longer than in the other family and in families previously described.[1] The enlarged regions are uniformly grey in photographs of GTL-banded chromosomes. The regions show moderately dark RGB-banding: i.e. they are not late-replicating. The enlarged regions remain faint after CBG-banding so they are not constitutively heterochromatic. The continuity of DNA through the regions has been shown using the Feulgen reaction. Ag-staining, N-banding, and in situ hybridization of rDNA failed to demonstrate ribosomal genes in the segment. The segment was also DAPI/distamycin negative. Carriers are designated 46,XXorXY,hsr(9)(p12) because we interpret these variants to be due to regions of repeated tandem duplication of small chromosomal segments similar to homogeneously staining regions found in tumours, and in cultured oncogenic cells.

In 1977 Professor Jacob Wahrman suggested that the long uniformly staining segments in the B-chromsome of Chortoicetes terminifera[2] might be homogeneously staining regions. The evidence now strongly favours this suggestion and indicates that the B-chromosome is derived from one of the smallest autosomes.

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MIC-5 MAPS BETWEEN *HPRT* AND *G6PD* ON THE LONG ARM OF THE HUMAN X CHROMOSOME

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MIC-5 is an X-linked gene determining the expression of a human cell surface antigen. The antigen, which has a limited cellular distribution, is detected by mouse monoclonal antibodies (R1 and R2) raised against human-mouse somatic cell hybrids which contained the long arm of the human X chromosome as their only identified human genetic material.[1] As a means of localizing *MIC-5* to a specific X chromosomal region, we have examined a large number of somatic cell hybrids for R1 and R2 reactivity. The hybrids chosen contained defined regions of the human X chromosome. Many of these hybrids were obtained by HAT selection after the fusion of irradiated human lymphocytes with an HPRT deficient hamster cell line.[2] The presence in these hybrids of unselected human X-linked markers, including *PGK* and *G6PD*, along with the selected *HPRT* marker, delineated the extent of the human X chromosome present. The results obtained suggest that *MIC-5* is located between *HPRT* and *G6PD* at a position Xq26-28. Both the location of *MIC-5* in this particular region, and its other properties which will be referred to briefly, have a number of interesting implications which warrant further investigation.

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Latitudinal variation in P-M hybrid dysgenesis in Australian populations of *Drosophila melanogaster*.

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As an initial assay for P-M hybrid dysgenesis in Australian populations of *Drosophila melanogaster*, I have scored gonadal dysgenesis in progenies of crosses to tester stocks. Tests were done on twenty isofemale lines each from five localities, from Cairns (16.9° S. Lat.) in the north to Cygnet, Tasmania, (43.2° S. Lat.) in the south. P-tests were crosses of individual males from the isofemale lines to females of Canton-S; M-tests were crosses of females to males of Harwich. The mean proportion among lines of F1 females with one or both ovaries atrophied in the P-test crosses ranged from >50% in the north to <5% in the south; the mean proportion in M-tests ranged from 11% or less in the north to nearly 50% in the south. The patterns of P- and M-test gonadal dysgenesis are reciprocally clinal with latitude; the biggest change occurs between Coffs Harbour (30.3° S. Lat.) and Cann River (37.5° S. Lat.). Some individual isofemale lines showed large variance between individuals in P- and M-tests, but no within-line gonadal dysgenesis was detected in progenies reared at 29°.

Electrophoretic variation of cyclic AMP phosphodiesterase in *Drosophila*.

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We have investigated the form I cyclic nucleotide phosphodiesterase (PDE) from *Drosophila melanogaster* and shown that whereas heads and male thoraces and abdomens contain high levels of Ca^{++}-stimulated enzyme, female thoraces and abdomens contain little Ca^{++}-stimulated activity. The electrophoretic pattern of form I PDE from these three sources has also been studied and reveals that heads, and male thoraces and abdomens, exhibit two bands of form I PDE both of which are stimulated by Ca^{++}. Extracts of female thoraces and abdomens, on the other hand, show only a single, faster running, band of PDE activity which is only marginally stimulated by Ca^{++}. Surveying wild-type strains of *Drosophila* has revealed that one strain, *Swedish*, shows altered electrophoretic mobility both of the head PDE bands as well as that from female thoraces and abdomens. The alteration is such that *Swedish* PDE runs more anodally than does *Canton-S* or *Oregon-R* PDE. The *Swedish* electrophoretic variation of the PDE from female thoraces and abdomens has been found to be recessive with respect to the *Canton-S* phenotype, but the variation is observed to re-emerge and segregate with the third chromosome in the F_2 generation. Mixing experiments using co-homogenization of heads with female thoraces and abdomens, yeild a single faster running band on electrophoresis. This band contains only Ca^{++}-insensitive PDE. Attempts to reconstruct this loss of Ca^{++}-sensitive PDE without electrophoresis have failed. The reults indicate that electrophoretic variation in the form I PDE is insufficient to allow one to locate the structural gene for this enzyme.

Genetic drift accounts for non-random and non-uniform patterns of isozyme variation in introduced populations of the giant toad, *Bufo marinus*.

Macro- and micro-geographical patterns of genetic variation are described for ten isozyme loci in introduced Australian populations of the giant toad, *Bufo marinus*. On a micro-geographical scale the patterns are found to be non-random in all cases. The pattern of non-randomness varies among loci, being clinal in two instances. The frequency variation on a macro-geographical scale is non-random at all but one locus (*Mpi*) and also varies among loci, in this case being clinal in four instances. The relative importance of natural selection and genetic drift in generating these patterns is investigated with the aid of the historical information on the spread of the species in Australia, and computer simulations incorporating previously determined values for effective population size and gene flow. In both cases the most reasonable explaination for the patterns of variation is that they have resulted from genetic drift occurring as the populations went through size bottlenecks at the time of their establishment. It seems that natural selection has played little, if any, role in generating the observed gene frequency patterns. These results emphasise the need for caution in interpreting geographical patterns of variation. They show that even where clinal patterns exist at some loci but not at others, this cannot be taken as evidence that these result from natural selection, unless the demographic histories of the studied populations are known, and are inconsistent with the alternative hypothesis that the patterns result from genetic drift.

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AN ECOLOGICAL GENETIC STUDY OF HOUSE MICE INHABITING A RICE FIELD.

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Feral and commensal populations of house mice appear to be variable in their social and breeding structures. Previous studies have described populations consisting of exclusive territories & others group territories. Other rapidly churning populations with apparently little strucure have also been described. A previous 4-year study on an irrigated rice farm suggested that spacing behaviour may play an important role in the self regulation of population density on such farms. Within-population social plasticity, with switching from exclusive to group territories, was proposed.

In the present study, variation at two blood protein loci was monitored monthly over a year to test for genetic subdivision of the population. Changes in gene and genotype frequencies at these loci were monitored to see how they correlated with qualitative and quantitative changes in the population.

Two populations one km apart were monitored. In each case the genetic structure remained essentially the same from one sample period to the next and there was no significant genetic difference between the populations. F-statistics were used to describe the genetic structure within each population; all F_{IS} values and the majority of F_{ST} values were not significant. The two populations appeared to be panmictic at both loci. Although there were marked fluctuations in population density (from n=5 to n=368) and significant qualitative changes over the year, the gene and genotype frequencies remained stable.

The Tpi polymorphism of D. melanogaster: the meaning of environmental correlates?

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Gene frequencies at the triosephosphate isomerase (Tpi) locus were considered with respect to environmental factors in an orchard population of Drosophila melanogaster. Associations at both the adult and larval stages were investigated. Among adults, seasonal changes in Tpi-F frequency were correlated with total monthly rainfall concurrent with and previous to the time of collection.

Larval resources (apples) were sampled from the orchard on two occasions. D. melanogaster and D. simulans were the predominant species emerging from the fruit. D. melanogaster were electrophoresed and their genotypes at the Tpi locus determined. Tpi gene frequency was compared to the numbers of both species emerging from each fruit. Tpi-F was significantly associated with both the number of D. simulans (SIM) and the proportion of D. melanogaster to D. simulans (PROP) emerging from each fruit.

To gain an indication of the biological state of the apple resources the parameters, fruit mass, liquid content and pH were measured for the second sample. Tpi-F frequency, SIM and PROP were significantly associated with fruit pH. Partial correlation analysis indicates that Tpi-F frequency is more strongly associated with SIM, and the association with fruit pH is indirect. Gene frequencies at the Tpi locus in D. melanogaster may be influenced by competition with D. simulans.

In Drosophila, climatic factors have been related to gene frequency patterns at many allozyme loci. Results for the Tpi locus suggest that these effects may be indirect, and that close observations of climate related field conditions may be of more importance in elucidating natural selection at these loci.

"Phenotypic plasticity or genetic variation in corals ?"

David J. Ayre

Australian Institute of Marine Science

Biochemical genetic evidence was used to show that the skeletal morphology of individual 'heads' of the coral Pavona cactus is determined by environmental and genetic factors. Within a population at Eclipse Island the predominant growth forms change along environmental gradients. The population consists of a number of spatially restricted clones. Two or more growth forms were found within small habitat patches, but clonemates all displayed similar morphologies. However, the growth form of one clone was found to change along an environmental gradient.

Cloning the promiscuous plasmid DNA primase gene in Tn5

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In order to test the role of the DNA primase gene (pri) of the IncP-1 plasmids in their promiscuous property, pri was cloned in the transposon Tn5 in E. coli. The TraI DNA, which includes pri, was first cloned in a wide host range vector. pri was sub-cloned from the recombinant plasmid by complete digestion with HindIII and partial digestion with Sau3A and ligated into the HindIII/BamHI region of pBR322. Successful cloning of pri was identified by temperature-suppression of an E. coli chromosomal primase mutation. Further sub-cloning of pri from this recombinant plasmid was again achieved by partial Sau3A digestion and insertion of fragments into the BamHI site in Tn5, located in a narrow host range vector. One of these Tn5 derivatives carrying pri was transposed to a temperature-sensitive, replication defective mutant of another conjugative IncP-1 plasmid and transferred into Pseudomonas aeruginosa at permissive temperature. The transposon was integrated into the latter's chromosome by raising the temperature to a non-permissive level. The integrated transposon was found to efficiently complement the transferability of mutants of the IncP-1 plasmid R18 with transposon Tn7 insertions in pri which had affected the host range of the mutants from P. aeruginosa to Pseudomonas stutzeri.

Role of the promiscuous plasmid primase gene in wide host range behaviour

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The DNA primase gene of the conjugative IncP-1 plasmid RP4 has been cloned into the wide host range, non-conjugative, multicopy vector R300B. As the recombinant plasmid was highly unstable in Pseudomonas aeruginosa, a subclone was constructed in a homologous, wide host range vector in Escherichia coli and mobilized into P. aeruginosa. Primase deficient mutants of the plasmid R18 (identical to RP4), obtained in P. aeruginosa by Tn7 insertion mutagenesis of the primase gene and found to be specifically unable to conjugationally transfer from P. aeruginosa to Pseudomonas stutzeri recipients, were complemented to wild type transfer frequency by the cloned primase gene. The restoration occurred regardless of whether the cloned gene was in the donor or recipient. This, and the fact that the mutants had normal transmissibility within P. aeruginosa, within P. stutzeri or from P. stutzeri to P. aeruginosa suggested that the plasmid primase is absolutely required for priming plasmid DNA transfer replication when plasmid transfer is attempted specifically from P. aeruginosa donors to P. stutzeri recipients.

Copper Resistance in *E. coli*

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University of Melbourne.

The copper resistance in *Escherichia coli* carried by a plasmid pRJ1004 is inducible. The level of resistance is proportional to the inducing dose of copper. We propose that the inducible plasmid coded copper resistance interacts with the normal metabolism of the cell to protect it against toxic levels of copper while maintaining the copper dependent functions.

GENETIC CONTROL OF PRE-ECLAMPSIA

D.W. Cooper

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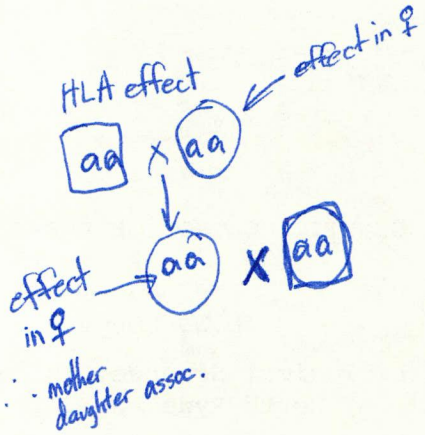
Pre-eclampsia (also called pregnancy induced hypertension, toxaemia, gestosis and some one hundred other names) is a common complication of pregnancy, particularly first pregnancy, in all human groups. In its severe form, it is associated with increased neonatal mortality. If allowed to progress to eclampsia (epileptic-like fits at or near the time of child bearing) it leads to many maternal deaths. Modern clinical practices have greatly reduced the incidence of eclampsia. Pre-eclampsia, however, remains the largest single problem which obstetricians in Western society must cope with, while in countries with less developed medical systems eclampsia and pre-eclampsia are both still major difficulties. The physiological cause of pre-eclampsia is not known; the most popular contemporary hypothesis is that an immunological aberration is involved. I shall summarise work done over the last five years with Scottish and American collaborators on the inheritance of pre-eclampsia. The data strongly suggest that its most troublesome form, severe or proteinuric pre-eclampsia, is due to a single recessive gene acting in the mother.


HLA effect
effect in ♀
aa
x
aa
effect in ♀
aa
x
aa
∴ mother daughter assoc.

IMMUNOGENETIC MARKERS IN PRE-ECLAMPSIA

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Pregnancy-associated hypertension has been shown to be a highly heritable condition. Data on the incidence in successive pregnancies and an unusual correlation with the HLA system suggest an immunological cause for this disease. If this is so it might be possible to find an association between specific immunogenetic markers and the condition. We have in mind the possibility of finding linkage disequilibrium between the putative locus or loci for severe pre-eclampsia and alleles at a marker locus. Our model is the well documented linkage disequilibrium between juvenile-onset diabetes (= insulin-dependent diabetes) and markers in the human MHC region, particularly markers at the Bf locus and the HLA A and B loci. Complement markers have been chosen for study because they exhibit a high degree of polymorphism. To date we have typed the complement components C_2, C_3, C_4, C_6 and Bf plus clotting factor XIIIB of pregnant hypertensive patients from the Royal North Shore Hospital. Significant increases in the frequencies of rare alleles at the C_3 locus have been found in this group of women, compared with a control group of normotensive pregnant women from the same hospital. The greatest departure from the control group occurs in patients with severe (proteinuric) pre-eclampsia. No significant variations from the normal gene frequencies have been found for the other complement markers studied.

DEVELOPMENTAL REGULATION OF GENE ACTIVITY IN MOUSE EMBRYONAL CARCINOMA CELLS

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The F9 line of mouse embryonal carcinoma cells can be induced to undergo differentiation by the addition of retinoic acid to the culture medium. The differentiation induced mimics steps in early mouse embryogenesis in which cells of the inner cell mass are converted to parietal endoderm via a primitive endoderm intermediate.

When the proteins from undifferentiated F9 cells and F9 cells treated with retinoic acid are compared by one- and two-dimensional gel electrophoresis, both increases and decreases in a whole range of proteins appear to be induced by the differentiation. In vitro translation studies indicate that these protein changes reflect alterations in the mRNA population of the cells on differentiation. Thus the F9/retinoic acid system is a useful one for studying -

(1) in vivo development in an in vitro system;

(2) processes involved in gene activation and inactivation;

(3) the role of specific promoter/enhancer sequences in controlling the activity of groups of genes.

We are primarily interested in the last two areas and we are approaching the question in two ways.

Firstly we are preparing cDNA libraries to differentiated and undifferentiated F9 cells and screening these for genes which show altered expression in the different cell types. This approach should provide cellular stage specific promoters which will be used in gene transfer studies to examine the molecular basis of stage specific gene expression.

Secondly we are using enhancer sequences, which are known to be inactive in early embryo cells but which are activated later in development in gene transfer experiments involving differentiated and undifferentiated F9 cells. If F9 cells truly mimic cells of the early embryo, then we expect these enhancers to be inactive in undifferentiated cells and active in retinoic acid treated cells. We can then examine the molecular basis for this regulation of enhancer activity.

SEX DETERMINATION IN SOME AUSTRALIAN *CHIRONOMUS* sp.

Dr Jon Martin and Dr Barry Lee
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University of Melbourne.

In the genus *Chironomus* there are no recognisable sex chromosomes, the sex of an individual being determined by a single gene or a small group of genes. The location of the sex determiners is variable both within and between species suggesting that a transposable element may be involved. A technique is suggested for isolating and cloning a sex determiner from these species.

Cytogenetic mapping of the male determiner in Lucilia cuprina.

D Bedo and G Foster

CSIRO Division of Entomology

Using Y-autosome translocations and recovering their non-disjunction products several karyotypes with deficient Y chromosomes were constructed. The sex and fertility of these flies provided information on the location of male determining and fertility genes in the Y chromosome. Flies which lacked most of the Y chromosome short or long arms were made first and proved to be fertile males. This suggests the male determiner lines in the centromeric portion of the Y chromosome common to both types of males. This was confirmed by constructing males lacking both the short and long arms of the Y chromosome. The male determiner is thus located in the centromeric portion of the Y chromosome offering a convenient vehicle for genetic manipulation of male determination in Lucilia.

PARTHENOGENESIS IN THE GEKKO *LEPIDODACTYLUS LUGUBRIS*

Max King[1], Georges Pasteur[2], Craig Moritz[3] and Charles Blanc[4]

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[2&4]Laboratoire de Génétique Ecologique, Univesité de Montpellier II, Montpellier, France

Cuellar and Kluge (1972), in a study of the tropical gekko *Lepidodactylus lugubris*, found that this species was diploid and an obligate parthenogen. A reanalysis of specimens throughout much of the species range indicates that there are both diploid (2n = 44) and triploid (3n = 66) clones present. C-banding studies suggest that the triploids are products of hybridization between males and diploid parthenogens. An electrophoretic analysis supports this finding, and indicates the likelihood of multiple hybrid origins. The unusual distribution of the triploids, coupled with the presence of males in certain areas, indicates the probable centre of origin.

A Highly Variable Sex Chromosome in *Gehyra purpurascens*

C. Moritz. Department of Population Biology, R.S.B.S., Australian National University

A karyotypic survey of the gekkonid lizard *Gehyra purpurascens* has revealed a distinctive sex chromosome system. G-banding has shown that the Z chromosome of males is derived from a tandem fusion of two acrocentric chromosomes of a presumed ancestral 2n = 44 *Gehyra*. Through the application of G, N and C banding, a total of six morphs of the W chromosome have been identified. These differ by paracentric and pericentric inversions and, in one case, by a centric shift. The possible reasons for such extensive variation in the W chromosome are considered and it is suggested that increased mutability of the W chromosome may be a causal factor. In contrast to earlier speculations, this example demonstrates that sex chromosomes can evolve without significant changes in the amount of C-band heterochromatin.

Why do drones produce such a small amount of sperm ~1 μl.
when they are relatively so big?

ON BEING THE RIGHT SIZE: MALE CONTRIBUTIONS AND MULTIPLE MATING IN SOCIAL HYMENOPTERA

R. H. Crozier School of Zoology, University of New South Wales, Kensington, N. S. W. 2033

A number of hypotheses for the occurrence of multiple mating by queens of social Hymenoptera are reviewed in the light of Cole's (1983) observation that polyandrous species tend to have larger colonies than single-mating ones. Most of these hypotheses cannot be definitively excluded, but only three of them appear sufficiently plausible and predictive to be useful guides to further research. All three involve the amount of genetic variation within colonies. The hypotheses, and their predictions, are: that caste-determination has a genetic basis and hence polyandry allows fuller expression of the potential caste system in each colony (species with more complex caste differentiation should be more often polyandrous than species with simpler caste systems), that polyandry maximises the production of divergent worker genotypes and hence the range of environmental conditions that a colony can tolerate (broader-niched species should be more often polyandrous than species with narrower niches), and that the reduction of the variance of diploid male production, under the heterozygosity sex-determination model, favors polyandry when sexuals are produced late during colony growth (queens in species reproducing during the exponential phase of colony growth should tend to mate once, but queens should tend to be polyandrous in species with reproduction occurring further along the colony growth curve).

ANALYSIS OF CONTINUOUS VARIATION OF HAMULI NUMBER IN *APIS MELLIFERA* BY MEANS OF A DIALLEL.

B. Oldroyd [1] [2] and C. Moran [2].

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N.S.W. 2006.

A 9 x 9 partial diallel cross was made between inbred lines of honeybees (*Apis mellifera*) using artificial insemination. The lines represented three races and three synthetics. In one experiment, bees were sampled directly from the brood nests of the maternal colonies. In a second, combs of very young larvae or eggs were removed from the maternal colonies and reared to pupation in standardised feeder hives.

Counts were made of the hooks (hamuli) on the right-hand wings of each worker bee, and a fixed-effects model diallel analysis conducted for each data set.

Large general and specific combining ability effects were detected for both rearing conditions. However, reciprocal effects were only significar where the bees had been maternally reared. This indicates that the reciprocal effects observed in the maternally reared data were environmental rather than cytoplasmic in origin.

Both the combining ability analyses, and examination of the results of individual crosses revealed significant heterosis for this character. However, the direction and magnitude of heterosis was highly variable.

$\log_e y = -2.7 + 24x - 0.82x^2$

$R^2 = 20\%$

$n = 23$

Signif x^2 term for single gene.

Single gene - variance maximal at mean.

The Physiological Approach to Animal Genetics Illustrated by Research on the Inheritance of Susceptibility to Bloat in Cattle

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The genetics of quantitative characters depend upon a mathematical approach based on a partitioning of the observed variance and this forms the basis of animal breeding technology. Our more recent knowledge of the nature of the gene and more recently the development of techniques for direct manipulation of DNA, have yet to be incorporated into animal breeding theory or technology.

A major reason for this is a lack of knowledge of the physiological pathways between the genes and the traits defined as economic.

Direct selection on bloat susceptibility has resulted in dairy cows which are resistant to bloat. The practical application and understanding of the genetics of this result will be simplified by a knowledge of the physiological pathways concerned. The experimental approach to study these, and some results from it, will be described.

Quantitative Analysis of Sheep and Non-Sheep Populations of *Lucilia cuprina*.

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Different geographic populations of *Lucilia cuprina* have long been considered to be ecologically similar, particularly in South Eastern Australia, with sheep being regarded as the essential resource for the maintenance of these populations. However, recently it has been found that within a geographic locality *L. cuprina* can be collected from areas presumably isolated from sheep, such as refuse tips.

A detailed quantitative analysis of population samples from Northern N.S.W. and Victoria has been undertaken to assess the level of population differentiation at the phenotypic level both between geographic areas and between sheep and non-sheep populations. In addition the genetic structure of populations has been defined, using biometrical analysis techniques.

Results suggest that tip flies may be other than mere transients and that the differentiation may reflect differing patterns of adaptation and selectional history of the populations.

? additive effects for fitness traits in all pops.

POSTERS

The Mouse Glandular Kallikrein Gene Family

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Glandular kallikreins are a family of mammalian serine proteases thought to be important in the maturation of biologically active peptides. For example, kallikreins have been shown to activate nerve growth factor, epidermal growth factor, and vasoactive kinins. Although different kallikreins show a high degree of substrate specificity, they are found to cross-react immunologically and to share very similar physical properties. We have found that mouse glandular kallikreins are encoded by a family of highly homologous genes linked within a single locus on chromosome 7.

Restriction mapping and hybridization analysis of 65 different genomic clones has indicated that there are at least 23 non-allelic kallikrein genes. Each gene spans a region of 4.5 kb and consists of 5 exons separated by 4 introns. To date, we have found one cluster of at least 10 genes, with intergenic spacer regions of 3.5 to 7.0 kb, and another cluster of at least 4 genes. Using nucleotide sequence analysis, we have been able to show that 3 of the genes in this second cluster encode the α- and γ-subunits of nerve growth factor, and the major renal kallikrein, respectively.

We hope to identify the products of further kallikrein genes by a combination of limited sequence analysis, the use of specific oligonucleotide probes, and gene transfer into mammalian cell lines. Information on the linkage relationships between different kallikrein genes, as well as their function and patterns of tissue-specific expression, should provide some interesting insights into the evolution of this multigene family.

The Mitochondrial Genome of the Common Brushtail Possum, *Trichosurus vulpecula*.

Nigel Farrar

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Although the mitochondrial genomes of several eutherian mammals have been extensively characterized, no work has yet been published on the mitochondrial DNA (mtDNA) of metatherians. The mtDNA of the common brushtail possum *Trichosurus vulpecula* has been chosen as a model for the study of marsupial mtDNA mainly due to the abundance of this animal. As a base on which to construct a genetic map, a physical map of the cleavage sites of nine restriction endonucleases is presented. The consensus of single and double digestion data shows the mitochondrial genome of *T. vulpecula* to be 16.5 kilobase pairs (kb) $\pm$ 0.5 kb. This is comparable to the size of eutherian mtDNAs and suggests a similar economy of sequence and gene organization.

MULTIPLE INSEMINATION IN THE LOGGERHEAD TURTLE (*Caretta caretta*)

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ABSTRACT

Genotypic ratios within clutches of loggerhead turtle (*C. caretta*) embryos, from the Mon Repos rookery (Queensland), provided strong evidence that females of this species mate with a number of males in mass courtship. One third of all clutches provided evidence of multiple insemination. Clutches from two females indicated that these *C. caretta* individuals may have mated between nestings, during the 1982/1983 breeding season. During a nesting season a female loggerhead lays 4-6 individual clutches, each consisting of approximately 112 eggs. The large number of males contributing to any individual clutch suggests that each clutch may be a repository for a considerable proportion of the total population genetic variation. This is significant to the genetic structure of small rookeries of this species.

R. Koebner, Waite Agricultural Research Institute

'Use of biochemical markers to screen for rare recombinants between wheat and alien chromatin.'

The presence of the Ph gene, located on chromosome 5B of Triticum aestivum, does not permit non-homologous chromosome pairing either between homoeologues within polyploid wheat or between wheat chromosomes and alien homoeologues introduced into wheat as addition or substitution lines. However, a mutation at the Ph locus has led to the isolation of a line where this pairing restriction has been relaxed.

Non-homologous pairing in phph backgrounds occurs, although probably at a low frequency. When an alien segment to be recombined with wheat is marked by two or more well spaced genes which can be screened for simply and efficiently in large numbers of progeny, rare recombinants can be isolated by selecting genotypes where the linked alien genes have become separated.

A chromosome arm of cereal rye, marked by a storage protein gene close to the centromere, and a terminal heterochromatic knob has been used as a model system to illustrate the technique. In three separate phph lines, the frequency of non-parental types ranged from 5.8 to 9.6% of selfed progeny, while two control populations in Ph- background yielded 1.6% and 2.1%, reflecting the level of aneuploidy in the progeny.

Expression of Mouse Glandular Kallikreins

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Department of Genetics and Centre for Recombinant DNA Research, Australian National University.

Glandular kallikreins are serine proteases which act on kininogen substrate to release the peptide hormone, kinin. The proposed physiological roles of glandular kallikreins include the regulation of blood pressure, local blood flow and electrolyte balance. Glandular kallikreins have been localised, using immunological techniques, in the salivary gland, kidney and pancreas, and their secretions.

A multigene family coding for mouse glandular kallikreins has been characterised (Mason et al., 1983). Encoded proteins which are expressed in the salivary gland include α and γ subunits of nerve growth factor (NGF), epidermal growth factor (EGF)-binding protein, and γ-renin, all of which possess 70-85% amino acid sequence homology with glandular kallikreins. To determine which of these genes is expressed in other tissues, in particular the kidney, we have constructed and screened a cDNA library from mouse kidney. From the unamplified library of 350,000 clones, 40 hybridised to a general kallikrein probe, and using more specific probes, 3 were shown to be full-length cDNA. One full-length cDNA clone was sequenced and an oligonucleotide (30-mer) corresponding to a variable region of the sequence was chemically synthesised. The remaining clones were analysed using this oligonucleotide probe and, combined with sequencing data, the results suggest that only one member of the kallikrein gene family is expressed in the kidney. Partial sequence analysis of kallikrein λ clones from a genomic library showed that the sequenced kidney cDNA corresponds to the gene mGK-6. Hybridisation of the oligonucleotide probe to λ clones containing all of the kallikrein genes demonstrated that it is specific for mGK-6. Therefore the 30-mer probe is an mGK-6 specific probe.

Dot blots of RNA from various mouse tissues demonstrated that kallikreins are expressed in many locations. However, only RNA from the kidney, pancreas and, to a lesser extent, the salivary gland, hybridises to the specific mGK-6 probe. RNA from the cell line AtT20, derived from a mouse pituitary tumour, also hybridises to the mGK-6 probe, suggesting that specific cells in the pituitary may also synthesise this kallikrein. Other members of the kallikrein family, as yet unidentified, are expressed in the brain, lungs, liver, adrenal glands and aorta.

The gene mGK-6 is in a group of 4 contiguous genes, 2 of which code for the α and γ subunits of NGF. Gene transfer experiments will be used to investigate the regulation of tissue specific expression of these different glandular kallikreins.

Mason et al., Nature 303:300-307, 1983

DEVELOPMENT OF THE LATERAL THORACIC SCLERITES OF *DROSOPHILA MELANOGASTER*

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Embryonic development in *Drosophila* proceeds by the subdivision of the embryo into groups of cells. One class of cells, the imaginal discs, are the precursors of most of the external structures of the adult fly. By the use of radiation-induced mitotic recombinants cells in a disc can be marked and their development to the final structure followed. Flies which are heterozygous for markers that alter either the colour or structure of the cuticle or the morphology of the bristles are used so that any mitotic recombinant clones are phenotypically distinguishable.

This study investigated the relationship between the lateral thoracic sclerites and compartments in the thorax of *Drosophila melanogaster*. Gamma rays were used to induce mitotic recombination in heterozygous larvae from various crosses at defined stages of development. Examination of the resulting clones revealed that clones are restricted to individual sclerites from the 4 hour stage in development. Clonal restriction may indicate a relationship between the thoracic sclerites and compartments of the lateral thorax.

Genetic Structure of Stands of the Bracken Fern, Pteridium esculentum.

J.A. Thomson, C.M. Shearer and R. Byatt,
School of Biological Sciences, University of Sydney, N.S.W., 2006.

Changes in land use patterns and in the frequency of bushfires appear to have favoured the spread of bracken fern [Pteridium esculentum (Forst.) Nakai] since land clearing commenced in Australia, especially on the eastern and western slopes of the Great Dividing Range. Two features of bracken populations to attract attention in relation to this spread are the longevity of individual stands (Oinonen, 1967, Acta forest. Fenn. 83 (1): 1) and the rarity with which new sporelings are observed in the field (Conway, 1953, J. Ecol. 41 : 289; O'Brien, 1963, Ann. Bot. (NS) 27 : 253).

The present analysis, based on electrophoretic comparisons covering a number of enzyme loci, provides information on rates of spread of individual bracken stands, and on the genetic structure of stands in contrasting ecological situations. In ecosystems where bracken spread is rapid and coverage extensive, genetic diversity in many apparently continuous stands is typically too great to be readily accounted for by somatic mutation (see e.g. Whitham and Slobodchikoff, 1981, Oecologia 49 : 287). It is concluded that sporeling establishment is probably important over long time periods, even though it is an occasional event probably favoured by local small-scale conditions little related to the factors favouring rapid vegetative spread.

The effects of development time, and of demethylating agents, on variegated position effect in *Drosophila melanogaster*.

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Variegated position effect refers to the mosaic expression of a gene juxtaposed near to heterochromatin by rearrangement. Its molecular mechanism is quite unclear, but the suggestion has been made that deacetylation of histones is involved.

We have studied the expression of the white eye gene in the In (1) w^{m4} stock (homozygous for an X chromosome inversion) by visual estimation of the percent wild type ommatidia in the eye. We have found that differences in development time of flies reared at different temperature, or in media of different pH, are inversely correlated with percent wild type expression. At all development times, males showed more pigment expression than females.

We also discovered that flies reared on media containing butyric or propionic acid, or especially dimethylsulphoxide, showed much greater pigment expression than control flies, although they developed more slowly (and would therefore be expected to show less). The suppression of position effect by butyrate and propionate has been described previously (Mottus *et al*, Molec. Gen. Genet. 178, 465) and attributed to interference with histone deacetylation. However, since DMSO has no known effect on histone modification (Christman *et al*, Eur. J. Biochem. 81, 53) and all three drugs are known to induce hypomethylation of DNA, we suggest that DNA methylation should be considered as a mechanism for variegated position effect, notwithstanding the reported absence of methyl groups from bulk *Drosophila* DNA (Urieli-Shoval *et al*, Febs. Letts. 146, 148).

Transfer, characterization and expression of the *Rhizobium trifolii* infection genes in *Rhizobium* and non-*Rhizobium* backgrounds.

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Two different self-transmissible *Rhizobium trifolii* Sym plasmids (pBRIAN and pPNI) were transferred to four bacterial species, *Pseudomonas aeruginosa*, *Azospirillum brasilense*, *Corynebacterium nebraskense* and *Lignobacter* strain K17. *Corynebacterium* and *Azospirillum* strains carrying plasmid pPNI caused root thickenings, thick and short root (Tsr^+) and root hair distortions (Had^+) on white and sub-clover plants. *Pseudomonas* and *Lignobacter* strains carrying pPNI formed pseudonodules mainly around developing lateral roots on clovers.

Two plasmids, pKT230 (high copy number vector) and pRK290 (low copy number vector) which contain a 5.2Kb BglII fragment that carries the hair curling (*Hac*) genes from *R.trifolii* strain ANU843, were transferred to different bacterial species (*E.coli*, *Agrobacterium tumefaciens*, *Azospirillum brasilense*, *Lignobacter*, *R.meliloti* Hac^- and *R.trifolii* Hac^- mutants).

In all bacterial species investigated so far, the Hac^+ phenotype of the *R.trifolii Hac* genes was found to occur on clover plants. Other legumes such as lucerne, vicia and siratro were also tested with these different transconjugants for infection gene expression.

Karyotypic Variation in Huntsman Spiders (Sparassidae:Arachnida)

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Chromosomally, the family Sparassidae is highly homogeneous, with the majority of the Australian species possessing 40 autosomes and $X_1X_2X_3/O$ sex determining system. The karyotype is wholly telocentric. C-banding studies on *Olios*, *Delena* and *Isopoda* species have shown that all chromosomes contain centromeric heterochromatin and high levels of polymorphism for telomeric heterochromatin. Apparent differences exist between species in the timing of condensation of the X-chromosomes at meiosis and mitosis, and the degree of association between the X's at different stages of meiosis. Of particular interest were findings in the colonial species *Delena cancerides*, where the karyotype is characterised by multiple Robertsonian fusions, resulting in a male diploid number of 21. Several of these fusions are maintained in a permanently heterozygous state, which results in the formation of a chain of chromosomes during male meiosis. The multiple association of non-homologous chromosomes is perpetuated through the presence of an X-autosome fusion. This mechanism should result in females homologous for the fusions, and normal bivalent formation during oogenesis. A chain of 9 chromosomes in male meiosis predominates in eastern Australia, although lower order chains are present in some populations. The X-chromosomes segregate together, but no association between them is evident after diplotene.

Genetics of Colour Polymorphism in the St John's Wort Beetle *Chrysolina quadrigemina*

by: John Tomasov
Department of Genetics & Human Variation
La Trobe University.

The chrysomelid beetle *Chrysolina quadrigemina* was introduced into Australia, from France, in the early 1920's in an effort to control the noxious weed *Hypericum perforatum* (St John's Wort). The beetle is currently widespread through-out North-Eastern Victoria and Eastern New South Wales but is only moderately successful as a control agent. *C.quadrigemina* occurs in four intergrading colour forms : bronze, green, blue and purple. Proportions of these forms vary with different collection sites both within Australia and between Australian and overseas populations, many of which have been derived from Australia. There is some evidence to suggest that the different colour morphs may have associated ecological characteristics.

The possible genetic basis of the colour polymorphism is being investigated. Results of crosses involving the three common colour morphs (bronze, blue and green) are best explained by a model involving two unlinked loci. At the first locus the "bronze" allele (b) is recessive to the "coloured" allele (B). A dominant allele (G) at the second locus acts to modify the effect of the B allele such that when both G and B are present the individual is phenotypically green. An individual homozygous recessive at the second locus (gg) and carrying the B allele is phenotypically blue. The intergradation in colour, particularly between blue and green morphs may account for some inconsistencies in progeny colour ratios. The system may be of more general interest for investigators of epistatic interactions between loci affecting fitness in natural populations.

Characterization of Sym plasmid-specific repeated DNA sequences in *Rhizobium trifolii*

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Rhizobium trifolii is able to infect the root hairs of clover plants (*Trifolium*) and induce the formation of nodules. Within these nodules, the bacteroid form of *R.trifolii* reduces (fixes) atmospheric nitrogen to plant-assimilible ammonia via the bacterial enzyme nitrogenase.

The bacterial partner in this symbiosis carries genetic determinants for both nodulation and nitrogen-fixation functions. These determinants map about 16 kilobases (kb) apart on a large Sym (*symbiotic*) plasmid in *R.trifolii*. A subcloned 14 kb region of this 180 kb Sym plasmid carries the genetic determinants of host specificity and nodule induction.

It has been found that a DNA sequence, in the promoter region of the *nifHDK* operon (encoding the nitrogenase enzyme subunits), is repeated at least six times in the genome. The repeated sequence copies appear to be located exclusively on the Sym plasmid and are conserved, in sequence and copy number, in all 20 isolates of *R.trifolii* examined. The *R.trifolii* repeated sequence shows no detectable hybridization to total DNA isolated from *R.meliloti*, *R.leguminosarum*, *R.japonicum* and several species of *Rhizobium* which nodulate tropical legumes.

DNA sequence analysis reveals that one copy of the repeated sequence is contiguous with the *nifH*-coding region. Sequence and hybridization analyses indicate that at least two classes of repeated sequence may be present in *R.trifolii*. These classes consist of a 200 bp sequence common to all the repeats and a second class containing an additional contiguous 150 bp of sequence which includes the N-terminal region of *nifH*. Analogous reiterated DNA sequences have also been demonstrated in *R.meliloti* and we have shown their presence in *R.leguminosarum*. These sequences may play a fundamental role in symbiotic nitrogen fixation, possibly as common promoter sequences which allow the host-regulated, coordinate expression of symbiotic genes.

LOCALIZATION OF THE Igh LOCUS TO THE DISTAL REGION OF CHROMOSOME 12 IN THE MOUSE USING **IN SITU** HYBRIDIZATION

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The structural gene cluster encoding murine immunoglobulin heavy chains has been localized to the distal half of mouse chromosome 12 using hamster/mouse somatic cell hybrids.[1]

We have been able to confirm this location using a recombinant plasmid containing 10.5 kb of DNA flanking the Igh gene Cγ1 on the 5' side[2] as a probe for *in situ* hybridization.

Because of the similarity of shape of mouse chromosomes, photography of G-banded preparations was necessary before hybridization.

The probe was labelled with 5-I^{125}dCTP using nick-translation. Dextran sulphate was included in the hybridization mix to increase the formation of a network of the probe at the locus of the gene.

Autoradiography with Kodak NTB-2 required 16 weeks exposure.

Analysis of 17 successful cells showed a clear peak of grains over chromosome 12 and, within the chromosome, over the distal region.

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ORGANIZATION AND PRIMARY STRUCTURE OF NITROGENASE GENES IN THE PARASPONIA RHIZOBIUM STRAIN ANU289

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Parasponia Rhizobium strain ANU289 effectively nodulates a wide range of tropical legumes and also the non-legume Parasponia.

The structural genes for nitrogenase have been isolated from a lambda genomic library of ANU289 DNA by hybridization to the cloned nitrogenase genes of Klebsiella pneumoniae (Scott et al.,1983). Restriction analysis has shown that the gene encoding the Fe-protein (nifH) is separated from the genes encoding the Mo Fe-protein (nifD and nifK) by at least 10kb of DNA. This arrangement is in contrast to that seen in free-living nitrogen-fixing bacteria and fast-growing Rhizobium strains in which nifHDK are contiguous. The gene for nifK lies immediately to the 3' side of the nifD gene suggesting that they are encoded on the same operon.

Analysis of DNA sequence data has allowed the prediction of the amino acid sequence for the nitrogenase sub-units. The nifH gene product is highly conserved when compared to the amino acid sequences of all other known Fe-proteins, containing 5 cysteine residues which are conserved in all of these sequences. The nifD and nifK gene products show strong homology to other known Mo Fe-protein sequences, both having 5 cysteine residues in conserved positions (Hase et al., 1984 and Mazur et al.,1982).

The 5' ends of the nifH and the nifDK transcipts have been mapped by S1-nuclease and primer extension experiments. The nifH transcript is preceded by a leader sequence of 155 bp while the nifDK coding region has a leader sequence of 38 bp. These sequences show regions of homology 5' to the transription initiation site as shown below:

	-30	-20	-10
nifDK	GCGC	TGGCA	TGCTCG TTGC AGTCTTGTT
nifH	GTGT	TGGCA	TGGCGA TTGC TGTTGAGTT

This homology is found in the promoter region of all other nitrogenase genes studied in slow-growing rhizobia, is similar in the fast-growing rhizobia, and is also similar to the promoter consensus sequence observed for nitrogen fixation operons in Klebsiella pneumoniae (Beynon et al.,1983). Conservation of these regions suggests that the regulation of nitrogenase gene expression in symbiotic organisms may occur through a similar mechanism to that determined for asymbiotic systems.

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GROWTH CHARACTERISTICS AND DNA SYNTHESIS IN DIPLOID PLATYPUS CELL LINES

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Cell lines have been derived from the webbing tissue of two female platypuses (Ornithorhynchus anatinus; Monotremata, Mammalia). These lines have been maintained for up to 80 cell generations and remain diploid and fibroblast-like.

The platypus lines grow optimally at 32°C, the body temperature maintained by the platypus (Grant, 1983). At 37°C, subcultured cells fail to attach to the culture surface; cells already attached and in log phase at 32°C die rapidly when transferred to 37°C.

At 32°C, the population doubling times are 41-43h. (cf. $\leq$ 24h for most mammalian fibroblast lines grown at 37°C). The G_1, S and G_2 phase times are all about double those usually observed for mammalian lines. These data are discussed with reference to phase and cycle times determined for Chinese hamster cells growing at 32°C and 37°C.

MITOSIS IN MAMMALIAN CELL HYBRIDS - WHY ARE CHROMOSOMES SEGREGATED?

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Interspecific somatic cell hybrids from which chromosomes of one parent are preferentially lost have been exploited to great advantage in gene mapping studies. However, the underlying mechanism of chromosome segregation remains poorly understood. One suggested theory is that centromeres of the segregant parent fail to engage in functional attachments with the mitotic apparatus.

We have tested this hypothesis by viewing mitotic cells having a polar orientation and looking for a non-random arrangement of segregated and non-segregated chromosomes. By avoiding the use of colchicine and hypotonic treatments the arrangement of chromosomes on the periphery of the metaphase plate remains intact. Chromosomes which are detached from the spindle fibres would be expected to fall inside or outside this radial metaphase configuration. Differentially stained hamster-human and hamster-marsupial hybrids were studied. In both cases the hamster is the retained parent. Human chromosomes can be distinguished using G-11 staining and marsupial chromosomes by a fluorescent labelling technique using chromomycin A3. In neither case was a non-random arrangement of the segregating chromosomes found. This suggests that the centromeres of segregating chromosomes are able to form functional attachments with the mitotic apparatus.

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