MKTee

GENETICS SOCIETY OF AUSTRALIA

37TH ANNUAL MEETING ABSTRACTS

30 JUNE – 3 JULY 1990

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GENETICS SOCIETY OF AUSTRALIA

Animal biotech.
1, 73-77
1990

J.D.S
72 3190-3196

Medrano JF & Shannon L. 1989.
milk protein typing of bovine mammary gland tissue used to generate a cDNA library.

37TH ANNUAL MEETING ABSTRACTS

Lake International
141 Brebner Dr
Westlake 356 4444

7.15 pm

GENETICS SOCIETY OF AUSTRALIA

37TH ANNUAL MEETING
ADELAIDE, SOUTH AUSTRALIA

PROGRAMME

SATURDAY, 30TH JUNE

1:00 - 4:00 p.m.	Amersham Non-Radiactive Workshop (Enrolled Participants Only)
2:30 - 7:30 p.m.	Coffee and Registration

Session 1: Gene Mapping

Chair:	***Dr. Marianne Frommer***
5:30 - 6:00 p.m.	The Human Genome Project *Grant R. Sutherland*
6:00 - 6:30 p.m.	Comparative Gene Mapping in Cattle; Conservation of Synteny with Mice and Humans *J. E. Womack*
6:30 - 6:50 p.m.	Progress Toward a Bovine Genetic Map *D.J.S. Hetzel. W. Barendse, R.D. Drinkwater, R. Hediger & S.S. Moore*
6.50 - 7.10 p.m.	Mendelian Factors underlying Quantitative Traits: Chromosomal Locations, Phenotypic Effects and Evolutionary Relationships. *A. Paterson, J.W. DeVerna, J.D. Hewitt, D. Zamir, E.X. Lander & S.D. Tanksley*
7.10 - 7.30 p.m.	Construction and Use of a Yeast Artificial Chromosome Library to Isolate Fatty Acid Desaturase Genes from the Higher Plant *Arabidopsis thaliana* *S.I. Gibson, E. Grill & E.R. Somerville*
7:30 p.m.	Mixer

SUNDAY, 1ST JULY, 1990

Session 2: Genetics and Technology

Chair:	***Dr. Judy Ford***
9:00 - 9:15 a.m.	Welcome ***Senator Graham Maguire***
9:15 - 10:00 a.m.	Genetics in Private Enterprise *Ken C. Reed*
10:00 - 10:30 a.m.	Coffee

Session 3: Genomic Conservation and Evolution

Chair:	*Dr. David Smythe*
10:30 - 11:15 a.m.	Molecular Phylogeography and Principles of Genealogical Concordance *John C. Avise*
11:15 - 11:35 a.m.	Genetic Variation in Swamp Buffalo (Bubalus bubalis) and Native Goat (Capra hircus) Populations of Southeast Asia *J.S.F. Barker, T.K. Mukerjee, S.G. Tan, O. Selvaraj, M. Sekaran & J. Panandam*
11:35 - 11:55 a.m.	Evolutionary Dynamics of Mitochondrial Gene Duplications in Parthenogenetic Lizards *C. Moritz, A. Heideman & E. Zevering*
11:55a.m. - 12:15p.m.	Genetic Diversity and Interspecific Larval Density Effects Between the Cactophilic Drosophila Species D. aldrichi and D. buzzatii *R.A. Krebs & J.S.F. Barker*
12:15 - 12:35 p.m.	Allozyme Variation and Chromosome Change in the Stylidium caricifolium Species Complex *D.J. Coates & S.A. Carstairs*
12:35 - 12:55 p.m.	Genetic Variation of Acacia melanoxylon *J. Playford, C. Bell, G.F. Moran & R. Appels*
12.55 - 2:30 p.m.	Lunch

Session 3b: Genomic Conservation and Evolution (cont)

Chair:	*Dr. Jean Mayo*
2:30 - 2:50 p.m.	Genetic Divergence in the Old World Screw-worm Fly, Chrysomya bezziana *D. Bedo*
2:50 - 3:10 p.m.	Reduced Sequence Conservation in the argA Genes of two Closely Related Pseudomomas Species *S. Dharmsthiti & V. Krishnapillai*
3:10 - 3:30 p.m.	On the Origins and Functions of Some of the Tandem Repeats from the Primates and Water Birds *J.C.S. Fowler, X. Zhu & L.A. Burgoyne*
3:30 - 4:00 p.m.	Coffee

Session 4: Genetics of Mating Systems

Chair:	*Professor Ross Crozier*
4:00 - 4:20 p.m.	Borage Borago officinalis Does Not Have a Multi-factorial Self-incompatibility System *C.R. Leach & O. Mayo*
4:20 - 4:40 p.m.	Genetics Studies on the Tammar Wallaby (Macropus eugenii) *D.W. Cooper, R.A.H. van Oorschot, P.G. Johnston, W.E. Poole, L.A. Hinds, C. Collett & T.K. Bell*
4:40 - 5:00 p.m.	Cytogenetics of Natural Hybrids within a Diploid-tetraploid Complex of Frogs *M.J. Mahony*
5:00 - 5:20 p.m.	Population Genetics of the Green Turtle, Chelonia mydas, in Australia *J. Norman*
5:20 - 5:40 p.m.	Comparative Studies of the Pattern of Chiasmata Distribution at Meiosis in Male and Female Marsupials *D.L. Hayman, J.C. Rodger & M.J. Smith*

Sunday Evening free

MONDAY, 2ND JULY, 1990

Session 5: Developmental Genetics

Chair:	*Dr. Tony Howells*
9:00 - 10:00 a.m.	Genes Controlling the Establishment and Elaboration of Segmental Pattern in the Drosophila Embryo *P. Ingham*
10:00 - 10:20 a.m.	Studies of the Molecular and Biological Roles of the "Rough" Homeodomain *R. Saint, J. Garwood, J. Lake, P. Moretti & T.J. Lockett*
10:20 - 10:40 a.m.	Genetic Analysis of a New Homeotic Mutation in Drosophila melanogaster *A. Elizur, M.P. Williams, J. Carlson & R.B. Saint*
10:40 - 11:10 a.m.	Coffee

Session 6: Genomic Organization

Chair:	***Dr. Alex Dobrovic***
11:10 - 11:30 a.m.	Adventures with Multiple Peaks from In Situ Hybridization: The Cases of Human Ubiquitin, Cyclin and Glutathione S-Transferase, and Mouse 11-2, Gene Probes *G.C. Webb*
11:30 - 11:50 a.m.	Organization of the Genome of Parasitic Trichostrongylids of Sheep *M.J. Callaghan & K.J. Beh*
11:50a.m. - 12:10p.m.	Evolution of Mammalian Sex Chromosomes and Sex Determination *J.A. Marshall-Graves, J.M. Watson & J.A. Spencer*
12:10 - 12:30 p.m.	The Highly Conserved Histone H2A Variant is Essential in Drosophila *A. van Daal & S.C.R. Elgin*
12:30 - 12:50 p.m.	A Search for "his-3" Recombinator in Neurospora by Gene Disruption Using Repeat Induced Point Mutation *D.E.A. Catcheside, F.J. Bowring & P.J. Yeadon*
12:50 - 2:20 p.m.	Lunch

Session 7: Molecular Studies of Genes and Chromatin

r:	***Dr. Robin Holliday***
- 2:40 p.m.	Isolation of a Genomic Clone Partially Encoding Kangaroo Hypoxanthine Phosphoribosyltransferase: Identification of Active-X-Specific Methylation in the Kangaroo HPRT Gene *A.A. Piper, J.A. Martorana, M.K. Swanton, A.M. Bennett & D.W. Cooper*
40 - 3:00 p.m.	A Model of Protamine Binding to Chromatin *A. Arellano*
3:00 - 3:20 p.m.	Sequences from Lucilia cuprina with Homology to Retrotransposons from Drosophila melanogaster - Lucilia Transposable Elements? *H. Perkins & T. Howells*
3:20 - 3:40 p.m.	A Molecular Analysis of "Cinnabar": An Eye Pigmentation Gene that Encodes Kynurenine 3-Monooxygenase in Drosophila melanogaster *W.D. Warren & A.J. Howells*
3:40 - 4:00 p.m.	Coffee

Session 8: Genetics of Human Diseases

Chair:	***Professor Henry Bennett***
4:00 - 4:20 p.m.	Population Genetics and Counselling for the Fragile X Syndrome Under the X Inactivation Model of Laird *J. A. Sved*
4:20 - 4:40 p.m.	Association of HLA DQ and DR RFLPS with Disease Progression in Human Immunodeficiency Virus Infection *J.A. Donald, K. Rudman, D.W. Cooper*
4:40 - 5:00 p.m.	Severe Pre-Eclampsia/Eclampsia Maternal Susceptibility Genes are not Linked to HLA *A.N. Wilton, D.W. Cooper & S.P. Brennecke*
5:00 p.m.	Annual General Meeting
7:15 p.m.	Conference Dinner

TUESDAY, 3RD JULY, 1990

Session 9: Agricultural Applications of Genetics

Chair:	***Dr. Jeremy Timmis***
9:30 - 9:50 a.m.	Selection of the Dieldrin Resistance Locus in Overwintering Populations of the Australian Sheep Blowfly *J.A. McKenzie*
9:50 - 10:10 a.m.	Biochemistry and Physiology of Esterases in a Wild Type and An Organophosphate Resistant Strain of Australian Sheep Blowfly *A.G. Parker & J.G. Oakeshott*
10:10 - 10:30 a.m.	Laboratory Selection of Dieldrin and Diazinon-Resistant Strains of the Australian Sheep Blowfly Following Mutagenesis *K.A. Smith, A.G. Parker & J.A. McKenzie*
10:30 - 10:50 a.m.	An Arabinose Kinase-Deficient Mutant of Arabidopsis thaliana *O. Dolezal & C. Corbett*
10:50 - 11:10 a.m.	A Rapid Procedure for Producing Wheat Chromosome Additions to Rye *M. Baum & R. Appels*
11:10 - 11:40 a.m.	Coffee
11:40a.m. - 12:40p.m.	M.J.D. White Address:
Chair:	***Professor Michael Hynes***

Genetic and Molecular Studies of Flax and its Rust
J. Timmis

1:00 p.m. Barbeque

POSTER PRESENTATIONS

1. Mutations affecting inflorescence development in Arabidopsis thaliana; *by J. Alvarez, C.L. Guli and D.R.Smyth*

2. Genetic Mapping of the Rye (*Secale cereale*) genome; *by M. Baum and R. Appels*

3. Population Structure of Orange Roughy Haplostethus atlanticus; *by M. Black, P.I. Dixon and P.R. England*

4. Genetic Diversity in the Cycad Macrozamia riedlei; *by M. Byrne*

5. Meiotic Chromosome Pairing in Hybrid Water Buffaloes; *by K. Dai, A. Dollin and C. Gillies*

6. Synaptonemal Complex Analyses of Three Different Robertsonian Translocations in Sheep (Ovis aries); *by K. Dai and C. Gillies*

7. Molecular Studies of Philadelphia Chromosome Positive and Negative Leukaemia using the Polymerase Chain Reaction; *by A. Dobrovic, J. Hardingham, J.H. Wan, D. Kotasek, R.E. Sage, R. Seshadri, E.H. Januszewicz and A.A. Morley*

8. The Relationship Between Echidna and Platypus - New Data from DNA Studies; *by D. Edwards and M. Westerman*

9. Use of Molecular Genetic Markers to Detect Residual Tumour Cells in Stem Cell Collections from Blood and Bone Marrow; *by J. Hardingham, A. Dobrovic, T. Gooley, D. Kotasek, R.E. Sage*

10. Embryo Sexing in Livestock by Simultaneous PCR Amplification of Y-Chromosomal and Autosomal DNA; *by C.M. Herr, K.I. Matthaei and K.C. Reed*

11. Cloning and Characterization of a Gene Necessary for Post-blastoderm Cellular Proliferation; *by G. Hime and R. Saint*

12. FcγRII RFLPs in Systemic Lupus Erythematosus; *by E.C. Jazwinska, C. Olive, P.M. Hogarth, P.A. Gatenby, S.W. Serjeantson*

13. Asynchronous Expression of Milk Protein Genes During the Different Phases of Lactation in the Tammar Wallaby; *by R. Joseph, C. Collet, and K. Nicholas*

14. Studies of Gene Amplification in a Cell Line Derived from a UV Induced Skin Tumour in the Mouse; *by R. Lan, C. Moran, H. Qin and C.H. Gallagher*

15. Regulation of Copper Metabolism and Resistance in Escherichia coli; *by B.T.O. Lee, N.L. Brown, A. Bergemann, J. Camakaris and D.A. Rouch*

16. DNA Genotyping for Pedigree Validation of Cattle; *by K.I. Matthaei, D. A. Mann and K. C. Reed*

17. Interactions of Behavioural and Lethal Alleles at the stoned locus in Drosophila melanogaster; *by J. Merakovsky, T.Z. Petrovich and L.E. Kelly*

18. Can Genetic Drift During an Ancient Bottleneck Account for the Present Incidence of Cystic Fibrosis?; *by G. B. Peters*

19. Genetic Markers for Individual Acute Lymphocytic Leukaemias: A Sensitive Test, Using the Polymerase Chain Reaction (PCR); *by P.J. Sykes, J. Condon, M.J. Brisco and A.A. Morley*

20. Characterization of the Drop Gene of Drosophila melanogaster; *by R. Tearle and R. Saint*

21. Genotyping of the A and B Variants of Cattle b-lactoglobulin (BLG) using Restriction Fragment Lenth Polymorphisms (RFLPs); *by M.K. Tee, C. Moran and F. Nicholas*

22. Binding of Proteins from Embryonic & Differentiated Cells to a Bidirectional Promoter Contained within a CpG Island; *by C. Tyndall, F. Watt, P. Molloy and M. Frommer*

23. Detection by In Situ Hybridization of Two Sites of Insertion of a Sheep Intermediate Filament Gene in Transgenic Mus musculus; *by G.C. Webb, A.T. Correll, J.H. Ford, G.E. Rogers and B.C. Powell*

24. Human Genome Variation: The Use of RFLPs and PCR in Forensic Science; *by K. Williams, V. Dunaiski and C. Fowler*

25. Hypomutable and Hypermutable strain of *E. Coli* - A simple and general method to select them.
by G.W. Grigg

26. A Model of Protamine Binding to DNA and Chromatin.
by A. Arellano

MUTATIONS AFFECTING INFLORESCENCE DEVELOPMENT IN ARABIDOPSIS THALIANA

John Alvarez, Catherine L. Guli and D. R. Smyth

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

A large series of recessive mutants with modified inflorescence structure have been isolated following EMS mutagenesis of Landsberg *erecta*. We have investigated three classes. The first have inflorescences with more branching than wild type and more cauline leaves. Individual flowers are also abnormal, with more sepals in irregular locations around an often abnormal gynoecium. Some mutants also have a few petals and stamens. At least three of these mutations are allelic with *leafy* (Haughn, G.W. & Somerville, C.R. 1988 Dev. Genet 9, 73-89). The *leafy* locus is closely linked to *yi* on chromosome 5.

A second class has several normal flowers on each flowering stem before development terminates with a compound flower. This flower often has two or three gynoecia surrounded by irregular numbers of sepals, petals and stamens. We have had many independent occurrences of this mutation which we call *triple-flower*.

The last class also has many fewer flowers per stem than wild type. In this case, though, the inflorescence terminates in a short, undifferentiated outgrowth of the apical meristem. The flowers produced usually have fewer sepals and stamens but more petals than the wild type, and a pin-shaped gynoecium with reduced or no ovaries. The standard mutation of this class, which we call *pinoid*, is not allelic with *pin-formed* (Goto, N. *et al.* 1987 AIS 23, 66-71).

All these mutants are being characterized and their loci mapped. We anticipate that a molecular description of their action when in wild type form will help reveal the gene cascade involved in setting up decisions in early inflorescence development.

CHEF

A MODEL OF PROTAMINE BINDING TO DNA AND CHROMATIN

Alejandro Arellano

Dept. Entomology, University of Adelaide,
Waite Agricultural Research Institute, S.A.

The protamines are the most basic proteins known and are found associated with the DNA in the sperm head. They condense the DNA to such an extent that it can be transferred to the ova without damage.

Whereas the chromatin organization in somatic cells has been well characterized as being formed by nucleosomes and superstructures of them, the sperm chromatin remains totally unknown despite many efforts to reveal its organization.

However, in order to understand the structure of the complex formed by DNA and protamines, a prior knowledge of the molecular conformation of both partners is essential. Thus, while the DNA conformation under physiological conditions is accepted to be the B-form, an absolute disagreement is continuing in relation to protamine structure.

Using secondary structure prediction, measurements of binding affinity of protamine fragments to DNA and fluorescence polarization, we have suggested a globular conformation for protamines, rather than a random coiled one. This finding, together with nuclease digestion experiments on DNA-protamine and chromatin subunits-protamine complexes, lets us propose a model of protamine binding to DNA and chromatin.

- ↑ protamine ⇒ larger DNA fragments protect from micrococcal nuclease.
- Bind at the linker then to nucleosomal.

- Fish protamines mainly arg ⇒ for DNA binding?
(Random coiled peptide w/o struct) – actually has α-helical struct.
- Use 2° struct predictn for protamine: protamines from fishes similar by β-struct + coil struct.
Reason: Seq similar.
- found protamine globular. N + C terminals close together.
- Protamine-DNA interaction = co-op one?

- Phylogeny - Geographic concordance also in crabs, seaside sparrow, american oyster.
Not eel

MOLECULAR PHYLOGOGRAPHY AND PRINCIPLES OF GENEALOGICAL CONCORDANCE

John C. Avise
Department of Genetics, Biological Sciences Building, University of Georgia, Athens, GA 30602, U.S.A.

In higher animals, mitochondrial (mt) DNA evolves rapidly, and exhibits a uniparental, non-recombining mode of inheritance. These features make observed sequence differences in mtDNA well suited for estimation of matriarchal phylogeny on a microevolutionary (intraspecific) scale. The geographic distribution of branches in such gene genealogies constitutes the phylogeographic pattern for a species. I will summarize evidence that both freshwater and marine species in the southeastern United States exhibit a remarkable degree of mtDNA phylogeographic concordance, suggesting the strong influence of historical biogeographic factors in shaping the genetic architecture of a regional fauna. Such phylogeographic concordance at particular loci among species, and/or phylogeographic concordance among loci within an organismal pedigree, are strong indicators of significant, historical population subdivisions that go beyond the idiosyncratic and transient gene genealogical structures expected under isolation by distance alone.

- 16 → 24kb ~ 37 final genes
no introns + spacer sequences
(in transcribed genes)
- Rapid evolution at mol. level
2-4% sequence / million yr.
- Mutation - silent mutation,
single base Δ,
some deletions, additions.
- maternally transmitted.
- use RFLP's patterns to construct
possible phylogeny.
- Also get Geographic/Genotypic
associations
eg fish in SE USA, align
parsimony network & Geographical
⇒ Associations

PROGRESS TOWARDS A BOVINE GENETIC LINKAGE MAP

W. Barendse, S.M. Armitage & D.J.S. Hetzel

Molecular Genetics Group, CSIRO, Division of Tropical Animal Production,
Box 5545, Rockhampton Mail Centre, Queensland 4702

A genetic linkage map is a powerful tool for mapping genes of unknown primary product using a procedure known as Reverse Genetics. As part of a concerted international effort, we are constructing a genetic linkage map for cattle consisting of known genes and polymorphic DNA markers. Our initial aim is to be able to define chromosome segments conserved between humans and cattle so that the large number of placements from the human map can be extrapolated to the bovine map.

Linkage mapping is being carried out within a group of selected reference families: these families are a resource being shared with other research groups. Large DNA stocks from two and three generation families of *Bos indicus* x *Box taurus* or *Bos indicus* strain crosses have been established. To date, 16 full sib families comprising 179 animals have been sampled. The number of offspring in sibships varies from 10 to 52.

Recombination frequencies (q) derived from Maximum Likelihood Estimates (MLE) of linkage using LODSCORES of comparisons between restriction fragment length polymorphisms (RFLPs) have been estimated. Linkage data on the following genes have been collected, viz, Growth Hormone (GH), protein kinase C A polypeptide (PKCA), Homeobox 3 (HOX3, Gelsolin (GSN), the K-RAS 2 oncogene (KRAS2), Collagen 2 A 1 (COL2A1), mammary tumor virus homolog (INT1), a-2 macroglobulin (A2M), and a lactalbumin (LALBA). These genes are thought to be located on Bovine chromosomes 5 and 19. We show that there is some conservation between humans and cattle in the ordering of these loci. We also show that close linkage can be excluded for some of the genes from chromosome 5.

GENETIC VARIATION IN SWAMP BUFFALO (*BUBALUS BUBALIS*) AND NATIVE GOAT (*CAPRA HIRCUS*) POPULATIONS OF SOUTHEAST ASIA[1]

J.S.F. Barker[a], T.K. Mukerjee[b], S.G. Tan[c], O. Selvaraj[b], M. Sekaran[b] and J. Panandam[b]

a. Department of Animal Science, University of New England, Armidale, NSW
b. Institute of Advanced Studies, University of Malaya, Kuala Lumpur, Malaysia
c. Department of Biology, University Pertanian Malaysia, Serdang, Malaysia

Swamp buffalo and native goats are animals of prime importance in Southeast Asia. Throughout this region, animals of each species are phenotypically similar, and no specific breeds or strains are recognized. However, given the geographical expanse of the species' distributions, the large number of island populations (Indonesia, Philippines, Taiwan, Sri Lanka), and their primary use by small-holder farmers which would not be conducive to long-distance migration, genetic differentiation may have occurred as a result of genetic drift and/or differential selection.

Biochemical polymorphisms are being studied to determine the extent of genetic differentiation among populations. The objective is to sample blood from 50 animals from each of a number of populations in Malaysia, Thailand, Philippines and Indonesia (and Australia for buffalo) and to assay for variation at about 70 loci using cellulose acetate and polyacrylamide gel electrophoresis. Assay systems have been developed so far for 45-50 loci in each species. To date, 11 of 27 loci have been found polymorphic for buffaloes, and 15 of 31 for goats. Preliminary results will be presented using seven and 12 loci respectively in buffaloes and goats.

In both species, departures from Hardy-Weinberg equilibrium frequencies are common. Most show an observed deficiency of heterozygotes, consistent with inbreeding, but for two loci in goats (Albumin and Transferrin) observed frequencies of heterozygotes are greater than expected. Hierarchical F-statistics show for buffalo less differentiation among populations within a country than among countries, while for goats differentiation is primarily among populations within countries. Dendrograms derived from genetic distance estimates, however, show for both species that genetic distances are not simply related to geographical distances.

1 Work supported by Australian Centre for International Research (ACIAR Project 8364)

GENETIC MAPPING OF THE RYE (*SECALE CEREALE)* GENOME

M. Baum & R. Appels

CSIRO, Division of Plant Industry, P.O.Box 1600. Canberra, ACT 2601

Relatively little work has been carried out on the detailed genetic mapping of rye (*Secale cereale)* due to the outcrossing nature of diploid rye and thus the limited availability of fertile or semifertile inbred rye lines. The "International workshop on rye chromosome nomenclature and homoeology relationship" in 1983, formally initiated the mapping of rye by designating a nomenclature for loci and chromosome arms (Sybenga,1983). In addition inbred rye lines and a translocation set of rye lines was made available. The arrangement of 12 genes in 4 linkage groups of which 2 groups were assigned to specific chromosomes (Schiegel and Mettin, 1982) as well the assignment of 32 polymorphic isozymes loci (Wehling,1986) and morphological markers (DeVries and Sybenga,1984) have been reported. The incorporation of a DNA marker into a genetic linkage map (including isozymes and seed storage protein markers) was first reported by Lawrence and Appels (1986) for chromosome 1R. The same F2 populations described by Lawrence and Appels were used for mapping more isozyme markers and RFLP markers in the present study.

Enrichment cloning using lines of rye 1R added to wheat was used to isolate new sequences for chromosome mapping. The DNA from a Chinese Spring wheat/1R addition line was digested with Sau3A and mixed with an excess (100x) of randomly sheared Chinese Spring DNA. The mixture was denatured and renatured and ligated to pUC118 digested with BamH1. A total of 5000 clines were analysed and 775 (15%) were found to carry rye (or wheat + 1R) specific repetitive sequences with sequence analysis proving that the expected rye heterochromatic sequences were recovered in the experiment. Other repetitive sequences were also recovered and are still being characterised. Low copy number sequences (not necessarily rye specific) were also recovered and these were shown to be polymorphic in the rye parents used to generated F2 segregating progeny and are currently being incorporated into the genetic map discussed below.

To produce a genetic map for rye which incorporates biochemical and DNA markers, two rye populations from the crosses rye2 2a x rye 26 and rye 2a x rye 14 (Lawrence and Appels, 1986) were analysed. Examples of useful RFLPs will be shown and the current map, constructed using MAPMAKER, will be presented. RFLPs which were utilised included clones from diverse sources (mainly wheat and rye) as well as the ones isolated form rye itself.

References.

Lawrence G.J. and R. Appels 1986: Mapping the nucleolus organiser region, seed protein loci, and Isozyme loci on chromosome 1R in rye. Theor Appl Genet 61:742-749

Schlegel R. and D. Mettin 1982: The present status of chromosome recognition and gene localisation in rye, S. Cereal L. In:Aufgabenund Entwicklungstendenzen der Roggenforchung und Roggenzuchtung, Teil 1, 131-152. Taf-Ber, 198, Akad.Landwirtsch-wiss DDR,Berlin.

Sybenga J. 1983: Rye chromosome nomemclature and homoeology relationships. Z. Pflanzenzuchig.

De Vries J.N. and J. Sybenga 1984: Chromosomal Location of 17 Monogenically Inherited Morphological Marker in Rye (Secale cereale L.) Using the Translocation Tester Set Z Pflanzenzuchtg. 92,117-139

Wehling P. 1986: Genetische Analuse und chromosomale Lokalisation von Isoenxymloci bein Roggen, Diss. Universitat Hannover

GENETIC DIVERGENCE IN THE OLD WORLD SCREW-WORM FLY, CHRYSOMYA BEZZIANA

D.Bedo

Division of Entomology, CSIRO, Canberra

Trichogen cell polytene chromosomes were studied from populations samples in South Africa, the Middle East, South East Asia and Papua New Guinea. In addition the chromosomes of hybrids made between a mass reared colony in Papua New Guinea and flies from these localities were analysed. No fixed chromosomal differences were detected between any localities. A small amount of polymorphism, mainly involving band and telomeric heterochromatin was found.

Asynapsis of the chromosomes was measured and compared between populations and hybrids with the Papua New Guinea strain. Asynapsis levels up to 20% per chromosome were typical of the populations samples. Individual chromosomes have characteristic asynapsis levels, normally with very high variance. In most cases little difference was observed between populations. However hybrids tend to have elevated asynapsis levels. This is especially marked in hybrids between Papua New Guinea and South Africian flies which show very high asynapsis levels in all chromosomes. These results suggest some genetic divergence exists between populations, especially those at the extremes of the geographic range of C. *bezziana.*

POPULATION STRUCTURE OF ORANGE ROUGHY *HAPLOSTETHUS ATLANTICUS*

M. Black, P. I. Dixon and P. R. England

Orange Roughy *Haplostethus atlanticus* occur in continental slope waters of the Atlantic and Pacific oceans. Fish mature when they are 33 to 35 cm in length. Dense spawning aggregations were recently discovered in Australian waters. Due to escalating fishing industry interest in the resource, research has been directed at developing a management strategy that will ensure a sustainable yield of Orange Roughy. It is important to determine whether the Australian population comprises one interbreeding unit or several subpopulations and if it is related to the New Zealand population. Isozyme electrophoresis was used to examine the relatedness of fish caught throughout the species' known range off South Australia, Victoria, Tasmania, New South Wales and New Zealand. Nine polymorphic enzyme loci were used in the study. These were Aat, Est, Gpi 1, Gpi 2, Idh, Mdh, Me, Mpi and Pgm. Allele frequencies were analysed with pairwise comparisons using the G-statistic to determine whether the populations were subdivided geographically. Three subpopulations of Orange Roughy were indicated: New Zealand, separated by four loci; South Australia, separated by six loci and Eastern Australia including Tasmania. A maximum likelihood phylogenetic tree was constructed using Felsenstein's CONTML program. The dendrogram separates New Zealand and South Australia with longer branch lengths relative to the other localities.

GENETIC DIVERSITY IN THE CYCAD *MACROZAMIA RIEDLEI*

Margaret Byrne.

Department of Botany, The University of Western Australia, Nedlands W.A. 6009

Macrozamia riedlei is a dioecious, wind-pollinated cycad native to southern Western Australia. A survey of 9 isozyme systems in the pollen of the species revealed 14 consistently interpretable loci of which 13 were polymorphic. Analysis of these 14 loci in 15 populations of *Macrozamia* showed that the species maintains most of its allelic diversity within populations. There is a general increase in mean percentage of polymorphic loci and mean number of alleles per locus in a north-south direction through populations. Across all populations and within populations there is a significant excess of heterozygotes compared to that expected under Hardy-Weinberg equilibrium. This survey revealed that *Macrozamia riedlei* is a highly polymorphic species that undergoes selection for heterozygous individuals in the adult plants.

ORGANIZATION OF THE GENOME OF PARASITIC TRICHOSTRONGYLIDS OF SHEEP

M.J. Callaghan and K.J. Beh

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The aim of our work is to characterize the organization and the structure of the genome of the major parasitic nematodes of sheep, *Trichostrongylus colubriformis*, *Haemonchus contortus* and *Ostertagia circumcincta*, with a view to developing improved methods of diagnosis and to facilitate rational drug or vaccine design. The first step in achieving this aim was to isolate, clone and characterize repetitive sequences in the parasite genome and to develop diagnostic probes. Repetitive sequences have been isolated from the DNA of each species by shotgun cloning genomic DNA into pUC-18 followed by colony hybridization using labelled genomic DNA as a probe. Repeat sequences specific for each species have been subjected to Southern blot, dot blot and DNA sequence analysis. Plasmid pTc15 contains a 1.2 kb insert that hybridizes specifically to the DNA of *T. colubriformis*. This repeat appears to exist in the *T. colubriformis* genome as a unit of ~320 bp flanked by Eco*R1* recognition sites. Plasmid pHc44 contains a 136 bp insert specific for *H. contortus*. In the *H. contortus* genome, this sequence is also flanked by Hind*111* recognition sites. The plasmid pOc1 contains a 1.1 kb insert specific for *O. circumcincta* DNA. The repetitive unit of this sequence in the *O. circumcincta* genome is ~40 bp and is bounded by Hind*111* recognition sites. Southern hybridization to partial Hind*111* or Eco*R1* digests of worm DNA has shown that each repeat exists as a tandem array in its homologous species.

Further characterization of the genome was achieved by cloning selected genes present in single copies in the parasite genome. Differential screening of uterine and intestinal specific cDNA libraries of *H. contortus* using uterine or intestinal cDNA probes resulted in the isolation of genes which are expressed in a tissue specific manner and therefore represent potential targets for drugs or antibodies. These tissue specific cDNAs are currently being characterized.

In conclusion, our results show that sheep parasitic Trichostrongylids contain significant amounts of repetitive DNA sequences arranged throughout the genome often in tandem arrays. Some of these repeats are specific for each species and are therefore potentially useful for differential diagnosis. Genes expressed in a tissue specific manner have been isolated from *H. contortus* and are being assessed as potential drug or vaccine targets.

- Cog have 2 alleles - ↑ freq
↓ freq

↑ freq allele: rec in his 3 locus
in heterozygote
- rec occur only if cog is ↑ freq
on same side
Shows that cog necessary for rec.
Have recognition site for protein binding?
- Only 3' portion of his 3 clones can transform Neurospora

A SEARCH FOR THE *HIS*-3 RECOMBINATOR IN NEUROSPORA BY GENE DISRUPTION USING REPEAT INDUCED POINT MUTATION

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Discovery of a polymorphism in the *cog* site close to the *his*-3 locus in Neurospora[1] and the demonstration that it is *cis* acting, identified an initiation site for recombination at *his*-3 regulated by alleles of the unlinked locus *rec*-2. The genetic data do not permit choice between plausible models for this control[2]. We seek to identify the sequences involved in the initiation of recombination at *his*-3 as a step towards defining the mechanism of recombination control.

Fragments of cosmids covering a 55 kb segment of chromosome I flanking *his*-3 have been subcloned into a plasmid carrying hg^R and used to transform Neurospora to hygromycin resistance. The resulting duplications are subject to repeat induced point mutation (RIP) in the dikaryotic phase preceding meiosis, leading to transition mutation of a substantial proportion of G-C base pairs to A-T within and confined to the duplicated DNA[3]. In the appropriate crosses, disruption of *cog* or other sequences involved is expected to abolish recombination between *his*-3 alleles.

The orientation of *his*-3 with respect to the genetic map has been investigated by determining which *his*-3 mutants are transformed to prototrophy with clones carrying half of the *his*-3 promoter. This contrasts with the case of gene conversion within the *arg4* locus of yeast where the *arg4* promoter region has been shown to be a recombinator[4]

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ALLOZYME VARIATION AND CHROMOSOME CHANGE IN THE *STYLIDIUM CARICIFOLIUM* SPECIES COMPLEX

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The *Stylidium caricifolium* species complex consists of six taxa restricted to the south west of Australia. Marked chromosome differences occur between five of the taxa while two *S. nungarinense* and *S.* sp. Chiddercooping are chromosomally equivalent. Morphological intermediates between *S. caricifolium* and *S. affine* were found in an ecological transition zone between these two taxa. These transition populations generally showed increased chromosomal polymorphism in association with a *S. affine* karyotype.

Thirty five populations covering the six recognized taxa and a further two closely related taxa were investigated for allozyme variation at 16 polymorphic loci. Differentiation among populations within taxa and among taxa was examined using gene frequency and genetic distance data. The average Nei's genetic distance among populations of *S. affine* (D-0.110) and *S. caricifolium* (D=0.125) was relatively high particularly as both taxa are insect pollinated obligate outbreeders. Transition populations between *S.affine* and *S. caricifolium* shared alleles common to both taxa suggesting a probable hybrid origin although the possibility that they represent a zone of primary integradation cannot be discounted. With the exception of *S.* sp. Chiddercooping these data supported the morphological and chromosomal data which indicated five genetically distinct taxa.

Low levels of gene flow between populations and high levels of inbreeding are characteristic of populations of all taxa despite the fact that they are insect pollinated and primarily outcrossing. These conditions provide a ready means for chromosome repatterning, rapid populations differentiation and speciation.

GENETIC STUDIES ON THE TAMMAR WALLABY, (MACROPUS EUGENII)

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The tammar wallaby (Macropus eugenii; 2n=16) is the macropodid marsupial most widely used for biological research in Australia. A detailed understanding of the physiology of its reproduction has been achieved over the last two decades. This knowledge can be used to produce at least half a dozen offspring per year from the one female. This, combined with its small size, makes it useful for genetic work. In 1986/87 a pedigree colony of tammars was established at Macquarie, this paper will give an overview of some of the work done since then.

Crosses between animals from different parts of Australia have established that there is probably one species of tammar, despite large genetic distances between them. Linkage between protease inhibitor (Pi) and phosphoglucose isomerase (PGI) has been found, with an indication that female recombination is not as restricted as in *Sminthopsis crassicaudata*. Two intersexual animals (one tammar, one grey kangaroo) will be discussed in terms of current theories of mammalian sex determination.

MEIOTIC CHROMOSOME PAIRING IN HYBRID WATER BUFFALOES

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The water buffalo of Asia (*Bubalus bubalis* L.) has been classified into two types, the larger Murrah (River) breed from India and Pakistan which is used mainly for milk, and the smaller Swamp breed from Southeast Asia which is used for draught and meat. Cytological examination has revealed that the River type is 2n=50, whereas the Swamp type is 2N=48 (the indigenous Swamp buffalo of Sri Lanka is unique in being 2n=50). Attempts are being made in a number of Asian countries (particularly Malaysia) to combine the characteristics of the two types by hybridization. The resultant 2n=49 hybrids are relatively fertile, and backcross and F2 progeny have been obtained with chromosome numbers of 48, 49 and 50.

The chromosome number difference between the two breeds is the result of a tandem fusion of chromosomes 4 (submetacentric) and 9 (acrocentric) of the River type to form a large metacentric found in the Swamp type. We will illustrate by means of synaptonemal complex spreads the formation at pachytene in the F1 spermatocytes of trivalents, and present evidence concerning the nature of the chromosomal rearrangement involved. We will also illustrate other prophase I chromosome pairing behaviour which may be of significance for fertility of the F1, backcrosses and F2, including interaction between the trivalent and the XY pair in 50% of cells from one backcross bull.

We acknowledge the collaboration of the staff of the Faculty of Veterinary Medicine and Animal Science at the University Pertanian Malaysia, Serdang, Selangor.

SYNAPTONEMAL COMPLEX ANALYSES OF THREE DIFFERENT ROBERTSONIAN TRANSLOCATIONS IN SHEEP (*OVIES ARIES*)

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Three different Robertsonian translocations of domestic sheep Massey I (M I), Massey II (M II) and Massey III (M III), have been described by Bruere and co-workers from Massey University, N.Z. The component arms of the translocation chromosomes are as follows: M I, chromosomes 5 and 26; M II, chromosomes 8 and 11; M III, chromosomes 7 and 25. It is possible to separate them from each other by morphology of the translocation products and Ag-staining technique: t2 is metacentric, t1 and t3 both are submetacentric but the chromosome 25 in t3 is an Ag-staining NOR chromosome.

The synaptonemal complexes prepared by settle and spreading techniques from these three translocation heterozygotes have been analyzed. The difference between the settle and spreading techniques is significant, the former results in many more zygotene cells and information, the latter gives more pachytene ones and saves times. Translocation chromosomes show a pairing delay at the centromeric region. In the zygotene stage the unpaired ends tend to associate with the unpaired ends from X, Y, or other autosomes. it is important to substage zygotene and pachytene in considering the pairing behaviour and configurations of both XY and translocation pairs. Meanwhile, one ram previously expected to be M I, has been shown by lymphocyte chromosome analysis to be a mosaic with 2n=53 and 2n=52. Synaptonemal complex analysis revealed that there is a karyotype of 53 XY t1 in half of the spermatocytes, but the other half were 52 XY t1 + a second (as yet unidentified) translocation.

REDUCED SEQUENCE CONSERVATION IN THE *argA* GENES OF TWO CLOSELY RELATED *PSEUDOMONAS* SPECIES

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The *Pseudomonas* species, *P. aeruginosa* and *P. putida* are genetically very closely related. To verify whether the high conservation observed at the chromosomal level also applied at the gene level, the DNA of their *argA* genes which occur within the chromosomally conserved segment, was sequenced and compared with each other and also with that of *E. coli*. Although the deduced amino acid sequences of all three genes had two regions of very high homology of 75-92% and 86-93%, suggesting they are evolutionarily related, the *Pseudomonas* genes had unexpectedly low levels of overall homology at the nucleotide and protein levels of 65% and 42%, respectively. This could be due to the reduced functional importance of the protein in arginine biosynthesis. Secondly as the 42% homology between the two *Pseudomonas* proteins was not higher than the 41-44% homology between each of them and that of the E. *coli* protein, it suggests that the *Pseudomonas* genes could have evolved from different ancestral genes. Additionally homology between the *Pseudomonas* genes at the protein level was lower than that at the nucleotide level. This suggests that the *Pseudomonas* genes could be duplicate genes which have evolved independently into the contemporary *Pseudomonas* genes

- Pseudomonas' genome = 58 Kb
- Both Conserved regions may correspond to the functional regions of the 3 genes.

MOLECULAR STUDIES OF Philadelphia CHROMOSOME POSITIVE AND NEGATIVE LEUKAEMIA USING THE POLYMERASE CHAIN REACTION

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The Philadelphia chromosome is a result of the reciprocal translocation t(9:22)(q34:q11). The molecular consequence of the translocation is the fusion of the *ABL* and *BCR* genes. The hybrid *BCR-ABL* gene is found on the Philadelphia chromosome and is critical in the development of chronic myeloid leukemia (CML). We have developed a technique to detect the hybrid *BCR-ABL* gene by PCR of reverse transcribed RNA and have used this technique to investigate the following areas.

1. The influence of *BCR* exon 12 on the course of CML: PCR readily distinguishes two forms of the *BCR-ABL* message which differ by the presence or absence of the 75 base pair *BCR* exon 12. Patients with exon 12 have been found to have a significantly shorter chronic phase. The PCR test therefore may have definite prognostic implications.

2. Correlation between bcr rearrangements and PCR: A major advantage of PCR is that it is diagnostically useful to detect the *BCR-ABL* rearrangement and thus confirm the diagnosis of classical CML even in the absence of the Philadelphia chromosome. Rearrangement of the bcr region of the *BCR* gene has been the other alternative in this case. The correlation between bcr rearrangement and PCR in over 60 cases of Philadelphia positive and negative CML was very good. Several bcr+ PCR- patients were seen. The reasons for this are currently under investigation.

3. Philadelphia chromosome positive and negative ALL: The Philadelphia chromosome also occurs in a proportion of adult ALLs. The molecular event that we see is one of two types. The first type is identical to the CML type(s) and the patient may be a CML with an unidentified acute phase. The second involves joining of *ABL* to *BCR* exon 1 and can be identified with the addition of another primer. It is an open question as to how often either of these two molecular events occurs in Philadelphia negative ALLs.

4. Detection of leukaemic cells following transplantation: In collaboration with hospitals from Adelaide, Melbourne and Sydney, we have analysed samples from CML patients who have been transplanted in order to detect residual leukaemic cells. We can detect one leukaemic cell in a hundred thousand normal cells. This has allowed us to detect residual leukaemic cells at up to three years post transplant. This raises important questions about the nature of remission and PCR is likely to have important consequences in predicting and even preventing relapse.

AN ARABINOSE KINASE-DEFICIENT MUTANT OF *ARABIDOPSIS THALIANA*

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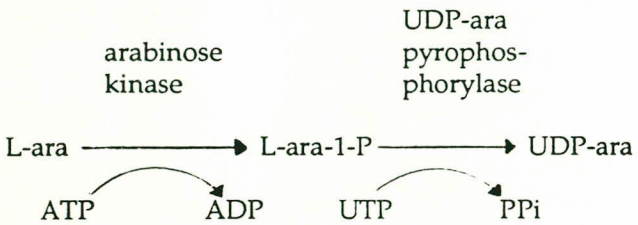
In plants sugar nucleotides are the precursors for polysaccharide biosynthesis. Many sugar nucleotides, for example, UDP-arabinose, are synthesized via a *de novo* pathway from UDP-glucose. However, free sugars released by polysaccharide turnover are utilized via *salvage* pathways. The arabinose salvage pathway, for example, is as follows:



Wildtype *Arabidopsis* seedlings are insensitive to high levels (50mM) of exogenous L-arabinose. An L-arabinose-sensitive mutant has been identified fortuitously when testing a number of M3 seed families for growth in the presence of various sugars.

The arabinose-sensitive phenotype is specific for L-arabinose, is semi-dominant with respect to the wildtype and exhibits a Mendelian segregation pattern. Linkage studies place the locus on chromosome 4.

In vivo and *in vitro* metabolism of ^{3}H-L-arabinose by mutant and wildtype seedlings or their extracts has been examined. Incorporation of ^{3}H-L-arabinose into polysaccharide material is reduced in the mutant compared with the wildtype and is accompanied by an accumulation of ^{3}H-L-arabinose itself rather than any metabolite. This suggests a defect in arabinose kinase activity. In vitro assays support this hypothesis. Enzyme assays of crude plant extracts have demonstrated a deficiency in arabinose kinase activity in the mutant.

ASSOCIATION OF HLA DQ AND DR RFLPS WITH DISEASE PROGRESSION IN HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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The disease spectrum in people infected with the Human Immunodeficiency Virus (HIV) ranges from the production of antibody in asymptomatic individuals through lymphadenopathy and autoimmune disease to full blown AIDS with opportunistic infections or Kaposi's sarcoma. A number of studies have examined whether genetic factors, in particular HLA genes, contribute to the variation in disease course and outcome in people exposed to HIV. Several associations have been reported, in particular between different HLA DR alleles and clinical type of full blown AIDS, as well as between the haplotype A1 B8 DR3 and rate of disease progression.

We have typed a cohort of 139 N.S.W. haemophiliacs for HLA D region genes by means of restriction fragment length polymorphisms (RFLPs) detected by HLA DQ and DR gene probes. Disease progression was studied in the 65 HIV antibody positive patients, who were infected by contaminated clotting factor before 1985. Strong associations were found between disease progression in HIV infected patients and allelic DNA fragments revealed by a DQα gene probe. A 5.7 kb fragment was reduced in frequency and a 5.0 kb fragment increased in frequency ($p<0.005$) in the faster progressing group, as measured both by immunological and clinical criteria. The maximum relative risk for disease progression associated with the 5.0 kb fragment was 3.8. These results correlate with DR types, which we deduced from the RFLP patterns revealed by DRβ gene probes. Disease progression was associated with a decrease in DR4, an increase in DR5 and an increase in the Dr3 subtype found in the A1 B8 Dr3 haplotype ($p<0.05$).

THE RELATIONSHIP BETWEEN ECHIDNA AND PLATYPUS -NEW DATA FROM DNA STUDIES

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The exact phylogenetic relationship of the extant monotremes has, due to the lack of fossil records, been uncertain. Based on amino acid sequence data for three globin molecules, an early Tertiary monotreme radiation has been suggested. DNA-DNA hybridizations have been used to test this conclusion. This technique uses the total genetic information present in the unique sequence portion of the respective genomes. Two Australian and one South Americian marsupial species were used as outgroups. Differences between the unique DNA sequences of the two Australian monotremes, the platypus and the short-beaked echidna, were found to be consistent with a divergence date in the late Cretaceous - early Tertiary period.

GENETIC ANALYSIS OF A NEW HOMEOTIC MUTATION IN DROSOPHILA MELANOGASTER

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Following a P-element mutagenesis search for antennal defects[1] a dysgenic mutant exhibiting a variety of developmental defects was generated and named extra antennal bristles (eab). This mutant exhibits several recessive phenotypes including an increase in the number of bristles in the second antennal segment, thickening of the arista, roughened eyes, notched wings and reduced viability. Genetic mapping and complementation studies have shown eab to be X-linked (7E5) uncovered by Df(1) RA2 and allelic to 1(1)14-160[2].

A series of additional alleles were generated by introducing eab into a dysgenic background. Flies with more extreme phenotypes were generated. Most notable in the more extreme alleles is the homeotic transformation of the arista into a leg type structure.

Lethal phase studies have shown that some of the new eab alleles have an embryonic as well as an adult phenotype while others are completely lethal. Cutical preparations of these embryos show an absence of cuticular structures suggesting that eab could be involved in formation of the hypoderm.

In situ hybridization of P-element sequences to polytene chromosomes showed the presence of a P-element at the site of the eab gene in one of the original eab lines. although two other lines had presumably lost the P-element from this site.

A Genomic DNA library has been prepared from the eab line carrying a P-element at position 7E (as well as approximately 15 elements in other chromosomal positions). 50 P-carrying clones have been isolated and are currently being tested for their genomic location.

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ON THE ORIGINS AND FUNCTIONS OF SOME OF THE TANDEM REPEATS FROM THE PRIMATES AND WATER BIRDS

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Studies of the high-copy number tandem repeats of the water birds (ducks and geese) and the primates, have led to, or at least supported, some common conclusions about the role and origins of these major constituents of genomes.

In the case of the human Satellite III, the extreme purity of some of the sequences, sometimes for megabases, the fact that lower molecular weight subsets (4-17kb) exist in multiple copies of exactly the same length and well defined length in clusters and appear to be Mendelianly inherited, together argue for a replicative mechanism of generation and argue for extreme youth. The alternative mechanisms would not seem to readily generate such a spectrum of properties. Unequal crossover would be expected to cause the equal lengthed subsets to exchange pieces and become unequal in length, a telomerase-type mechanism would not generate clusters with such regular size members, transpositional mechanisms would be expected to produce interspersed repeats rather than tight clusters. While unequal crossover and transposition may well operate to some degree, it is argued that they are not the major mechanism. The major mechanism is probably a multiple initiation-replication event that produces a localized unstable polyteny as its first product and this is then resolved into a stable set of tandem repeats.

This implies that replicative "explosions" are frequently, albeit intermittently, occuring in these components of the genome and this is in accord with comparisons between primates and is also in accord with the results of comparisons of the major tandem repeats in the water birds. In these avian genomes it appears that a number of the tandem repeats are held in common between all species but that a definite species may be characterized by one new class of tandem repeats that has only expanded recently. In the case of the human being a similar observation has been made, the species characteristic class of sequences from humans being satellite II (not satellite III).

All this leads to the conclusion that the tandem repeats are a very dynamic component of the genome and supports the old suggestions that they may be commonly involved in speciation in some way.

CONSTRUCTION AND USE OF A YEAST ARTIFICIAL CHROMOSOME LIBRARY TO ISOLATE FATTY ACID DESATURASE GENES FROM THE HIGHER PLANT *ARABIDOPSIS THALIANA*

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The small genome size (100,000 kb) and short generation time (5-6 weeks) which have made *Arabidopsis thaliana* an increasingly popular model organism for conducting plant genetic studies also make it an ideal organism from which to isolate genes using the techniques involved in chromosome walking. Chromosome walking in *Arabidopsis* should be facilitated by the availability of a restriction fragment length polymorphism (RFLP) map constructed by workers from the laboratories of E. Meyerowitz and H. Goodman. This map consists of over 200 RFLP markers so that the average distance between RFLP markers in the genome should be about 500 kb.

In order to facilitate chromosome walking in *Arabidopsis*, we have constructed four independent *Arabidopsis* genomic DNA libraries in a yeast artificial chromosome (YAC) vector. The number and average sizes of the clones contained in these libraries are as follows: 1) 2300 clones of 150 kb; 2) 2100 clones of ~120 kb; 3) 9600 clones of ~50-100 kb; and 4) 6700 clones of ~100 kb. An eventual goal of this lab and several others is to use these YAC libraries to construct a physical map of the *Arabidopsis* genome.

We are currently using this library to walk to several *Arabidopsis* loci involved in fatty acid metabolism. Mutations at one of these loci, the *FAD2* locus, result in plants that contain high levels of mono-unsaturated, and low levels of di-unsaturated, 18 carbon fatty acids. Plants containing the tightest mutant allele of the *FAD2* gene show a greatly decreased tolerance to growth at low temperatures. The *FAD2* locus has been accurately mapped with respect to the *Arabidopsis* RFLP map. YAC clones hybridizing to the RFLP marker closest to *FAD2* have been isolated and are being used to construct a "mini-DNA library" in a plant transformation vector. Clones from this library will be used to try to complement the fatty acid composition phenotype of the *FAD2* mutant. In addition, end probes have been generated from the isolated YAC clones and are being used to identify adjacent YAC clones in case the *FAD2* gene is not present on the originally isolated group of YAC clones. Hopefully, the isolation of this gene, and of other genes involved in fatty acid metabolism, will lead to a better understanding of fatty acid metabolism and will allow the eventual manipulation of oil content and quality in crop plants.

HYPO-MUTABLE AND HYPERMUTABLE STRAIN OF *E.COLI* - A SIMPLE AND GENERAL METHOD TO SELECT THEM

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Thymine-requiring strains of *E.coli* derived by the aminopterin selection method require 25μg/ml of thymine to grow at the normal (*thy+*) rate. When seeded on a broth agar medium containing thymine concentrations < 25μg/ml they form thin flat colonies containing numbers of papillae on their surface. The papillae arise from bacteria in the colony having a second mutation - at either the deoxyribomutase (*drm-*) or deoxyriboaldolase (*dra-*) locus which reduces the thymine requirement for optimal growth to about 2μg/ml. The double mutants grow much faster than the *thy-* parental strain when the thymine concentration is limiting.

This characteristic can be used to select strains having a high or low spontaneous mutation rate by plating bacteria on a broth agar medium which has ≤ 25μg/ml thymine and searching amongst the colonies which appear for those deficient in papillae or those having a surfeit of papillae. Those having no papillae are hypomutators and those having an excess of papillae are hypermutators.

Since the character being scored results from any sort of forward mutation (rather than a reverse mutation) the method does not, in principle at least, select one sort of non-mutator (such as one defective in base substitution) against any other (such as frameshift mutation).

USE OF MOLECULAR GENETIC MARKERS TO DETECT RESIDUAL TUMOUR CELLS IN STEM CELL COLLECTIONS FROM BLOOD AND BONE MARROW

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Twenty seven leukaemia and lymphoma patients have undergone peripheral blood stem cell and/or bone marrow harvesting for future autologous transplantation. Knowledge of the presence of minimal residual tumour cells in the stored stem cell collections determines whether transplantation will proceed. The presence of minimal disease pre-transplantation may necessitate additional chemotherapy, or the reappearance of these cells after transplantation may predict clinical relapse. Detection of residual tumour cells is therefore of paramount importance.

The polymerase chain reaction (PCR) and Southern blot hybridization techniques are used to search for tumour markers in presentation specimens from these patients. The tumour specific marker is then used to detect tumour cells throughout the course of the disease and in all the stem cell and bone marrow harvests. In preliminary investigations a molecular disease marker has been found in 20 patients. PCR detected the translocation t(14;18) in 6 non-Hodgkins lymphoma (NHL) patients, and the translocation t(9;22) in 4 chronic myeloid leukaemia (CML) patients. Southern blotting detected immunoglobulin heavy chain gene rearrangements and T cell receptors β gene rearrangements in another 10 NHL patients.

At present, 5/9 autologous transplant patients have relapsed. Contaminating tumour cells have been found retrospectively in three patients' infused stem cell collections. The testing of other patients stored cells is currently underway.

Molecular techniques, especially PCR, hold great promise in their ability to detect residual tumour cells in those patients in whom a molecular marker is identified.

COMPARATIVE STUDIES OF THE PATTERN OF CHIASMATA DISTRIBUTION AT MEIOSIS IN MALE AND FEMALE MARSUPIALS

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Recombination levels observed in females of the marsupial *Sminthopsis crassicaudata* are very much lower than those observed in the males[1]. Paralleling these observations, chiasmata distribution at meiosis in the female shows a pronounced distal localization which is absent from the male.

These observations are at variance with the generalization that levels of recombination in the heterogametic sex are lower than those in the homogametic sex.

Comparative observations of meiosis in males and females of three further marsupial species[2,3,4] including one from South America, have been made in order to determine whether this unusual result is present in other marsupials. The results of these studies and of casual observations in two further Australian species will be reported.

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EMBRYO SEXING IN LIVESTOCK BY SIMULTANEOUS PCR AMPLIFICATION OF Y- CHROMOSOMAL AND AUTOSOMAL DNA

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A.B. Technology Pty. Ltd. c/o Department of Biochemistry, Faculties, Australian National University, P.O. Box 4, Canberra ACT 2601

The potential for genetics and molecular genetics in livestock production has been underscored by our development of a procedure for rapidly and unequivocally determining the sex of embryos during transfer. Translation of the necessary technology from esoteric laboratory techniques to the routine of production industries has not been without its difficulties, nor its rewards.

The sexing assay is invasive, requiring removal of a small number (2-10) of cells from the embryo for detection of Y-chromosomal DNA by polymerase chain reaction (PCR). One of the two pairs of PCR primers corresponds to sequences separated by approximately 100 bp that are conserved in a ruminant Y-chromosomal repeat, the second to sequences in a major autosomal satellite that are separated by approximately 200 bp. Cycling between appropriate temperatures allows amplification of the intervening Y-specific and autosomal (control) sequences to a level where they are detectable by ethidium staining following agarose gel electrophoresis. Embryonic cells containing a Y chromosome are indicated by two bands - one of approximately 140 bp and one (the control) of approximately 240 bp; embryos lacking a Y chromosome are indicated by the single band of approximately 240 bp. The assay, applicable to all three domestic ruminant species, requires about 3 hours to complete and has been found to be 100% accurate (excepting human error).

Essential refinements to previous embryo splitting technology have increased speed and minimized the reduction of embryo viability during biopsy. Indeed, the system developed for biopsy is now used routinely for artificial twinning - 45 blastocysts can be split within an hour, incurring minimal damage to the embryo. We commonly observe just a 5-10% reduction in pregnancy rate (per half embryo) compared with unsplit whole embryos.

Perhaps the most significant implication of our twinning and sexing technologies is that embryonic marker assisted selection (MAS) has come of age. While the economic and genetic consequences of MAS for sex are significant, this is but the first in an unlimited range of potential applications of similar technology.

PROGRESS TOWARDS A BOVINE GENETIC MAP

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A genetic linkage map is a powerful tool for mapping genes of unknown primary product using a procedure known as Reverse Genetics. As part of concerted international effort, we are constructing a genetic linkage map for cattle consisting of polymorphic DNA markers. Our initial aim is to be able to define homologous chromosomal segments between humans and cattle so that the large number of placements from the human map can be extrapolated to the bovine map.

Linkage mapping is being carried out within a group of selected reference families: these families are a resource for mapping to be shared with other research groups. Large DNA stocks from two and three generation families of *Bos indicus* X *Bos taurus* or *B indicus* strain crosses have been established. To date, 16 full sib families comprising 124 animals have been sampled. The number of offspring in these sibships varies from 10 to 44.

Recombination frequencies (q) derived from Maximum Likelihood Estimated (MLE) of linkage using LODSCORES of comparisons between restriction fragment length polymorphisms (RFLPs) have been estimated. Linkage data on the following genes have been collected, viz., Growth Hormone (GH), protein kinase C a polypeptide (PKCA), Homeobox 3 (HOX3), Gelsolin (GSN), the K-RAS 2 oncogene (KRAS2), Collagen 2 a 1 (COL2A1), mammary tumour virus homolog (INT.1), a-2 macroglobulin (A2M), and a lactalbumin (LALBA). These genes are thought to be located on bovine chromosomes 5 and 19. We show that there is some conservation between humans and cattle in the ordering of these loci. We also show that close linkage can be excluded for some of the genes from chromosome 5.

CLONING AND CHARACTERIZATION OF A GENE NECESSARY FOR POST-BLASTODERM CELLULAR PROLIFERATION

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The gene *pebble* (*pbl*) was uncovered during a screen for zygotic lethals on the third chromosome of *Drosophila melanogaster*[1]. Embryos homozygous for the *pbl* mutation show only a faint ball of cuticle, lacking any definable structure[2,3]. The *pbl* gene does not appear to have a pattern forming roll, but lack of *pbl* product leads to cessation of cell proliferation following blastoderm formation[3].

We have used feulgen staining of embryos to reveal the presence of multinucleate cells early in embryogenesis. Late in development cells of mutant embryos are fewer in number and larger in size. Our interpretation of these observations is that the mutant continues to proceed through the nuclear aspects of the cell cycle, but is incapable of septation. This would result in the multinucleate cells (seen early) of which the nuclei presumably fuse to form large polyploid nuclei (seen late).

Genetic analysis of *pbl* mutants has localized the gene to subdivision 66B on the left arm of chromosome 3. We are currently mapping several markers in this region relative to *pbl* and characterizing a yeast artificial chromosome containing 330 kb of DNA from subdivision 66B[4] to further define the molecular position of *pbl*.

1. Jurgens G., Wieschaus E., Nusslein-Volhard C. & Kluding H. (1984) Wilhelm Roux's Arch 193: pp 283-295
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4. Garza D., Ajioka J.W., Burke D.T. & Hartl D.L. (1989) Science 246: pp 641-646

GENES CONTROLLING THE ESTABLISHMENT AND ELABORATION OF SEGMENTAL PATTERN IN THE *DROSOPHILA* EMBRYO

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The segmental organization of animal bodies, whilst long considered a distinguishing anatomical feature of the Arthropoda, is increasingly being recognized as a fundamental developmental strategy employed by both invertebrates and vertabrates.

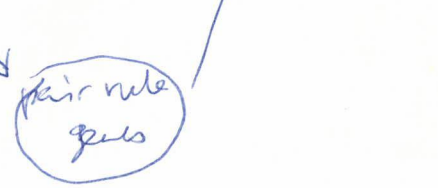
In *Drosophila*, the allocation of cells to discrete segmental primordia occurs at an early stage during embryogenesis, as nuclei respond to a molecular prepattern of positional information generated during the first two hours of development. This prepattern can be visualized in the periodic distributions of pair-rule gene products along the antero-posterior axis of the blastoderm embryo. A central goal of recent research has been to elucidate the mechanisms which control these periodic transcription patterns.

It is now clear that the early embryo is sequentially subdivided into successively smaller domains by the region specific activation of particular zygotically expressed genes in response to the graded distributions of maternally encoded transcription factors[1]. The primary targets of the maternal gene products are the gap genes which are activated in broad overlapping non-periodic domains. Different combinations and levels of these gene products then in turn interact with specific regulatory sequences upstream of the pair-rule genes *hairy* and *even-skipped*, resulting in their polarized periodic expression patterns. The pattern and phase relationship of these two genes plays a fundamental role in regulating the expression of other pair-rule genes, and through them, the segment polarity and homoeotic genes which control cell patterning in individual segments.

During this initial phase of development, the embryo develops as a syncitium. Cells first form after the prepattern has been established, so that the initial patterning events are independent of intercellular communication. After the cellularization of the embryo, pattern is elaborated in each of the segments, a process which requires interaction between cells. These interactions are mediated by the segment polarity genes and consistent with this function, several of these genes have been found to encode components of signal transduction pathways.

1. Ingham, P.W. (1988)Nature 335: pp 25-34

- pair-rule gene have stripe expr.
- maternally encoded gene eg bicoid activates other zygotic genes eg bicoid mRNA localise anterior, but product diffused from ant. → post.
- Upstream of pair-rule genes have regulatory sequences for interaction ∈ gap genes. (like deletion analyses)
- wingless product — patched



FCγRII RFLPs IN SYSTEMIC LUPUS ERYTHEMATOSUS

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b. Research Centre for Cancer and Transplantation, University of Melbourne, VIC
c. Royal Prince Alfred Hospital, Camperdown, NSW

Systemic lupus erythematosus (SLE) is considered to be the prototype immune complex disease and much of the major organ damage is a result of immune complex deposition. It has thus been proposed that these patients may be unable to process immune complexes effectively through a defect which may be primary or acquired as a result of the disease process. The human receptors for immunoglobulin G (FcγR) have a diverse range of functions in immune processes including the clearance of immune complexes. Our aim was to investigate the existence of RFLPs in the FcγRIIα locus and to determine whether particular FcγR/RFLPs were significantly associated with predisposition to SLE.

We identified FcγRII RFLPs in *Taq*I and *Msp*I restricted genomic DNA and found their distribution in SLE did not differ significantly from control. Our results also indicated that part or all of the FcγRIIα locus is duplicated in some individuals (both SLE patients and healthy controls) indicating possible numerical heterogeneity in FcγRIIα like genes.

A SYNCHRONOUS EXPRESSION OF MILK PROTEIN GENES DURING THE DIFFERENT PHASES OF LACTATION IN THE TAMMAR WALLABY

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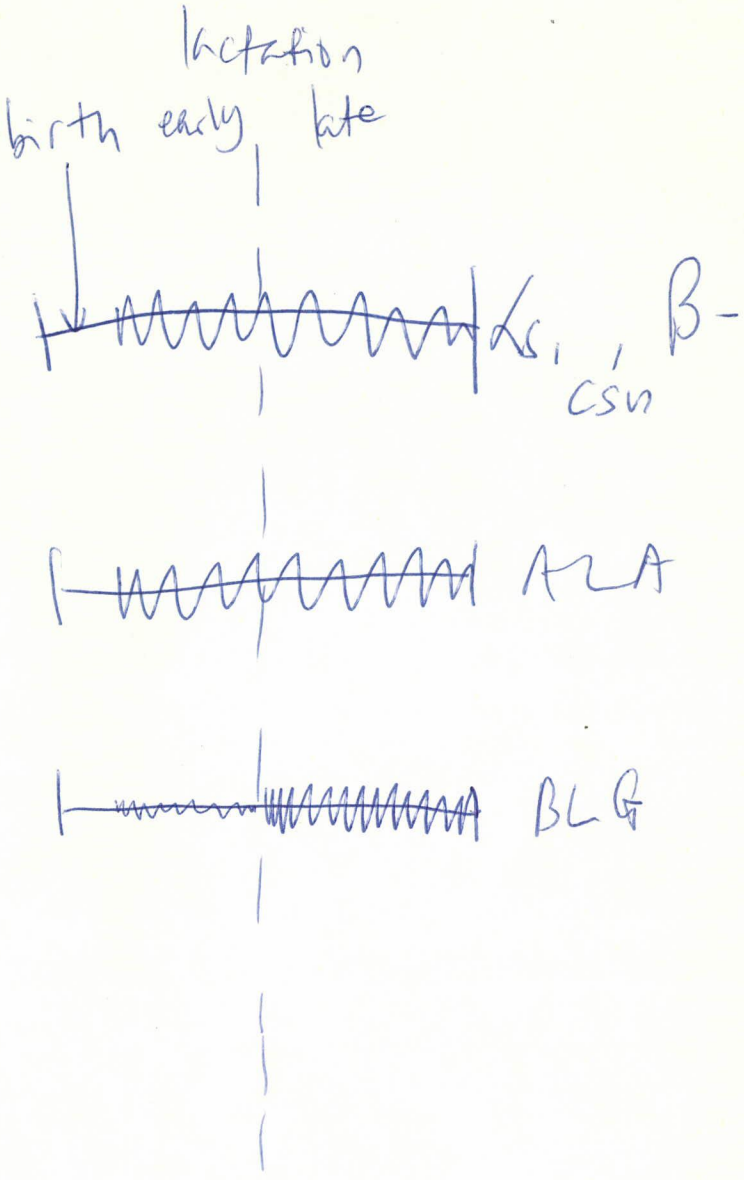
The reproductive strategy of marsupials has evolved differently to that of eutherians with greater emphasis on lactation rather than gestation during development. The neonate is immature and organogenesis continues within the pouch. Milk composition and production alters to accommodate the changing nutritional requirements of the young in the transition to herbivory. In the tammar wallaby (*Macropus eugenii*), two types of milk are secreted. A dilute "early" milk is secreted for about the first 180 days until organogenesis is complete and a lipid-rich "late" milk for the remainder when the young exits the pouch and undergoes rapid growth.

An unusual feature of macropod marsupials is their capacity to suckle two young of different developmental stages simultaneously. A neonate can attach to one teat and receive early milk while a young that has vacated the pouch but continues to suckle receives late milk from an adjacent gland. Histological studies of early and late glands of a dual lactating tammar show their morphology to be different with distended alveoli in late phase. This contrasts eutherian lactation where development of the mammary tissue and milk composition and production is synchronous for each of the glands.

Total RNA from early and late mammary glands was hybridized with cDNA clones encoding the tammar milk protein genes α-lactalbumin, β-lactoglobulin and late-lactation protein (LLP). The levels of α-lactalbumin and β-lactoglobulin mRNA in the late phase gland increased markedly compared to the levels in early gland. LLP mRNA was not detected in the early gland but was present in the late phase gland. Increased levels of α-lactalbumin and β-lactoglobulin mRNA in late phase gland probably reflects the two- to three-fold rise in milk production and the increased ratio of volume of epithelial cells to connective tissue.

The capacity of the epithelium in tammar mammary glands to synthesize two types of milk in adjacent glands simultaneously suggests that the two glands are controlled independently. Control may be either by developmentally regulated changes in responsiveness of the epithelial cells to circulating hormones or by an intrinsic (autocrine/paracrine) mechanism.



GENETIC DIVERSITY AND INTERSPECIFIC LARVAL DENSITY EFFECTS BETWEEN THE CACTOPHILIC *DROSOPHILA* SPECIES *D. ALDRICHI* AND *D.BUZZATII*

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Two distinct *Drosophila* species utilize the patchily distributed *Opuntia* cactus of northern New South Wales and southeast Queensland. *Drosophila buzzattii* is present throughout this region, but *D. aldrichi* is restricted to the warmer, northern part of the cactus range, where both species co-occur and may utilize the same necrotic cladodes. Although inhabiting a wide geographical range, genetic variation and population differentiation are relatively low in *Drosophila buzzattii*. Analysis of 20 enzymes in six populations of *D. aldrichi*, however, suggests this species is genetically more variable (8 loci show common polymorphisms) and differentiation among populations is greater. The smaller size of *D. aldrichi* populations may account for the greater diversity among populations.

Because these two species utilize identical resources in nature, *D. aldrichi* may be restricted in its range by competition with *D. buzzattii*. That *D. aldrichi* persists in the north is most likely a function of greater temperature tolerance. Larval competition for limiting food in the laboratory at 25° C affects adult size and development time for both species but only *D. aldrichi* suffered a decline in survival. Three genetically diverse *D. aldrichi* populations (determined by electrophoresis) and three simultaneously collected sympatric *D.buzzattii* populations were used to identify genetic variation for temperature tolerance and larval competitive ability of both species.

STUDIES OF GENE AMPLIFICATION IN A CELL LINE DERIVED FROM A UV INDUCED SKIN TUMOUR IN THE MOUSE

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The primary aim of the study is to investigate oncogene activation in ultraviolet light induced skin carcinogenesis in the hairless mouse model. Proto-oncogenes are activated by three major mechanisms: amplification, point mutation and rearrangement.

We have observed double minute chromosomes in a cell line derived from a mouse skin squamous cell carcinoma induced by UV radiation. Double minute chromosomes are one of the karyotypic abnormalties indicative of gene amplification. Given that amplified genes are overexpressed, we initially screened for overexpression of oncogenes using a reverse dot blotting procedure which uses radioactive labelled cDNA reverse transcribed from total cellular poly-A+ RNA to screen a panel of oncogenes spotted on a membrane. Out of 16 oncogenes surveyed, only *myc* appeared overexpressed in the reverse dot blot. Northern hybridization confirmed that *myc* is highly expressed in the cell line. Southern blotting was then used to individually screen all 16 oncogenes for amplification. An additional 2 oncogenes, and 3 other genes which have been found to be amplified in tumours were also screened. No evidence was found for amplification. As more than 40 oncogenes have been discovered up to date and new oncogenes are continually being discovered, it has not been feasible to screen for amplification of all the oncogenes by Southern hybridization. However, it is now feasible to clone the amplified sequences due to the development of a new technique termed in-gel renaturation-SINE hybridization, which has been used for detection and cloning of amplified genes associated with multiple drug resistance in mammalian cells and the *gli* gene amplified in a human glioma.

BORAGE *BORAGO OFFICINALIS* DOES NOT HAVE A MULTI-FACTORIAL SELF-IMCOMPATIBILITY SYSTEM

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Crowe[1] claimed that borage has a polygenic self-incompatibility system that operates by embryo abortion. A single borage flower can set from 0 to 4 seeds. Open pollinated borage sets an average of less than 2.5 seeds under field conditions. Hypothetical models of polygenic self-incompatibility can predict a much higher level of ineffective pollination than oligogenic self-incompatibility[2]. We set up crosses to detect systematic patterns in seed set. None was detected. Seed set declined approximately proportionally with increasing closeness of inbreeding. Even selfs of plants produced from sibs, however, set an average of more than one seed per flower[3].

Cytological studies showed that there is no embryo abortion.

Some 20 isozyme loci were assayed in samples from some 14 separate acquisitions. Only three systems were polymorphic, and then only with a small number of alleles. Several samples were monomorphic at all loci. These results are not consistent with a mainly outbreeding reproductive system.

Crosses using isozyme markers showed that open pollinated plants in the population were largely selfed.

We conclude that borage has no self-incompatibility system.

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2. Mayo O. & Leach C.R. (1989) Quantitatively determined self-incompatibility. I. Theoretical considerations. Theor. Appl. Genet. 77: pp 375-378
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REGULATION OF COPPER METABOLISM AND RESISTANCE IN *ESCHERICHIA COLI*

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Copper is an essential micronutrient, but is toxic in excess. In *Escherichia coli* a plasmid-borne determinant (*pco*) confers inducible resistance to copper. In addition to this resistance *E. coli* must have a basal level of copper metabolism. A series of chromosomal mutants in copper metabolism (*cut*) have been isolated and characterized in terms of uptake-accumulation and export. The *pco* determinant does not produce any detectable resistance in two types of chromosomal mutants, namely, *cutA* (an uptake mutant) and *cutD* (an efflux mutant); in addition there is a trans-acting regulatory function (*cutR*). The nucleotide sequence of the plasmid-borne copper regulatory gene (*pcoR*) shows a high degree of homology with the sensor/regulator type of "signal transduction" sytems. A plasmid promotor clone which is copper inducible in the absence of functional plasmid genes has been isolated of potential copper sensor mutants. We have developed a Cu model of the regulatory system and the interactions between the chromosome (*cut*) genes and the plasmid coded resistance (*pco*) genes.

CYTOGENETICS OF NATURAL HYBRIDS WITHIN A DIPLOID-TETRAPLOID COMPLEX OF FROGS

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b. Department of Zoology, University of Western Australia

Four of the ten species of the genus Neobatrachus are bisexual tetraploids. The distribution of these species is broadly allopatric with some regions of parapatry. Conversely, the diploid species show a variety of distribution patterns; some species have broad zones of sympatry, others are parapatric and others are allopatric. When the distribution of the diploid and tetraploid species are considered together the pattern is complex.

Natural hybridization between two of the tetraploid species has been detected using chromosome markers in a region of parapatry. The resultant F_1 tetraploid hybrids are viable and fertile, and backcross individuals have been detected. There is some evidence that these backcrosses have reduced fecundity. Hybridization between diploid and tetraploid species has been detected at three locations and the resultant F_1, triploid hybrids are viable but show greatly reduced fecundity. In both these situations the distribution pattern of the species that hybridize is parapatric, and the number of hybrids detected is relatively high. Hybrids between diploid species have also been detected. The finding of triploid hybrids resulting from the crossing of two diploid species, suggests that hybridization may have been involved in the original formation of polyploids. This does not imply that the polyploids must be allopolyploids.

- Interesting inactivation.
- Highly conserved in eutherian
- Comparative mapping on human vs marsupials. Difference 150-170 my
- In situ + somatic cell hybrids:
  Difficult to make hybrids as marsupial Xsomes don't retain.
  Screen hybrids for isozymes ⇒ Xsome assigned.
  Now use Southern.
- All long arm genes are conserved. But not short arm eg 2 genes on X1 (marsupial)
- No X specific fragment for marsupial but on X1 + X5
- Short arm represent ancestral state?
  Mouse/man same for X, but not marsupials.
  Possible that genes on short arm in human are originally autosomal.
  Some genes in short arm don't affected by inactivation.

EVOLUTION CHROMOSOMES A

Jennifer A. Marshall Jame

a. Department of Genetics University, Bundoora

b. Present address Beckma Centre, Duarte. Califo

We have use hybrids and *in situ* h probes to localize g monotreme species.

Comparison content in eutheri mammals has reve equivalent to the ma conserved on the X contradiction of conservation seems chromosome inac conserved region monotreme X, whi subject to inactivati

A large pa by genes on the sh from the X in bo instead, these gen autosomes. The re is therefore reveal chromosome in explaining some o regions of incom Xp). Since the X-l sex determining g and, like the othe marsupials and m gene serves a sex c

DNA GENOTYPING FOR PEDIGREE VALIDATION OF CATTLE

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Embryo transfer and artificial insemination are the most dramatic results of genetics applied to livestock production, accelerating both vertical gain in studs and lateral dissemination of superior traits through the production industries. The absence of physical joining of a sire with a dam, however, leads to an increased probability of incorrect parentage assignment. Although blood typing (generally for BoLA antigens) allows pedigree confirmation with a high degree of probability, it can not offer definitive proof of alternate parentage.

DNA genotyping for allelic RPLPs offers a solution. We have applied this approach in response to requests by cattle breeders faced with a variety of probems: blood typing had disqualified the sire or dam; embryo transfer calves were not true to breed; an untyped semen donor was no longer available; embryo donor dams died before being blood typed; and so it goes....

DNA was purified and restricted *in situ* from semen, peripheral blood lymphocytes or post-mortem tissue tissue homogenates that had been set in small blocks of low melting temperature (LMT) agarose[1]. Blocks containing equal amounts of digested DNA from the progeny and all possible sires and dams were melted and applied to agarose gels from parallel electrophoresis and alkaline Southern blotting[2]. The transfer membranes were hybridized with radio-labelled probes of cloned human polymorphic DNA sequences.

The most informative probe available for routine parentage assignment was found to be the 3'A-globin VNTR[3], which usually gave clear evidence of Mendelian inheritance of allelic polymorphisms for a variety of restriction fragments. Nevertheless much work remains to be done at both the population and molecular levels.

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2Reed K.C. & Mann D.A. (1985) *Nucleic Acid Research* **13**, pp 7207-7221

3Higgs D.R., Goodbourn S.E.Y., Wainscoat J.S., Clegg J.B. & Weatherall D.J. (1981) *Nucleic Acid Research* 9; pp 4213-4224

SELECTION AT THE DIELDRIN RESISTANCE LOCUS IN OVERWINTERING POPULATIONS OF THE AUSTRALIAN SHEEP BLOWFLY

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The development of wandering larvae entering the ground was monitored over a 3 year period. Development was arrested in larvae placed in the ground in May/June.

The proportions of overwintering larvae that reached the adult stage of the life cycle were low (range 2.8-6.0%) compared to those at other times (52.8-80.0%). the developmental rate was greatest during the late summer months. In laboratory controls the developmental times from egg to adult (13.01-14.0 days) and the proportions reaching the adult stage (78.0-96.0%) were consistent throughout the experiment.

The larvae used in the experiment were of the F_2 generation of an original cross between pure-breeding strains that were resistant (*Rdl/Rdl*) or susceptible ($+/+$) to dieldrin. The frequency of the *Rdl* allele in adults emerging from the overwintering population was significantly lower (0.90-0.15) than at other times (0.44-0.52) when the results were similar to laboratory controls (0.43-0.53).

Samples of pre-pupae placed in the ground in May 1988 were removed at 30-day intervals. A consistent decline in the proportion reaching the adult stage and in the *Rdl* frequency of these populations was observed with increasing time in the ground. Laboratory trials, in which pre-pupae were held at 8°C for periods of up to 11 weeks, showed similar trends to those observed in the fields studies.

INTERACTIONS OF BEHAVIOURAL AND LETHAL ALLELES AT THE STONED LOCUS IN *DROSOPHILA MELANOGASTER*

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The *stoned* neurological mutation of *Drosophila melanogaster* exhibits an unusual jump response to a light off stimulus, which is correlated to an enhanced off-transient in the electroretinogram and increased protein phosphorylation of neuronal substrate. Several lethal and behavioural alleles, which are viable as *stoned* heterozygotes, have been characterized with respect to these phenotypes. A suppressor mutation (*sustn*) which suppresses both the behavioural component of the *stoned* phenotype as well as the reduced viability of *stoned* alleles, also reduces the enhanced off-transient of the *stoned* electroretinogram. This suggests a causative relationship between the increase in the amplitude of the off-transient and the increased jump frequency in *stoned* flies.

The cyclic AMP-dependent phosphorylation of a synaptosomal protein is increased in *stoned* extracts. Furthermore, this protein exhibites low levels of phosphorylation in the *tan* mutation, which has no off-transient. By constructing double mutants, the interactions between *stoned* and other mutations affecting the nervous system and second messenger cascades (such as *tan* and *dunce*) have been examined. These interactions are discussed at the molecular, physiological and behavioural levels.

EVOLUTIONARY DYNAMICS OF MITOCHONDRIAL GENE DUPLICATIONS IN PARTHENOGENTIC LIZARDS

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Mitochondrial DNA (mtDNA) from triploid parthenogenetic geckos of the *Heteronotia binoei* complex varies in size from 17.2 to 27.6 kilobases (kb). Comparisons of long and short genomes using restriction endonucleases revealed a series of tandem direct duplications ranging from 1.2 to 10.4 kb. This interpretation was supported by transfer-hybridization experiments which also demonstrated that coding sequences were involved. Some of the duplications have been rapidly modified by deletion and base substitution, but no other rearrangements were detected. Analysis of the phylogenetic and geographic distribution of length variation suggests that duplications have arisen repeatedly within the parthenogenetic form which is itself of recent origin. The absence of duplications from the mtDNA of the sexual populations of *H. binoei* reinforces the previously observed correlation between nuclear polyploidy and duplication of mtDNA sequences. In comparison to the genomes of sexual *H. binoei* and most other animals, the mtDNA of these parthenogenetic geckos is extraordinarily variable in length and organization.

POPULATION GENETICS OF THE GREEN TURTLE, *CHELONIA MYDAS*, IN AUSTRALIA

Janette Norman

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Green turtles exhibit an unusual pattern of reproductive behaviour involving long-distance migrations from feeding grounds to nesting sites, an irregular remigration cycle (3-6 years for females and 1-3 years for males), and strong nest site fidelity. Female green turtles usually return to the same location to nest within and between seasons, which has led researchers to propose that females are returning to their natal beach. A consequence of this behaviour should be a restriction on female-mediated gene flow between rookeries. This was examined using mitochondrial DNA restriction site analysis.

Results so far indicate the presence of at least three discrete populations characterized by fixed or nearly fixed restriction site patterns and low levels of within population variation. There are fixed restriction pattern differences between east and west coast populations of a similar magnitude to that found between Atlantic and Indo-Pacific populations which have presumably been separated for 3 million years[1]. The two major populations on the east coast of Australia show a significant difference in the frequency of mitochondrial DNA haplotypes. These results are consistent with the natal homing hypothesis.

1. Bowen, B.,Meylan, A., and Avise, J. (1989) Proc. Natl. Acad. Sci. 86:573-576

STUDIES OF THE MOLECULAR AND BIOLOGICAL ROLES OF THE *ROUGH* HOMEODOMAIN

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The *rough* gene offers advantages for the analysis of the biological role of the homeodomain. Genetic transformation with this gene is possible because it is small (less than 7kb). Homozygous *rough* mutants are viable, so transformed genes can be examined in a fly which lacks the normal *rough* gene product. We have made a series of constructs in which the *rough* homeodomain is replaced by homeodomains of varying relatedness. We are currently analyzing the results of replacing the normal *rough* gene with the *in vitro* mutagenized gene.

The *rough* gene of *Drosophila melanogaster* is required for formation of the proper pattern of photoreceptor cells in the developing eye disc of *Drosophila melanogaster* and is expressed in a pattern that is consistent with the mutant phenotype, i.e. in the region of cellular pattern formation in the developing compound eye[1]. Tomlinson et al[2] have postulated that the *rough* gene is required for the expression of an intercellular signal that induces differentiation in neighbouring cells. We report here the use of the *rough* gene to further characterize molecular and biological properties of the homeobox group of genes.

CHEF

One common feature of the homeobox group of genes in *Drosophila* is their ability to regulate their own expression. We have generated a point mutation which exhibits all the properties of a null mutation of the *rough* gene. Using *in situ* hybridization to tissue sections we have shown that the level of transcript in tissues expressing the mutated mRNA (but a non-functional *rough* gene) are similar to the levels found for the normal *rough* gene. If the gene is positively or negatively self-regulated, the mRNA levels would have decreased or increased accordingly. We propose that the function of the *rough* gene does not involve self-regulation, unlike the function of other homeobox genes characterized to date.

We have used gel retardation techniques to show sequence specific DNA binding activity of the *rough* homeodomain. In particular, we have engineered the expression of the homeodomain as a discrete peptide. Expression and purification of the product was confirmed by protein sequencing. The purified domain folds into a configuration that permits sequence-specific binding to both the *engrailed* and *Ultrabithorax* consensus binding sequences, raising questions about the biological specificity of the homeodomain.

- Rough : single base Δ ⇒ premature termination.
- Rough + ant-p differences (although homebox genes conserved (~60 aa)

differences imp+ in DNA binding?

- Rough domain has binding sequences - gel retardation assay

because α helix 3 sequence is highly conserved.

- After α Helix 3 region ⇒ this get fro. No specificity in Helix 3?

but Put rough into engrail domain ⇒ no function.

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BIOCHEMISTRY AND PHYSIOLOGY OF ESTERASES IN A WILD TYPE AND AN ORGANOPHOSPHATE RESISTANT STRAIN OF AUSTRALIAN SHEEP BLOWFLY

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One wild-type and one organophosphate resistant strain of *Lucilia cuprina* have been compared for developmental expression and tissue distribution of eight esterases. These data, combined with substrate specificities and inhibition studies determined for six of these esterases, revealed much similarity between the two strains. The only major difference found is the failure of one esterase (E3), in the resistant strain, to utilize a and b naphthyl acetates, E3 gained an increase in hydrolytic activity against organophosphates. Expression of E3 occurs in larval and late adult stages, which are exposed to insecticide treatments, and in digestive tissues, which would be the first line of defence following ingestion of insecticide. These observations lend support to the hypothesis that E3 is directly responsible for organophosphate resistance in *Lucilia*.

MENDELIAN FACTORS UNDERLYING QUANTITATIVE TRAITS: CHROMOSOMAL LOCATIONS, PHENOTYPIC EFFECTS AND EVOLUTIONARY RELATIONSHIPS

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The genetic basis of quantitative inheritance, once a hotly-contested question in the history of genetics, can now be systematically determined using genetic maps of linked DNA markers. In crosses between the domestic tomato (*Lycopersicon esculentum*) and two of its wild relatives, *L.chmielewskii* and *L.cheesmanii*, we have studied the genetic basis of quantitative variation in mass per fruit, soluble solids concentration and fruit pH. The chromosomal locations of individual "quantitative trait loci" (QTLs) can be determined, to a resolution of 10-30 centimorgans (cM) in two-generation crosses, and pinpointed to 1-3 cM in subsequent generations. Individual QTLs have been found to show additive, dominant, recessive or intermediate modes of inheritance. Individual QTLs have different degrees of influence on phenotype; only a small number of QTLs have large effects, while many QTLs have smaller effects. In two distantly-related *Lycopersicon* species, about half of the QTLs mapped in two-generation crosses fell in similar locations, suggesting that some of the same genetic factors may influence quantitative traits in the two species. This similarity in location of QTLs suggests that comparative QTL mapping may be feasible. In the future, simpler techniques for identifying and mapping genetic markers may facilitate gene mapping in a variety of organisms.

SEQUENCES FROM *LUCILA CUPRINA* WITH HOMOLOGY TO RETROTRANSPOSONS FROM *DROSOPHILA MELANOGASTER - LUCILIA* TRANSPOSABLE ELEMENTS?

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Various transposable elements from *Drosophila* have been used in an attempt to identify similar sequences in Australian sheep blowfly. Subclones (from 297, B104, mdg3, gypsy, copia, and the I-element) containing conserved domains from reverse transcriptase coding regions, as well as a full length hobo element, have been nick-translated and used to probe Southern blots of blowfly total genomic DNA at low stringency. The 297 and B104 derived probes gave the strongest signals after one week exposures and were used to screen a *Lucilia* genomic DNA library. The number of hybridized plaques on the library filters from both screens indicate a copy number in the vicinity of 50-100 per genome.

Of the numerous clones purified, DNA from six of the "297" clones have been looked at and appear to have related, though not identical, restriction patterns. Homology to the original *Drosophila* 297 probe has been localized to a particular band or bands of the various digests and is clearly limited in extent. The smallest hybridizing fragment so far isolated, a 1.4 kb *Hind* III fragment, has been subcloned and shown to hybridize specifically to the latter half of the reverse transcriptases, but does not include the "YXDD" box.

Sequencing of the 1.4 kb fragment is underway to determine its status as part of a potential reverse transcriptase gene.

CAN GENETIC DRIFT DURING AN ANCIENT BOTTLENECK ACCOUNT FOR THE PRESENT INCIDENCE OF CYSTIC FIBROSIS?

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In Caucasians, mutant alleles causing cystic fibrosis (CF) occur at frequencies of 0.016 to 0.025, the highest known for any human recessive lethal gene. In Asians and Africans, this frequency is less than 0.004[1]. In 1968, Wright & Morton[2] calculated a 0.12% chance that sampling error, i.e. genetic drift, might account for these between-race differences. This remains the accepted likelihood of such an event[3]. Since there are many hundreds of human lethal genes, a 0.12% chance should ensure that approximately one such lethal reaches a frequency equivalent to that of CF.

Recent data on DNA sequence allows insight into pre-historic fluctuation of human population size[4], events that should impinge upon the probability of genetic drift. While these data remain controversial, several lines of evidence suggest a temporary reduction in population size at the time of divergence of the Caucasian race. Given such data, it is possible to model effects of selection and drift acting over many generations. Such a model, based on Kimura's pseudo-random sampling procedure[5], can simulate gene frequency change in periods during and following a population bottleneck.

With a bottleneck of size N_e=5000 lasting 1000 generations (and $u=3.3 \times 10^{-5}$), the model suggests a 0.2% chance that the CF allele will reach p=0.02. However, in the subsequent expansion of the population, there is a much smaller chance (0 cases in 10,000 trials) that such a frequency would persist for more than 150 generations (or 3000 years). Varying the parameters within plausible limits does not alter this conclusion.

Since the putative bottleneck occurred much more than 3000 years bp[4], such an event is very unlikely to account for the present frequency of CF.

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ISOLATION OF A GENOMIC CLONE PARTIALLY ENCODING KANGAROO HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE: IDENTIFICATION OF ACTIVE-X-SPECIFIC METHYLATION IN THE KANGAROO HPRT GENE

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X-chromosome dosage compensation in eutherian and metatherian mammals is achieved by inactivating one X-chromosome in female somatic cells. In eutherians, X-inactivation is random (ie either the paternal or maternal X can be inactivated) and the inactive state is (at least for housekeeping genes) stably maintained in all somatic tissues. In metatherians however, the paternal X is preferentially inactivated in certain tissues. The greater stability of random (RXI) versus paternal (PXI) X-inactivation may be due to 5′ hypermethylation of housekeeping genes on the eutherian, but not the marsuplial inactive-X. However, since this hypermethylation occurs after X-inactivation has occurred, the underlying inactivation mechanism may be similar for both RXI and PXI. If so, the timing of differentiation events may dictate the choice of X to be inactivated, since PXI also occurs in eutherian extraembryonic membranes, which are the earliest differentiating euutherian tissues. Thus the eutherian paternal X may carry an imprint which initially directs its inactivation, but which is lost by the time the embryo proper develops.

In order to study metatherian PXI we have isolated a clone which partially encodes the X-linked gene hypoxanthine phosphoribosyltransferase (HPRT) from a *Macropus robustus*-λEMBL4 genomic library. Restriction fragments containing coding sequences were identified by hybridization with mouse HPRT cDNA. Following M13 subloning and sequencing of these fragments, exons 4, 6, 7 and 8 were identified by homology to mouse exons. Despite the large evolutionary distance between kangaroos and eutherians the HPRT coding sequences were extremely well conserved, the base pair homology with human, mouse and hamster sequences being ≥ 82% for all 4 exons. Most of the nucleotide changes were degenerate, with only two of the 91 amino acids coded for by these exons changing between kangaroo and human sequences. Comparison of kangaroo, human, mouse and hamster amino acid sequences indicates that the kangaroo sequence is closest to the ancestral sequence. The methylation state of the gene in male and female animals was also examined by digestion of genomic DNA with the methylation sensitive endonuclease Hpa II and probing with the isolated *Macropus* HPRT clone. At least two sites which appear to be methylated only when on the active X have been identified and further studies searching for additional sex-specific differential methylation sites by use of a second methylation sensitive endonuclease (Hha I) are in progress.

GENETIC VARIATION OF *ACACIA MELANOXYLON*. COMPARISON OF ISOZYME AND 5S rDNA LOCI FOR POPULATION GENETIC STUDIES

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Acacia melanoxylon (blackwood) is an Australian native furniture timber. It is distributed along the east coast of Australia from southern Tasmania to Atherton in Queensland. An electrophoretic comparison of variation at 30 presumptive isozyme gene loci was performed for 27 populations over the distribution of the species. Relationships between the populations were deduced using phenetic procedures.

Allozyme analysis showed two distinctly different groups (Nei 1978) genetic distance = 0.33) dividing the species into a northern and a southern group. Mean heterozygosity levels were high and averaged 0.179 but northern populations were much [illegible] (0.123) than southern populations [illegible] the percentage of polymorphic loci [illegible] high (55.3) but was 42.21 in the north and [illegible]4 in the south.

These results are compared with results of restriction fragment length variation in the 5S rDNA locus.

These data will be used to discuss the evolutionary relationships within *Acacia melanoxylon*, comparing isozyme and molecular analysis.

GENETICS IN PRIVATE ENTERPRISE

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"With the advent of recombinant DNA, a new biotechnology swept the scientific and commercial worlds. We were the neo-Prometheans bringing hope of feeding the world, providing new drugs and making older drugs more accessible, detecting pathogens, curing disease, building fitter plants and animals. Gosh, in time we could even build fitter humans." The fire ignited financial interest and enterprises were built on the visions of the brightest and the best - an entire new industry was born and many scientists welcomed the opportunities. New pressures, too, were imposed as those scientists met the realities of the market place. We found it was not enough to explore and explode into one or two exclusive niches, for competition at the frontier is intense and intellectual property is fragile. In time, the mundane imperative of short term cash flow imposes a crushing burden even on venture capital.

We are now at the crossroads. We have seen the rise and fall of countless companies and must ask if it is realistic to continue thinking of businesses based solely on biotechnology. And where does genetics fit in? As a discipline it provides the framework for the molecular biology on which biotechnology is now based. But it's no accident that we find the overwhelming majority of research and applied applications of genetics in publicly funded institutions. It's true that genetics finds immediate application in plant and animal breeding, in understanding and diagnosing human genetic disease, in the expanding areas of linkage mapping and "DNA Fingerprinting", in the study and treatment of pathogenic organisms, in recombinant processes involved in manufacture of pharmaceuticals and drugs, in protein engineering and transgenesis. But which of these fields constitute viable commercial enterprises? Which can promise extraordinary financial returns within ten or even five years and which can generate healthy profits in two?

The science of genetics will continue to underpin biotechnology and provide the knowledge crucial to many private enterprises. Genetic research, though, is expensive and its benefits distant, perhaps too distant for the commercial world. *Quo Vadis?*

THE HUMAN GENOME PROJECT

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The aim of the Human Genome Project is to generate the DNA sequence of the entire human genome within the next 10-15 years. There are a number of U.S. and international agencies involved in the Genome Project and a bewildering number of new abbreviations and acronyms. The roles of some of the agencies will be outlined, these include DOE, NIH, HUGO and CEPH.

The genome research being carried out in the author's Department will be summarized. There are two major projects. The first is the correlation of the physical and genetic maps of human chromosome 16. This project aims to divide this chromosome into about 50 intervals of average size 2Mb by the construction of human\mouse somatic cell hybrids containing different naturally occurring rearrangements of chromosome 16. This hybrid panel will be used to map cloned genes and anonymous DNA fragments, most of which are polymorphic, by Southern or PCR analysis. At least one polymorphism in each physical interval will be determined on the CEPH panel of families. The other major project is directed towards cloning the fragile X and depends upon somatic cell hybrid construction, *in situ* hybridization, PFGE and YAC cloning for physical mapping and linkage analysis in CEPH and fragile X families for genetic mapping.

POPULATION GENETICS AND COUNSELLING FOR THE FRAGILE X SYNDROME UNDER THE X INACTIVATION MODEL OF LAIRD

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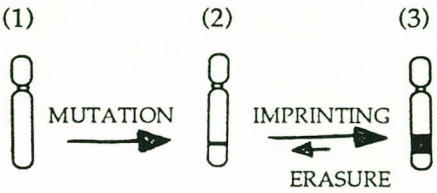
Laird[1] postulates that the fragile-X syndrome is caused by 'imprinting' of a mutant chromosome. The imprinting event results from a block to re-activation of an inactive (Lyonised) X-chromosome prior to oogenesis. There are three states of the X chromosome region under the model:



	Normal (X^+)	Non-imprinted (X^f)	Imprinted (X^{f*})
Males	Normal	Transmitting	Affected
Females	Normal	Imprinting	Semi-affected

The population consequences of this model can be calculated by extension of the theory for X-linked genes. Since fragile X males rarely reproduce, selection against the mutation must be opposed by a substantial force. It is argued that increased fertility of either class of fragile-X carrier females is unlikely to be sufficiently high to constitute a counter-balancing force, and therefore that a high mutation rate to the mutation must be postulated. Under these conditions, the expected equilibrium frequencies for the four classes of fragile X carriers are:

	Female	Male
Non-imprinted	$4u + 2v$	$2u + v/2$
Imprinted	$\frac{2u + v}{1 + s}$	$\frac{2u + v}{1 + s}$

where u and v are the mutation rates in females and males respectively, and s is the selective disadvantage of imprinted fragile X females. The results show that females who carry the imprinted chromosome should occur as often as males. However only about 20% of pedigrees are ascertained through affected females. The theory also predicts that a large percentage of fragile X mutations are newly arisen. It is argued that cloning of the gene, or perhaps very closely linked regions, will allow this prediction to be tested.

The Laird model can explain the variation in imprinting frequencies between different females (the Sherman paradox). The model postulates that there are very few precursor gonial cells, probably two, at the stage at which X-inactivation occurs. This variability in imprinting frequencies leads to some unexpected biases, due to the fact that females who imprint at high frequency are ascertained at a higher rate than females who imprint at low frequency. Overall, the expected frequency of imprinting among females in ascertained pedigrees is about 0.67, compared to a theoretical value of 0.5. It is estimated that transmitting males, who produce no affected offspring, and females who imprint at rate zero, are both ascertained with probability about 20%.

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GENETIC MARKERS FOR INDIVIDUAL ACUTE LYMPHOCYTIC LEUKAEMIAS: A SENSITIVE TEST, USING THE POLYMERASE CHAIN REACTION (PCR)

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There is great diversity in the human humoral immune system: a million or more immunoglobulin heavy chain (IgH) genes, each occurring only in a minute fraction of B-cell clones. A leukaemia is an expansion of one particular B-cell clone. The cells of a leukaemia will thus have a characterisitic IgH gene, which could be used as a genetic marker of that clone.

Using PCR, we amplified, cloned, and sequenced chain determining region 3 (CDR3), a hypervariable part of IgH genes, from four leukaemic patients. The sequences differed greatly from each other and from published sequences, showing the diversity of IgH genes, and suggesting that the IgH sequence could serve as a genetic marker of the individual patient's leukaemic clone. For one patient, we designed two PCR primers to amplify CDR3 of her leukaemic clone. These primers detected DNA from leukaemic cells diluted 1:10,000 in DNA from other lymphocytes (from peripheral blood). They also detected the leukaemia in DNA from the patients' bone marrow smears, in which standard histology failed to reveal any leukaemic cells (implying a population of 2% or less). These results show the high specificity and sensitivity of PCR for detecting traces of leukaemia. Such a test could warn early of recurrence of the disease after apparent cure. It could also improve monitoring of cytotoxic therapy.

LABORATORY SELECTION OF DIELDREN AND DIAZINON-RESISTANT STRAINS OF THE AUSTRALIAN SHEEP BLOWFLY FOLLOWING MUTAGENESIS

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Dieldrin and diazion-resistant strains fo Lucilia cuprina have been generated in the laboratory by screening Standard Wild-type flies after EMS mutagensis. Phenotypic characterisation of the homozygous dieldrin and diazinon-resistant strains showed the field selected and EMS-induced strains expressed similar responses t increasing concentrations of dieldrin and diazinon-resistant EMS-induced strains indicated the EMS-induced mutations lie in the vicinity of the field selected resistance locus in both cases, and are likely to be alleles of the field-selected loci.

GST 1
X1 near centromere on short arm

ADVENTURES WITH MULTIPLE PEAKS FROM *IN SITU* HYBRIDIZATION; THE CASES OF HUMAN UBIQUITIN, CYCLINE AND GLUTATHIONE S-TRANSFERASE, AND MOUSE 11-2, GENE PROBES

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A ^{3}H-labelled probe containing 2.2 human ubiquitin coding units hybridized *in situ* to numerous sites on the human chromosomes; most strongly to the short arm of chromosome 17 at bands 17p11.1-17p12 with significant secondary peaks at 12q24.2-25.32 and 2q21-24.1. A second probe containing the entire intron of UbB identifies 17p11.2 as the likely site of this polyubiquitin complex. A third probe containing the intron of one of the ubiquitin fusion genes hybridizes to a broad region of the distal end of 12q but most strongly, and most intriguingly, to 19p; this suggests that this intron is shared with a gene other than ubiquitin.

A human cyclin probe hybridizes to multiple sites, with the tallest peak, at 20p13, representing the gene and a secondary peak at Xp11.4 which is known to represent a pseudogene. A third peak at 11p15.1 is not in the literature but it is likely to be a second pseudogene.

for mitosis, after that degradated

A human GST-3 probe produces a primary peak over band 11q13, a localization predicted from the literature, but an unexpected secondary peak was found over bands 12p13-q14. These two localizations are of interest because of the unusual similarity of chromosomes 11 and 12 in humans.

common origin in mam & some similar genes.

In the mouse a probe for the T-cell growth factor gene, *11-2*, localizes to bands B-C on Chromosome 3 and there is a secondary peak over band A5 on Chromosome 11. The site on 11 is very close to that of the main cluster of murine cytokine genes and could indicate the presence of a *11-2*-related gene.

Lastly, *in situ* hybridization of many probes to human chromosomes shows a small peak of grains over bands 17q22-24. The significance of this peak remains unexplained.

DETECTION BY *IN SITU* HYBRIDIZATION OF TWO SITES OF INSERTION OF A SHEEP INTERMEDIATE FILAMENT GENE IN TRANSGENIC *MUS MUSCULUS*

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We investigated the male and female F1 progeny of a transgenic male *Mus musculus* created by injection of a sheep wool keratin intermediate filament type II (IF type II) gene into the male pronucleus of a fertilized ovum. Like his father, the male contained approximately 250 copies of the sheep gene and both of these males shared a phenotype of stable cyclic hair loss and regrowth (Powell and Rogers, 1990, EMBO J:9[5]: in press). The female contained only 5-10 copies of the sheep gene and showed a normal phenotype.

Chromosomes with 5BrdU incorporated into the pale G-bands were prepared from cultures of splenic lymphocytes stimulated with concanavalin A. Probes were labelled using nick translation and three ^{3}H-nucleotides to specific activities of 1-2.5 X 10^{8} cmp/ug. *In situ* hybridization was by standard methods and the slides were treated with Hoescht 33258 and 350 nm UV before staining.

Initially the probes used were i) intron 1 (900 bp) of the sheep IF type II gene in pGEM, ii) 450 bp of the 3′ non-coding region of a mouse K14 IF gene in pGEM and iii) pGEM as a vector-only control. Because of the presence of about 100 bp of pBR sequence in the transgenosed construct of the sheep genes, the vector-only control was not negative. The experiments were repeated using as probes: iv) a 600 bp cDNA sequence from the 5′ flanking region of the sheep IF type II gene and v) the above mouse K14 IF 450 bp insert.

The small number of copies of sheep IF type II genes in the female transgenic mouse were found to be inserted into Chromosome 2 near the centromere, probably at band 2C1. The larger number of sheep genes in her brother were inserted into Chromosome 11, probably at band 11A3. The location of the murine K14 IF gene were confirmed to be distal from the centromere on Chromosome 11, probably at 11D1-E1.1.

HUMAN GENOME VARIATION: THE USE OF RFLPs AND PCR IN FORENSIC SCIENCE

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A feature common to "satellite" sequences in the human genome is inter-individual variation in the number of unit repeats within a tandem array. This variation is found in the mega-base ("macrosatellites"), kilobase ("midi and mini-satellites") and the sub kilobase range ("micro-satellites").

"Mini-satellites" are used for Restriction Fragment Length Polymorphisms (RFLPs). Current systems use the biotin labelled probes, *YNH24* and *TBQ7*, with *Hae III* as the restriction enzyme. Our database for *YNH24* (locus D2S44) consists of 425 individuals and exhibits 49 alleles ranging in size from 1-9 kb. The database for *TBQ7*(locus D10S28) is currently being accumulated. Both systems allow for a high degree of discrimination and are ideal for paternity cases where the DNA isolated from case samples is of high quality and in sufficient quantity to perform the analysis[1].

Some "micro-satellites" are sufficiently small as to enable their efficient amplification by the polymerase chain reaction (PCR)[2]. The PCR technique enables analysis of forensic samples when the quantity and/or integrity of the DNA is limiting. We have amplified the 'AT' rich region 3' to the Apolipoprotein B gene on chromosome 2. Our data base consists of over 525 individuals which exhibit twelve codominant alleles ranging in size from 570-900 bps. Each allele has been isolated and remixed to form a control ladder. The authenticity of the amplification is assured by probing it with a biotin labelled probe flanking the tandemly repeating blocks[3]. Other VNTRs under investigation include the regions at loci D17S30, D1S58 and region 3' to the interleukin 6 gene[4,5,6]

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SEVERE PRE-ECLAMPSIA/ECLAMPSIA MATERNAL SUSCEPTIBILITY GENES ARE NOT LINKED TO HLA

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Pre-eclampsia is a disease of pregnancy involving high blood pressure, oedema, proteinuria and placental malfunction. Untreated it can proceed to eclampsia, epileptic like fits in the perinatal period. Seventeen families with multiple cases of pre-eclampsia or eclampsia were used to test the hypothesis that the maternal genes causing the condition are HLA linked as suggested by several reports of various associations of pre-eclampsia with HLA genotypes. Family members were typed for using RFLPs generated from DNA with Taq I restriction enzyme and a cloned DRβ gene. Lod scores were calculated for the family data using the LIPED program and the maternal susceptibility gene was excluded (lod score less than -2) from the region 10 cM either side of DRβ. If the maternal susceptibility gene is on chromosome 6 at all it must be well outside the HLA region. Analysis by the affected-sib pair method of the 24 affected sibs gives a chisquare of .15 (p=.93) confirming the result. A model with an HLA linked gene in the fetal genotype interacting with the maternal pre-eclampsia gene could explain these results and the reported HLA associations. On this model, the maternal gene might interact with an HLA gene product. The T-cell receptor β locus on chromosome 7 is such a candidate gene and was tested for linkage to pre-eclampsia/eclampsia with 15 families and close linkage was excluded.

COMPARATIVE GENE MAPPING IN CATTLE; CONSERVATION OF SYNTENY WITH MICE AND HUMANS

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Comparative gene mapping has become a powerful tool for the study of mammalian chromosome evolution. We have employed somatic cell techniques to map more than 150 genes in cattle. At least 140 of these genes were selected because of their location on the human map and 120 had also been mapped in the mouse. While all 29 bovine autosomal syntenic groups are represented by these genes, we have focussed attention on genes homologous to those on targetted human chromosomes, specifically HSA 1, 3, 4, 7, 8, 9, 10, 12, 15, 16, 17, 20 and 21.

Alignment of these bovine and murine genes with their respective homologues in the human map demonstrates extensive genomic conservation. Thirty-two rearrangements are necessary to account for the divergence of the mouse and human maps of these loci. Only 21 rearrangements, however, are necessary to account for the divergence of the cattle and human maps of the same loci. From this abbreviated comparative map, we surmise that as few as 100 chromosomal rearrangements may differentiate the bovine and human genomes.

Comparative mapping permits a more precise regional assignment of several genes on the human map. Moreover, it allows the development of new maps of mammalian species at an accelerated pace. The latter may prove extremely important in economically important animals such as cattle, in light of the extensive maps to be generated for humans and mice as part of the ongoing human genome initiative.

U16 ASS, LGB?

H X9 ASS

GENOTYPING OF THE A AND B VARIANTS OF CATTLE B-LACTOGLOBULIN (BLG) USING RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPs)

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Previous methods for BLG genotyping have involved native protein electrophoretic gels, where separation is based on amino acid charge differences of the protein variants in milk. A *Hph*I site in exon III and a *Hae*III site in exon IV[1,2] are polymorphic for the cattle BLG A and B variants. However, only the base substitution leading to the exon III polymorphism is likely to cause protein electrophoretic mobility shift, as it involves an amino acid charge change (Gly in B, Asp in A).

In the present study, 45 cows were genotyped for protein variants and both *Hph*I and *Hae*III RFLPs, 10 cows were genotyped for RFLPs only, and 4 cows were genotyped for protein variants only. The 45 cows come from at least 22 sire families, the 10 cows from a further 4 sire families, and the last 4 cows from a further 2 sire families; most of the cows are Holstein-Friesian.

There was complete correlation between the protein variant genotypes and the genotypes for each of the RFLPs. In the group of 10 cows, there was also complete correlation between the genotypes for the 2 RFLPs. Considering the data from all 59 cows (from at least 28 sire families), the frequencies of the A and B alleles were 0.45 and 0.55 respectively, and the genotypes were in Hardy-Weinberg equilibrium (P= 0.65).

As no intragenic recombination has been observed between these 2 polymorhic sites (1 kb apart in the 5 kb gene[2]), it may be possible to genotype AI sires using RFLPs of only one of the polymorphic sites. The *Hae*III RFLP is preferred for this purpose as the enzyme is 10 fold cheaper and it is technically easier to deal with the larger *Hae*III fragments (333 bp in A, 259 and 74 bp in B compared to *Hph*I fragments of 232 bp in A, 165 and 67 bp in B).

Previous studies have shown that cows with the BLG B allele produce better milk for cheesemaking[3,4], attributable to higher contents of total solids[3], fat and casein[3,5]. The beneficial effect of the B allele was also shown in the higher heat stability of milk at the pH of maximum stability[4]. In both respects, the availability of the bulls' genotypes may be useful to the dairy cattle breeding industry.

1. Alexander L.J., Hayes G., Pearse M.J., Beattie C.W., Stewart A.F., Willis I.M. and MacKinlay A.G. (1989) Complete sequence of the bovine β-lactoglobulin cDNA *Nucl.Acids Res.* 17(16) pp 6739
2. A.G. MacKinlay (Personal Communication)
3. McLean D.M., Graham E.R.B. & Ponzini, R.W. (1984). Effects of milk protein genetic variants on milk yield and composition. *J. Dairy Res.* 51: pp 531-546
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GENETICAL AND MOLECULAR STUDIES OF FLAX AND ITS RUST

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Genetical studies of flax (*Linum usitatissimum* L.) and its rust (*Melampsora lini* (Ehrenb.) Lev.) go back a long way (Flor, 1935 *et seq.*). The collection and testing of reactions of rust races on diverse flax varieties, and the subsequent development of a panel of essentially monogenic differential host varieties, facilitated Flor's study of the inheritance of the host/pathogen interaction. These inheritance studies gave rise to the "gene-for-gene" concept which suggested that, in the most specific cases, plant and pathogen genes controlling infection interact in complementary pairs.

Flor's work has been extended by further genetical research and flax and its rust now provide the best characterized relationship between a higher plant and an obligate fungal parasite.

Because of the paramount importance of obtaining an understanding of the cellular events controlling fungal infection, the flax/flax rust system has more recently become the target for molecular investigation. Capitalizing on the wealth of material and knowledge obtained from almost 60 years of formal genetics, molecular geneticists hope to clone and characterize fungal Avirulence genes and plant Resistance genes. No other comparable system is available and the hope is that flax and its rust will provide a Rosetta Stone for the understanding of fungal diseases of plants.

The genetical background of the system and the approaches being used to clone the interacting complementary gene pairs are the subjects of this talk.

- Natural inbred ⇒ homozygous.
- Some varieties have 1 or more R genes for rust.
- Gene-for gene concept: pathogen genotypes / host genotypes dependent.
- Have complementary genes for host/path. in controlling infection
- Virulent gene = recessive, can infect host.

- Model based transposon tagged to jump into R gene. Jumping low ∴ may need PCR detection. Maybe manipulate transposase levels.
- Or RFLPs.
- WIN: 4 closely linked genes (no rec) that recog particular path.
- Virulence not due to rec, but deletion.

BINDING OF PROTEINS FROM EMBRYONIC & DIFFERENTIATED CELLS TO A BIDIRECTIONAL PROMOTER CONTAINED WITHIN A CpG ISLAND

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b. CSIRO, Division of Biomolecular Engineering, Lab. of Molecular Biology, PO Box 184, North Ryde, NSW 2113

Associated with a large number of vertebrate genes are segments of DNA known as CpG islands. These are regions, of around 1 kb in length, which contain clustered CpG dinucleotides resulting from a very high G+C content and a lack of the characteristic CpG depletion of vertebrate genomes. CpG islands are found at the 5' ends of all widely-expressed genes and some tissue-specific genes.[1,2]

One such CpG island from the mouse genome (HTF9) contains the TATA-less promoters for a pair of overlapping, bidirectional transcripts which are found in polyA+RNA from all mouse tissues examined and which both appear to code for housekeeping proteins[3]. We have carried out DNaseI footprinting assays on HTF9 using nuclear extracts from a wide range of cell types - mouse L, NIH3T3-F9 & ES cells, and human HeLa & HepG2 cells. Our aims were to determine (i) the extent to which the very large CpG island comprises binding sites for tightly bound protein factors, and (ii) whether any unusual factors bind to HTF9 in undifferentiated cells, where the entire CpG island is maintained in an unmethylated stated in the presence of an active *de novo* methylase.

We have located and characterized several protected sites within the bidirectional promoter region of HTF9. Most of the protected sequences contain putative binding sites for previously identified transcription factors such as MLTF, CAAT-binding factors, AP1, AP2, Sp1 and ATF/CRE. We have carried out competition assays to further define the bound proteins. A novel feature of the promoter is the presence of a strongly protected region located exactly over start sites for the divergent transcripts. The factor binding to this region fractionates with single-stranded DNA binding proteins. We find significant differences between extracts from differentiated and undifferentiated cell lines in the relative
amounts of some factors. However, no additional binding sites are protected in undifferentiated F9 and ES cells.

We have analysed expression of the 2 transcripts arising from HTF9, and have found that the levels of both mRNAs are much higher in undifferentiated F9 cells, ES cells and testis than in differentiated cell lines and mouse tissues. However, this difference appears to be regulated entirely at the post-transcriptional level. By nucelar runoff assays we have found the same rate of transcription in all mouse cell lines assayed, including F9 and ES cells. Therefore, differences in transcription factor binding in different cell types do not appear to affect rates of transcription.

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2 Gardiner-Garden, M. & Frommer, M.(1987)*J.Mol.Biol* **196**: 261

3 Lavia, P. *et al*(1987)*EMBO J.* **6** 2773

THE HIGHLY CONSERVED HISTONE H2A VARIANT IS ESSENTIAL IN *DROSOPHILA*

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b. Dept. of Biology, Washington University, St. Louis, Mo., U.S.A.

Variants of the major histones have been documented for some time. The major histones are synthesized during the S-phase of the cell cycle only, their mRNAs are not polyadenylated and have short 5′ and 3′ untranslated regions, and the genes contain no introns. There is an H2A variant, originally identified in mammals, which is synthesized throughout the cell cycle. There is some evidence that this class of H2A variants is associated with transcriptionally active chromatin. The most compelling evidence comes from the fact that the *Tetrahymena* H2A variant is found exclusively in the transcriptionally active macronucleus and is absent from the transcriptionally inert micronucleus. cDna clones for this protein have now been obtained in chicken, sea urchin, *Tetrahymena*, *Drosophila* and mammals. These H2A variant proteins from different species are more closely related to each other (e.g. mammal and sea urchin are 98% similar) than any of the variants are to their major, cell cycle regulated H2As (60%).

In an effort to determine the function of this H2A variant, we have isolated the gene in *Drosophila*, an organism which is easily amenable to genetic analysis. The gene for the histone H2A variant in *Drosophila* (H2AvD) was isolated and sequenced. The deduced animo acid sequence is 98% similar to the chicken, sea urchin and mammalian and 85% similar to the *Tetrahymena* H2A variants, but only 59% similar to the major H2A. The unique gene contains three introns and the mRNA is polyadenylated. The position of the introns is conserved in *Tetrahymena* and chicken. The transcript is present in the first twelve hours of embryogenesis and in adult females. The protein is present at all developmental stages, indicating that the protein is quite stable. Immunofluorescence staining of polytene chromosomes with antibody to H2AvD reveals a banded pattern, distinct from the general chromosome staining seen with antiserum to the major H2A.

In situ hybridization places the locus on chromosome 3 at 97CD, which is separate from the histone cluster on chromosome 2. This has been further localized to the 97D1-5 region. An EMS induced lethal complementation group in the 97D1-5 interval was shown to contain a 311bp deletion within the H2AvD gene, which results in excision of the second exon. P-element mediated transformation using 4.5kb fragment containing the H2AvD gene results in rescue of this lethality, indicating that this variant histone protein is essential.

- Histone fn: to package DNA in compact structure.
- Histone variant - cell cycle ↑ exist terminal cycle.
- H2A is not polyadenylated ← functionally related?

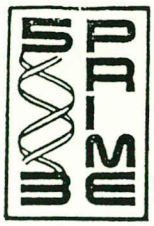
A MOLECULAR ANALYSIS OF *CINNABAR:* AN EYE PIGMENTATION GENE THAT ENCODES KYNURENINE 3-MONOOXYGENASE IN *DROSOPHILA MELANOGASTER*

W. D. Warren, and A.J. Howells

Department of Biochemistry, Faculty of Science,
The Australian National University, Canberra

The *cinnabar* locus of *Drosophila melanogaster* encodes the aromatic hydroxylase, kynurenine 3-monooxygenase. The synthesis of this enzyme is rigidly controlled during development and is directly involved in the production of the brown eye pigment xanthommatin. The cloning and characterization of this locus was undertaken to further our long term study of the coordinated regulation of the genes involved in xanthommatin biosynthesis.

In order to locate *cinnabar* sequences, cloned DNA isolated by chromosome walking in the 43E region was used to probe Southern blots of DNA from mutants having either chromosomal rearrangements or spontaneous mutations that disrupt *cinnabar* function. All of the changes detected in the spontaneous mutants clustered in a 5 kb region. Four cDNA clones isolated by homology to this region have been sequenced along with both strands of just over 8 kb of genomic DNA. Unfortunately only the shortest cDNA (768 bp) appears to be spliced. The others seem to be unspliced primary transcripts. The spliced cDNA spans just two exons, and has resulted from the removal of a 413 bp intron. It appears this clone carries only a central portion of the gene as there is no poly A tail and the two open reading frames identified in the genomic DNA through the splicing of this cDNA extend for several hundred bases both 5′ and 3′ beyond the ends of the cDNA. The full sequence from both open reading frames was then used to calculate a predicted amino acid sequence of 505 residues. When the sequence of this putative *cinnabar* peptide was compared to the protein sequence of other aromatic hydroxylases, a short region of significant homology was detected, indicating that this partial cDNA is indeed part of the *cinnabar* transcript.



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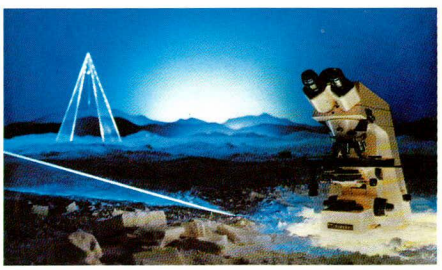

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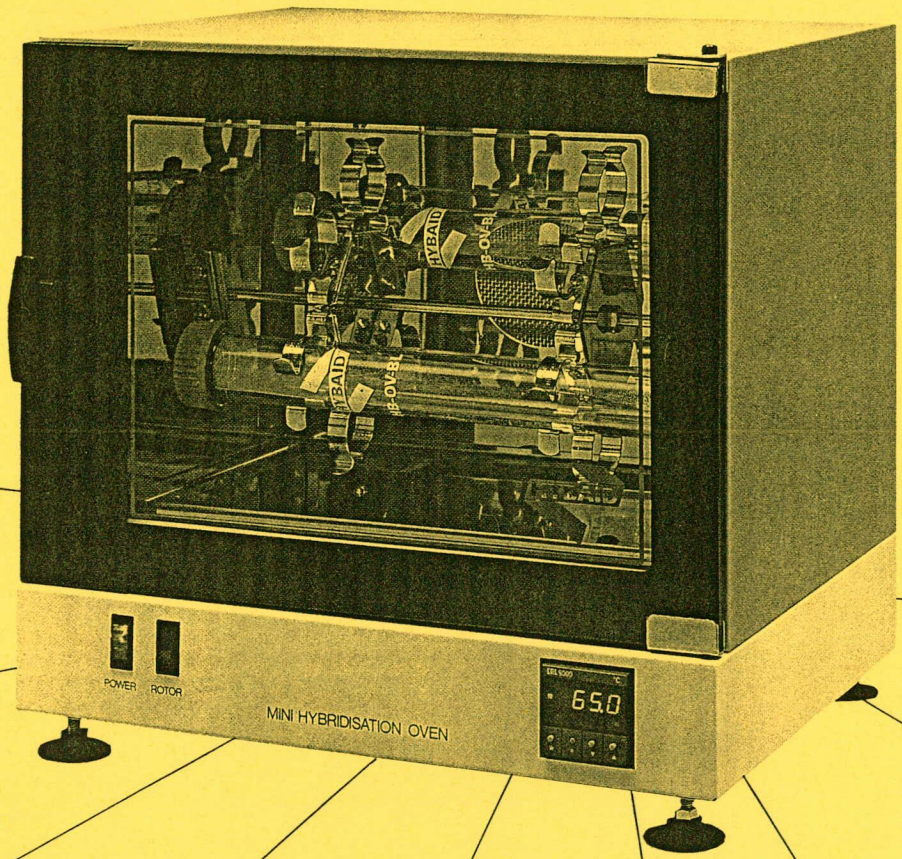

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The Vienna Times

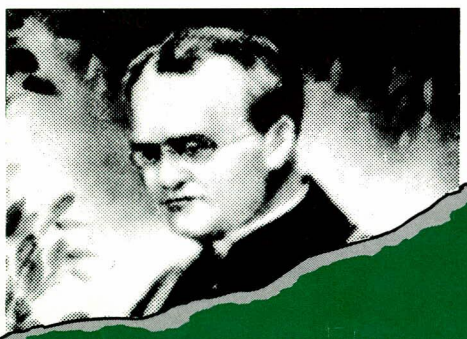
8th February 1865 — 8th March 1865

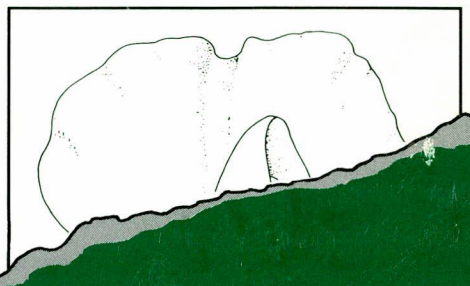
MENDEL'S LAUGHABLE HYPOTHESIS

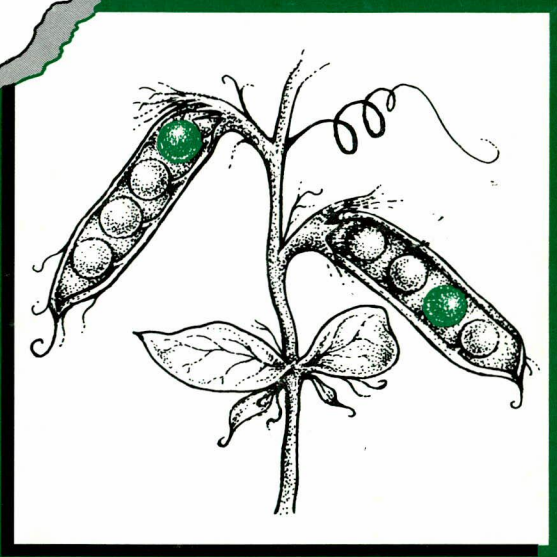
Gregor Johann Mendel (44), a former student of the University of Vienna, today presented an outrageous theorem on the nature of the inheritance to the Brunn Society Nature History.

His experiments conducted at the Brunn monastery under the guidance of Abbot Cyril Napp were carried out on ordinary garden peas.

Charles Darwin has already unsuccessfully carried out similar experiments with plants. Mendel's theory of dominant and recessive traits was laughed out of the room as was his law of segregation.







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