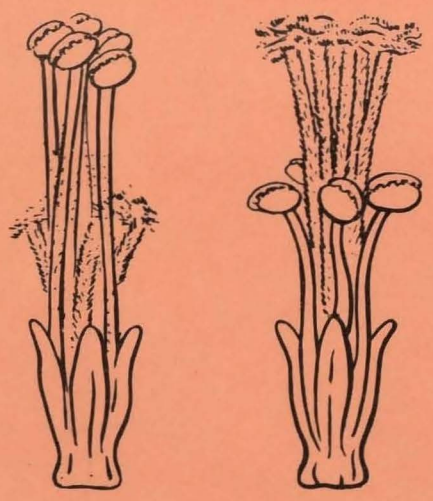
THE GENETICS SOCIETY OF AUSTRALIA

28th GENERAL MEETING

Held in conjunction with the

XIII INTERNATIONAL BOTANICAL CONGRESS



THE UNIVERSITY OF SYDNEY

23 - 24th August, 1981

CONTENTS

*I.B.C. SECTIONS INCLUDED ONLY IN MEETING PROGRAMMES

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GENERAL INFORMATION

REGISTRATION

The 28th meeting of the Genetics Society of Australia will be held in conjunction with the XIIIth International Botanical Congress. Registration fees for the meeting will be $40, which includes the right of attendance at I.B.C. scientific sessions plus the receipt of abstracts of genetical interest. Members who have enrolled for the I.B.C. pay a reduced fee of $5. The corresponding rates for students and those not in full-time employment are $14 and $2.

PAPERS

All contributed papers will be presented on Sunday August 23, in the Botany Lecture Theatre and the Robert Brown Lecture Room on the first floor of the Macleay Building (Botany/Genetics - see 16C on the map of Sydney University which has been included in the enrolment folder). Twenty minutes has been allowed for papers, which includes discussion time. Slide and overhead projection facilities will be available, and speakers are asked to ensure that slides are placed in the carousels by the start of the session of their talk.

POSTERS

These will be on display alongside the I.B.C. Section 7 (Genetical Botany) posters in Maclaurin Hall (16F). Members are requested to set up their posters on Saturday August 22, so that they are on display by the afternoon session 15.00 - 17.00, which has been designated by the I.B.C. as the official viewing time, although it clashes with the Section 7 symposium. The equivalent afternoon session on Monday August 24 was previously designated as the official time for G.S.A. members to view posters. This time now also clashes with the revised I.B.C. symposium times, so the viewing time for G.S.A. members has been changed to Monday 24 from 13.00 - 14.00, which is during the scheduled lunch break. Members presenting posters are asked to be present at this time.

I.B.C. PROGRAMME AND TIMETABLE

An abbreviated version of the I.B.C. programme is included in this printing for all G.S.A. members attending the meetings. This includes the programme and abstracts for all Section 7 (Genetical Botany) symposia and posters, as well as the programme for several other sessions judged to be of genetical interest. A complete I.B.C. programme and copy of the abstracts is available for perusal by members in Room 203, Macleay Building, during normal office hours.

All Section 7 symposia are to be held in the Lister Lecture Theatre of the Anderson Stuart Building (17H), while other symposia are to be held in the Carslaw Lecture Theatres (19K), and in rooms around the campus most of which are within easy walking distance.

BUSINESS MEETING

This will be held in the Botany Lecture Theatre on Sunday 23, shortly after the conclusion of papers. Sherry will be served before the meeting.

SOCIAL ACTIVITIES

Mixer: to be held on the Saturday 23 from 19.00 onwards, on level 6 of the Gunn Building (7E). Drinks and savouries will be provided.

Society Dinner: a Chinese banquet, to be held at the Shanghai Village Restaurant, in Dixon St, Sydney (see Haymarket district on the extended map). The cost is $17, which includes wine but not pre-dinner drinks. The time will be 19.30 on Monday August 23, not Tuesday 24 as stated in the I.B.C. official programme and in the first circular. Reservation and payment should be made when registering, the last time for acceptance being at Registration on Sunday morning.

MEALS AND REFRESHMENTS

On Sunday 23, there will be no food services available at Sydney University, so we will be collecting orders for take-away food for lunch. Coffee and tea will be available at all times.

The principal places for eating on campus during the week and on Saturday at lunch time are the Wentworth Building (19N) and Manning House (14G). In addition, arrangements are being made to register G.S.A. members as temporary members of Sydney University Staff Club (Mungo MacCallum Building - 15F), which has bar and dining room facilities during the week.

OTHER SERVICES

A bank and post office are conveniently located just opposite the Macleay Building on Science Rd (15D). Medical facilities are available at Student Health (Institute Building - 17Q).

GENETICS SOCETY VISITORS

Dr. Michael Bennett
Plant Breeding Institute,
Cambridge, U.K.

Dr. Richard Flavell
Plant Breeding Institute,
Cambridge, U.K.

Prof. Ken Frey
Agronomy Department,
Iowa State University, U.S.A.

Due to the shortened meeting this year, there will be no guest lectures at the G.S.A. meeting. However, each of the visitors is giving symposium talks, and will visit interstate centres after the meetings.

LOCAL COMMITTEE

For any queries, contact a member of the local committee:

Chris Gillies (Botany Annexe 201A, 692 2688)
Chris Moran (Gunn Building 512, 692 3553)
John Sved (Botany Annexe 208A, 692 2298)
John Thomson (Macleay Building 203, 692 2376)

PROGRAMME FOR SUNDAY AUGUST 23

REGISTRATION MACLEAY FOYER, 8.30 - 9.20

SECTION 1 BOTANY L.T. CHAIRPERSON: Dr. W.J. Peacock

9.20 M.J.D. White, E.S. Dennis, W.J. Peacock, N. Contreras and R. Honeycutt : Population Biology, A.N.U.
"Ribosomal DNA sequences in Warramba virgo and its parent species"

9.40 A.J. Hilliker and R. Appels : C.S.I.R.O. Plant Industry
"Biological effects of Drosophila X chromosome heterochromatin"

10.00 Brandon Wainright and Rory Hope : University of Adelaide
"Flow sorting of marsupial chromosomes: Results and applications"

10.20 Jennifer A. Marshall Graves : La Trobe University
"Derepression of genes on the inactive X chromosome by undermethylation"

10.40 - 11.10 MORNING TEA

SECTION 2a BOTANY L.T. CHAIRPERSON: Dr. D.W. Cooper

11.10 M.S. Johnson : University of W.A.
"Shell coil polymorphism in *Partula saturalis*: Behavioural reproductive isolation and positive frequency dependent selection"

11.30 N.D. Murray : La Trobe University
"Ecology and evolution in an isolated population of *Cactoblastis cactorum*"

11.50 P.A. Parsons : La Trobe University
"Central, marginal and colonizing populations"

12.10 P.R. Baverstock, M. Adams and M. Gelder : I.M.V.S., Adelaide
"Sex-linked isozymes in birds : Z-conservation and dosage compensation"

SECTION 2b ROBERT BROWN L.R. CHAIRPERSON: Dr. W.R. Scowcroft

11.10 David A. Jones : University of Adelaide
"Examination of the interaction between flax and its rust using two-dimensional electrophoresis of total protein"

11.30 Horst Lorz : C.S.I.R.O. Plant Industry
"Cell fragments and subprotoplasts as a tool in somatic plant cell genetics"

11.50 O. Mayo : Waite Institute
"Models of gene effects for quantitative traits"

12.10 R.C. Malik : University of New England
"Genetic differences in energy requirements of growing mice"

12.30 - 1.40 LUNCH

SECTION 3a BOTANY L.T. CHAIRPERSON: Dr. B.G. Rolfe

1.40 W. Zurkowski, M. Djordjevic, J. Shine and B.G. Rolfe : Genetics, A.N.U.
"Molecular properties of plasmids in rhizobia strains"

2.00 M. Djordjevic, W. Zurkowski, J. Shine, B.G. Rolfe and J. Badenoch-Jones : Genetics, A.N.U.
"Biological properties associated with plasmids in rhizobia strains"

2.20 J.E. Hughes, K.F. Scott, A. Chakravorty, P.M. Gresshoff, B.G. Rolfe and J. Shine : Genetics, A.N.U.
"Molecular cloning of symbiotic genes of *Rhizobium trifolii*"

2.40 G. Bender, Y. Cen, M.J. Trinick, N. Morrison, P.M. Gresshoff, J. Shine and B.G. Rolfe : Genetics, A.N.U.
"Transposon mutagenesis in slow growing *Rhizobium* strains which can nodulate both legumes and non-legumes"

SECTION 3b ROBERT BROWN L.R. CHAIRPERSON: Dr. D.L. Hayman

1.40 Peter Sharp : University of Adelaide
"Pairing of the sex chromosomes during male meiosis in marsupials with multiple sex chromosomes"

2.00 L. von Kalm and D.R. Smyth : Monash University
"Ribosomal RNA genes in Lilium"

2.20 G.B. Peters : Botany, A.N.U.
"Recurrence of centric fusions in familial lines of a grasshopper"

2.40 D.J. Coates and D.D. Shaw : Population Biology, A.N.U.
"Patterns of chiasma distribution in chromosomally distinct subspecies and their relationship to hybrid breakdown in *Caledia captiva*"

3.00 - 3.30 AFTERNOON TEA

SECTION 4a BOTANY L.T. CHAIRPERSON: Dr. E.S. Dennis

3.30 N. Morrison, M.J. Trinick, P.M. Gresshoff, J. Shine and B.G. Rolfe : Genetics, A.N.U.
"Genetic and biological properties of a fast-growing strain of Rhizobium which can nodulate both legumes and non-legumes"

3.50 K.F. Scott, B.G. Rolfe and J. Shine : Genetics, A.N.U.
"Comparison of the DNA sequences coding for the nitrogenase enzyme in free-living and symbiotic bacteria"

4.10 B.T.O. Lee, C.D. Dirckze and P.W. Atkinson : Melbourne University
"Plasmid effects on inducible repair in Pseudomonas aeruginosa"

4.30 A.M. O'Connell, D.J. Killingly, C.R. Byrne and K.D. Brown : Sydney University
"Structure and function of the tyrosine operon of Escherischia coli"

4.50 Michael Hynes, Catherine Corrick and Julie King : La Trobe University
"Cloning of the amidase gene of Aspergillus nidulans"

SECTION 4b ROBERT BROWN L.R. CHAIRPERSON: Dr. G.M.E. Mayo

3.30 John A. McKenzie and Stephen W. McKechnie : Melbourne University and Monash University
"Perturbation of gene frequencies in a cellar population of Drosophila melanogaster"

3.50 F. Kay Pearse and N.D. Murray : Melbourne University and La Trobe University
"Sex and variability in the common brown butterfly"

4.10 D.C. Vacek : University of New England
"Genetic differences in a cactophilic Drosophila for feeding and oviposition preferences on cactus yeasts"

4.30 D.J. Colgan and J.A. Sved : Sydney University
"Further studies on hybrid dysgenesis in Drosophila melanogaster"

4.50 R. Thomson, N.D. Murray and M. Westerman : La Trobe University
"Parasitic B-chromosomes : population and cytogenetical studies on the Australian Bushrat"

5.10 - 5.30 SHERRY

5.30 BUSINESS MEETING

POSTERS

C.R. Carter : University of N.S.W.
"Fowler's Gap arid zone Research Station"

P. Chandler, J. Zwar, T.J. Higgins and J. Jacobsen : C.S.I.R.O. Plant Industry
"Control of α-amylase gene expression in barley seeds by plant hormones"

M. Fischer : C.S.I.R.O. Plant Industry
"Tissue culture of cotton"

Y.J. Fripp : La Trobe University
"Mating system and cross-compatibility of races of *Epacris impressa*"

C.B. Gillies : University of Sydney
"Spreading maize synaptonemal complexes for electron microscopy"

D.L. Hayman : University of Adelaide
"G-banding studies in marsupials"

J.D. Murray, A.J.D. Bellett and A.W. Braithwaite : John Curtin, A.N.U.
"Effects of adenovirus type J infection on the DNA content and cell cycle time of rat embryo fibroblasts"

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Alan E. Stark : University of N.S.W.
"Properties of Haldane's model of assortative mating with dominance"

G.C. Webb, J. Halliday and M. Leversha : Children's Hospital, Melbourne
"Fra(X)(q27) in mentally retarded females"

ABSTRACTS

Please note that a postal strike prevented the receipt of some abstracts

RIBOSOMAL DNA GENES IN WARRAMABA VIRGO AND ITS PARENT SPECIES

M.J.D. White[1], E.S. Dennis[2], W.J. Peacock[2], N. Contreras[1] and R. Honeycutt[1]

[1] Research School of Biological Sciences, Australian National University

[2] Division of Plant Industry, C.S.I.R.O.

A study of the location of the ribosomal genes in Warramaba virgo and its bisexual relatives has confirmed at least two hybrid origins of the parthenogenetic species. The clones of the Standard phylad of W. virgo have their 18S+28S rDNA cistrons located in C-bands 4, 44 and 49, whereas the Boulder and Zanthus clones have them in C-band positions 74, 87.5 and 49. There is no visible C-band at position 49 in the Boulder and Zanthus clones and there is only a minor proportion of the ribosomal cistrons at this location. The differences between the clones of W. virgo support the view that the Standard phylad has arisen by hybridization between races of the bisexual species 'P169' and 'P196', different from those involved in the origin of the Boulder and Zanthus clones.

The genomic organization of the ribosomal cistrons, as shown by restriction enzyme analysis, is different in the Standard phylad and the Zanthus clone. There are also differences in the restriction enzyme patterns between the northern and southern races of P196. The restriction enzyme patterns of the southern race of P196 and of the Zanthus clone suggest that these are related, but the chromosomal locations and the repeat organizations of the ribosomal cistrons in the Standard phylad and the Boulder-Zanthus clones of W. virgo are not simply the additive products of any of the presently known populations of P196 and P169.

The N-banding technique stains all of the C-bands in which ribosomal genes are located, but it also stains some that do not contain ribosomal cistrons (e.g. C-bands 59.5 and 69). The Olert silver technique stains particular C-bands, none of which contain ribosomal cistrons.

FLOW SORTING OF MARSUPIAL CHROMOSOMES: RESULTS AND APPLICATIONS

Brandon Wainwright and Rory Hope
Department of Genetics, University of Adelaide, South Australia 5001.

Flow cytometry of isolated chromosomes is a procedure which permits individual metaphase chromosomes to be rapidly and automatically characterized and subsequently sorted. Chromosomes that are stained in aqueous suspension with an appropriate fluorochrome flow at high speed through a laser beam that excites the stain. The emitted fluorescence is measured photometrically and the accumulated data form a frequency distribution of chromosome fluorescence. The peaks of this frequency distribution represent groups of chromosomes of similar fluorescence; the peak mode is proportional to the chromosome fluorescence and the area under the peak is proportional to chromosome frequency of occurrence. Using this new approach to cytogenetics the frequency distribution serves as a karyotype. The use of a fluorescence activated cell sorter in conjunction with a flow cytometer enables chromosomes whose fluorescence falls within predetermined boundaries to be physically sorted.

Because of their small chromosome number and large variation in chromosome DNA content, certain marsupial species provide ideal material for flow cytometric chromosome analysis. Chromosomes from the Dasyurid marsupials *Sminthopsis crassicaudata* and *Antechinus rosamondae* were isolated from cultured cells, stained for DNA with ethidium bromide, and analysed for DNA content by a flow cytometer/sorter at rates up to 150,000 chromosomes per minute. For both species six distinct peaks were resolved that correspond well to the expected relative DNA content of each chromosome and the expected relative frequency of each chromosome type. Chromosomes were sorted from each peak and visual examination of each fraction indicated the purity of the sorted chromosomes.

Enriched or pure chromosome fractions can be analysed biochemically to provide information on the structure of DNA or chromosomal protein. They can also be used to map genes by chromosome-mediated gene transfer, or to examine sequence organisation of specific chromosomes by *in vitro* hybridisation.

DEREPRESSION OF GENES ON THE INACTIVE X CHROMOSOME Jennifer A. Marshall Graves, Department of Genetics and Human Variation, La Trobe University, Bundoora, 3083. Australia.

During early development of female mammals, one X chromosome becomes heterochromatic and genetically inert; this state is stably maintained during subsequent proliferation of somatic cells *in vivo* or in culture, but is reversed during oogenesis. In order to understand the mechanism of inactivation, many unsuccessful attempts have been made to reactivate genes borne on the inactive X.

Recently, re-expression of genes in other systems has been induced by treatment with the base analogue, 5 azacytidine, whose mode of action appears to be substitution of 5 methylcytidine in DNA, leading to permanent hypomethylation. An HPRT-deficient mouse cell line with a multiply marked inactive X (bearing the selected *Hpt+* allele) was treated with a range of 5-azacytidine concentrations (0-30μg/ml), and selected for re-expression of the *Hpt+* allele in HAT medium. A spectacular increase (from $<10^{-8}$ to 0.5%) in recovery of HPRT+ colonies was observed, suggesting a frequent derepression of this locus at least. Selected and unselected colonies are being typed for HPRT, Pgk-A and G6PD to confirm the origin of the selected marker and determine whether flanking markers have been coordinately derepressed.

Shell coil polymorphism in *Partula suturalis*: Behavioural reproductive isolation and positive frequency dependent selection.

M.S. Johnson
Department of Zoology, University of Western Australia.

Partula suturalis is unusual among land snails in being polymorphic for direction of shell coiling. Variation in chirality is due to a single pair of alleles with maternal inheritance. Polymorphic populations of *P. suturalis* occur in narrow transition zones between area effects for sinistral and dextral shells.

Direction of coil affects courtship behaviour, and no-choice mating experiments show that pairs with opposite coil mate less than half as frequently as do pairs with the same coil. The consequence is that fewer young are produced by mixed pairs.

Fertilities of dextrals and sinistrals are the same in a population composed of equal frequencies of the two morphs. However, among wild-caught snails from a population in which sinistrals occur at low frequency, the rare morph produces fewer young than the common morph. The effects of chirality on reproductive isolation apparently result in selection against the rare morph, accounting for the rarity of polymorphic populations.

ECOLOGY AND EVOLUTION IN AN ISOLATED POPULATION OF CACTOBLASTIS CACTORUM

N.D. MURRAY, Department of Genetics and Human Variation, La Trobe University, Bundoora, Vic. 3083.

Since its introduction to Australia to control prickly pear (Opuntia spp.), the moth Cactoblastis cactorum has established a persistent association with Opuntia. It now ranges more or less continuously from central Queensland to the Hunter Valley in New South Wales. In these areas the ecological stability of the interaction arises from moths preferentially laying eggs on particular plants, thus suiciding through larval starvation at high densities. The genetic and environmental factors leading to plant "attractiveness" have been investigated and identified as largely environmental: plant nutrition, size and spacing.

This report deals with an isolated population of Cactoblastis which has recently been discovered at Bulla, Victoria. Here the life-cycle has altered: a previously bivoltine cycle has become univoltine. In field studies the characteristics found to have changed include egg-laying choice criteria, and length of adult and egg stages of the cycle. As well, the extremely hot 1980-81 summer has resulted in a severe population crash in which only very fast or very slow developers have survived. These features reveal a demography which may help explain the very high levels of electrophoretic variation maintained in Cactoblastis populations, including the Bulla isolate.

The value of introduced organisms for studying rapid evolutionary change is emphasised.

CENTRAL, MARGINAL AND COLONIZING POPULATIONS

P.A. Parsons, Department of Genetics and Human Variation,
La Trobe University

There is a widespread literature on variation in central and marginal (potentially colonizing) populations based upon enzyme and chromosome polymorphisms. Most Drosophila data show a reduction of chromosome polymorphisms and of lethals and semilethals towards the margins, but no equivalent reduction in enzyme polymorphisms. Since these are genotypic assessments not directly relatable to the field situation, the somewhat unsatisfactory nature of these data from the interpretative point of view is understandable.

An ecologically marginal habitat from which colonists are derived can be regarded as one in which physical stresses of climatic origin tend to be variable and extreme, so that resources are unpredictable and short-lived. Prerequisites for genetic analysis are therefore phenotypes relatable to the r-K continuum of adaptive strategies. Ecological phenotypes (e.g. tolerance to environmental stresses, development time, and resource utilization variability) can be analyzed at the population level in D. melanogaster using isofemale strains as the starting material. Isofemale strain studies carried out at La Trobe on populations from different Australian localities are interpretable in terms of the r-K continuum.

It is highly likely that the genetic architecture of ecological phenotypes of marginal populations mainly comprise a few additive genes of relatively large effect. This is an architecture permissive of rapid adaptation to new habitats, provided that the appropriate genes are present. Discussions of speciation via the founder principle, a colonization event in itself, have invoked a similar explanation.

EXAMINATION OF THE INTERACTION BETWEEN FLAX AND ITS RUST USING TWO-DIMENSIONAL ELECTROPHORESIS OF TOTAL PROTEIN

David A. Jones
Department of Genetics, University of Adelaide, South Australia.

The interaction between flax (*Linum usitatissimum*) and flax rust (*Melampsora lini*) is controlled by specific genes for resistance in flax and by specific genes for pathogenicity in the rust. A unitary relationship, called "the gene-for-gene relationship", exists between these genes and may be stated as follows:

> For every resistance gene in the host there is a specific pathogenicity gene in the parasite.

This relationship is well characterized genetically, but the nature of the gene products and their mode of action is unknown.

Two-dimensional electrophoresis of total protein was undertaken in an attempt to isolate and identify resistance or pathogenicity gene products. Separation in the first dimension of this procedure is achieved by isoelectric focusing on tubular polyacrylamide gels and in the second by electrophoresis on discontinuous polyacrylamide slab gels in the presence of SDS (sodium dodecyl sulphate). Proteins are therefore separated on the basis of the independent parameters of pI (isoelectric point) and M_r (relative molecular mass), respectively.

Host and parasite were examined separately prior to the host-parasite interaction. Proteins from backcross lines of flax were compared to those from the near isogenic backcross parent. Similarly, proteins from homozygous virulent mutant rusts were compared to those from the heterozygous avirulent parent rust. Finally, these genetic stocks were used to compare proteins from incompatible and compatible flax-flax rust interactions.

Cell Fragments and Subprotoplasts as a Tool in Somatic Plant Cell Genetics

Horst Lörz, CSIRO, Division of Plant Industry, Canberra

The production of hybrid plants containing new combinations of cytoplasmic and nuclear characters is of special interest in plant genetics. Fusion of protoplasts and regeneration of the fusion products resulted in hybrid plants and, in rare events, in cybrid plants. Heteroplasmic cybrids contain the nuclear genome of one parent and the organellar genomes of both parents, homoplastic cybrids have the nucleus of one parent and the plastids of the other. Cybrid formation was achieved directly by fusion of protoplasts with cytoplasts.

Cytoplasts (or enucleated protoplasts) are produced by fractionation of isolated protoplasts in isoosmolal density gradients during high speed centrifugation. Cytoplasts have been isolated from cultured cells of Nicotiana tabacum, line SRI, which have a chloroplast coded streptomycin resistance. Streptomycin sensitive protoplasts of N. plumbaginifolia have been fused with the cytoplasts of N. tabacum. The selection for fusion products and cybrids was performed on streptomycin containing media at the tissue culture level. Analysis of the regenerated plants based on leaf morphology, flower morphology, isoenzyme patterns and chloroplast DNA restriction endonuclease pattern gave evidence for new cybrid plants with N. plumbaginifolia nuclear genome and N. tabacum plastids.

Cytoplasts, therefore, proved to be a useful tool in somatic plant cell genetics to transfer cytoplasmically inherited genetic characters separately from the nuclear genome.

Models of Gene Effects in Quantitative Inheritance

O. Mayo,
Biometry Section,
Waite Agricultural Research Institute,
University of Adelaide

The consequences of one modification of Fisher's classical additive model with equal gene effects will be considered. It is a model where gene effects decrease as a geometric progression. It seems first to have been considered by Fisher in a letter to "Student" in February 1933, and has more recently been proposed by Matthysse et al. (P.N.A.S. 1979). An attempt to determine its consequences and to fit it to some human data will be described.

GENETIC DIFFERENCES IN ENERGY REQUIREMENTS OF GROWING MICE

R. C. Malik
Department of Animal Science, University of New England, Armidale, N.S.W., 2351

Experimental evidence of genetic variation in feed consumption during growth period is available from a number of selection studies in mice. However, evidence of genetic variation in the energy requirements for maintenance heat production and the need for growth, mainly in terms of protein and fat deposition is not well documented. The division of the energy intake between maintenance and the requirement for protein and fat deposition changes continuously during the entire period of growth. The changes in energy partitioning are most rapid during the period of accelerated growth immediately following weaning and tend to stabilize as the animal approaches maturity.

The capacity to deposit protein during growth seems to be determined genetically. The appetite control has been related with the protein deposition capacity in a few recent studies. Therefore, a large proportion of the excess energy consumed relative to the needs for protein synthesis, unless the animal is able to reduce its energy intake as the lean tissue asymptotes, may well be deposited as fat. This would indicate that the fat deposition is controlled not only by the genotype of the animal but also by its physiological status and the level of feeding. Experimental results of partitioning the ingested energy into maintenance and growth components using lines of mice artificially selected for high and low body weight and an unselected line will be discussed in relation to these points.

MOLECULAR PROPERTIES OF PLASMIDS IN RHIZOBIA STRAINS

W. Zurkowski, M. Djordjevic, J. Shine and B.G. Rolfe

Genetics Department, Research School of Biological Sciences, The Australian National University, P.O. Box 475, Canberra City 2601.

Plasmids were detected in all the tested twenty eight *Rhizobium trifolii* strains. At least four classes of plasmids different in size could be detected. Class I, plasmids greater than 200 Mdal; class II, 95-125 Mdal; Class III, 40-65 Mdal; and class IV, small plasmids of molecular weight 3-7 Mdal. At least two of these classes of plasmids were found to co-exist in Rhizobium strains. Large plasmids of molecular weight of 95-125 Mdal, and ones greater than 200 Mdal, were detected in all the tested strains, whereas smaller plasmids were detected only in some strains. It was found that large plasmids of the class I and II, form non-integrated complexes with the chromosome in cells of *R. trifolii*. Analytical centrifugation of DNA preparations in CsCl buoyant density gradients, have shown a smaller content of (G+C) in all the tested plasmid DNA than in chromosomal DNA. A high frequency of loss of nodulation ability was observed in some strains after incubation of bacteria at elevated temperatures. It was shown that a correlation exists between the loss of the ability to nodulate and the loss of a large plasmid from *R. trifolii* cells. The main molecular mechanism for elimination of nodulation is the temperature-sensitive replication of the plasmid possessing nodulation functions. These functions were restored after introduction of the missing plasmid to the bacteria.

BIOLOGICAL PROPERTIES ASSOCIATED WITH PLASMIDS IN RHIZOBIA STRAINS

M. Djordjevic, W. Zurkowski, J. Shine, B.G. Rolfe and J. Badenock-Jones

Genetics Department, Research School of Biological Sciences, The Australian National University, P.O. Box 475, Canberra City 2601.

Recent evidence has unequivocally shown that large endogenous plasmid species in fast-growing *Rhizobium* strains encode many of the functions (but probably not all) involved in the *Rhizobium*-legume symbiosis. From molecular and genetic studies of various transposon induced mutants, and mutants where either a plasmid species has been lost or extensively deleted, we have concluded that genes involved in host specificity, the early steps of nodule induction (Roa: root attachment; Hac: hair curling; Inf: infection; Noi: nodule initiation; and Bar: bacteroid release) are all encoded on one symbiosis or sym plasmid. In addition, genes involved in nitrogen fixation are in at least some cases, linked to these early nodulation genes and hence are probably also encoded on the sym plasmid.

In our studies we have used two 130 Mdal Tn5-labelled self-transmissible sym plasmids. One plasmid, pJB5JI encodes the ability to nodulate peas; the other, pBR1AN encodes the ability to nodulate clovers. Both sym plasmids encode nitrogenase genes. By transferring these plasmids to various non-nodulating (Nod^-) deletion mutants and strains in which plasmids encoding Nod genes have been cured, one can confer on these recipients the ability to nodulate peas or clovers respectively. One transposon induced Nod^- mutant of *R. trifolii* can be complemented by either pJB5JI or pBR1AN and interestingly the ability to nodulate lucerne can be restored by either pJB5J1 or pBR1AN in a Nod^- plasmid deletion mutant of *R. melioti*.

MOLECULAR CLONING OF SYMBIOTIC GENES OF *RHIZOBIUM TRIFOLII*

J.E. Hughes, K.F. Scott, A. Chakravorty, P.M. Gresshoff, B.G. Rolfe, and J. Shine

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Several of the bacterial genes responsible for nodulation and nitrogen fixation on clover roots have been isolated by molecular cloning of *Rhizobium trifolii* DNA sequences. Transposon-induced mutants of *R. trifolii*, defective in various stages of the symbiotic process were identified by plant assay. Total DNA extracted from these mutants was cloned into *Escherichia coli* plasmids and clones carrying the transposon Tn-5, together with flanking *Rhizobium*-sequences, were selected by the antibiotic resistance carried on the transposon. These cloned mutant DNA fragments were then used as hybridization probes to identify the corresponding wild-type genes in a clone bank of *Rhizobium* DNA. To confirm the presence of the genes responsible for the mutant phenotype, the cloned wild-type DNA fragments were sub-cloned into the conjugative plasmid RP4 and introduced into the original mutant *Rhizobium* strain. Two such mutants have been fully investigated in this manner. In the first mutant carrying a recombinant RP4 plasmid a double recombination event is required to remove the transposon from the bacterial chromosome and restore the wild-type phenotype. In the second mutant the gene product from the wild-type DNA fragment carried on RP4 is sufficient to complement the mutation and produce a fully effective, nitrogen-fixing nodule. The ability to readily isolate such symbiotic genes by molecular cloning should permit a detailed analysis of the events leading to an effective N_2-fixing symbiosis.

TRANSPOSON MUTAGENESIS IN SLOW-GROWING *RHIZOBIUM* STRAINS WHICH CAN NODULATE BOTH LEGUMES AND NON-LEGUMES

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Strains of *Rhizobium parasponia* have the properties of slow-growing Rhizobia and can nodulate many tropical legumes and four species of *Parasponia*, a non-legume. Since strain ANU289 can express nitrogenase activity *in vitro* it is an ideal model for the study of nif gene function and the genetic control of nodulation of both legumes and non-legumes.

The genetic system of transposon mutagenesis has been successfully extended to the slow-growing Rhizobia. Various auxotrophic mutants were isolated and these reverted to prototrophy at different frequencies. DNA isolated from seven presumptive Tn-5 induced mutants was shown to contain a single copy of Tn-5 by specific hybridization with cloned Tn-5 DNA.

Seventeen mutants defective in symbiosis were obtained. One of them, mutant 155, which did not fix nitrogen *in vitro*, is unable to form nodules on the legumes, *Macroptilium atropurpurem*, *Vigna unguiculata*, and *Stylosanthes humilis*. It is possible that this Tn-5 induced lesion is in a gene central to the ability of strain ANU289 to both nodulate a wide range of legumes, and to reduce acetylene *in vitro*.

PAIRING OF SEX CHROMOSOMES DURING MALE MEIOSIS IN MARSUPIALS WITH MULTIPLE SEX CHROMOSOMES.

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A number of species of marsupial have multiple sex chromosomes due to translocations between the original sex chromosomes and autosomes. *Lagorchestes conspicillatus* has an $X_1X_2Y♂/X_1X_1X_2X_2♀$ sex chromosome system, while the other species have an $XY_1Y_2♂/XX♀$ system.

During male meiosis, these chromosomes form trivalents containing regions of both sex chromosome and autosomal origin. The pairing behaviour of these regions has been examined by conventional cytogenetic methods, and by both light and electron microscopy of spread synaptonemal complexes of pachytene spermatocytes.

RIBOSOMAL RNA GENES IN LILIUM

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All Lilium species have similar karyotypes, but the number and location of nucleolar organizing regions (NORs) is quite variable. There are two different NORs in henryi, three in longiflorum and three in speciosum, all in different locations. The NORs are the sites of 18S and 25S ribosomal RNA (rRNA) genes, as shown by silver banding or in situ hybridization using labelled complementary RNA made from cloned wheat ribosomal genes (generously provided by Dr Wayne Gerlach).

Unlike 18S and 25S genes, there is only one site for 5S rRNA genes in these three unrelated species. The site is on an apparently homoeologous chromosome, close to or within an intercalary C-band. However, in henryi and speciosum 5S genes are near the centromere in the long arm while in longiflorum they are much more distal. Also, in longiflorum they are located close to a NOR, which is not present in the chromosome of the other two species. It seems possible that 5S rRNA genes in Lilium, unlike the 18S and 25S genes, occur at only one, relatively constant C-banded location.

RECURRENCE OF CENTRIC FUSIONS IN FAMILIAL LINES OF A GRASSHOPPER

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Abstract. Single-pair matings of the grasshopper Atractomorpha similis (2N = 19♂ ; 20♀) were set up in the laboratory. Among the progeny, three families out of eleven exhibited a tendency toward chromosome rearrangement. All rearrangements involved centric fusion. From eight to ten different fusions were observed. These fusions resulted from one interstitial and 15 to 19 procentric chromosome breakage events. At least seven of the fusions were not transmitted from the parent generation. They arose spontaneously within the individuals in which they were observed. In one family, more than one fusion type arose within each of two individuals. It is concluded that these three grasshopper families have a transmissible tendency towards a particular type of chromosome rearrangement, namely centric fusion. Fusions observed involve at least seven of the ten chromosome pairs. Thus the tendency to procentric breakage is non-specific in respect of linkage group. This phenomenon is discussed in relation to the process of karyotypic orthoselection. The data presented are used in construction of a model of this process.

PATTERNS OF CHIASMA DISTRIBUTION IN CHROMOSOMALLY DISTINCT SUBSPECIES AND THEIR RELATIONSHIP TO HYBRID BREAKDOWN IN *CALEDIA CAPTIVA*

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Analysis of hybrids between two chromosomally distinct subspecies of the grasshopper *Caledia captiva* have shown that although the F_1 generation does not differ from the parental taxa in fertility or viability, the F_2 generation is completely inviable and backcross generations show a range in viability from 0-50%. This inviability results from embryonic breakdown due to arrested development during embryogenesis. It has been proposed that the hybrid inviability may be directly related to the major changes in recombination during meiosis in F_1 hybrids which break up coadapted gene complexes. This proposal has been investigated by studying patterns of recombination during male meiosis in the parental taxa and their F_1 hybrids.

The two taxa (Moreton and Torresian) differ by a series of pericentric rearrangements and by a complex series of interstitial and terminal C-bands which are only present in the Moreton taxon.

Pericentric heterozygosity results in a major change in the pattern of recombination in F_1 individuals. The cellular chiasma frequency is significantly reduced from 15.4 in the parental taxa to 12.9 in the hybrids. Chromosomal heterozygosity is also associated with a major repositioning of chiasmata in 7 of the 11 autosomal bivalents. As a consequence of these readjustments to the crossover positions, a large proportion of the F_1 gametes contain novel recombinant chromosomes. The types and proportion of these recombinant chromosomes expected in the backcross progeny can be predicted after mapping chiasma distributions. Evidence from chromosome studies on the surviving backcross embryos demonstrate a significant difference between observed chromosome types and those predicted from the recombination analysis in the F_1 progeny. This suggests that major changes in the pattern of recombination in the F_1 progeny and the resultant break up of the parental genomes is instrumental in generating the high levels of hybrid inviability among the recombinant F_2 and backcross progeny.

GENETIC AND BIOLOGICAL PROPERTIES OF A FAST-GROWING STRAIN OF *RHIZOBIUM* WHICH CAN NODULATE BOTH LEGUMES AND NON-LEGUMES

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The fast-growing *Rhizobia* strain, NGR234, is unusual since it was isolated from *Lablab purpureus*, a plant of the 'cowpea' group. Usually 'cowpea'-type plants are nodulated by classic slow-growing strains of *Rhizobia*. The host range of NGR234 is extensive amongst the 'cowpea' group of plants, and also includes plants usually nodulated by fast-growing *Rhizobia*, such as *Medicago sativa*, and plants which usually have strict symbiont requirements (*Acacia* and *Lucaena*). This fast-growing strain which has a broad host range amongst the legumes is also able to nodulate the non-legume, *Parasponia*.

A preliminary genetic and biological analysis of this strain is presented. A carbon source utilization analysis affords a comparison with slow-growing strains able to nodulate both cowpea plants and the non-legume, *Parasponia*.

The behaviour of 'suicide' plasmids used for transposon mutagenesis is discussed and the transfer of various other plasmids is presented.

COMPARISON OF THE DNA SEQUENCES FOR THE Fe-PROTEIN COMPONENT OF THE NITROGENASE ENZYME IN FREE-LIVING AND SYMBIOTIC BACTERIA

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A DNA fragment carrying the *Klebsiella pneumoniae* nifK, D and H genes was isolated from the mutant strain UNF841 (Tn5::nifK) by molecular cloning into the *E. coli* plasmid pBR325. The nucleotide sequence of the nifH gene, which encodes the Fe-protein (Dinitrogenase Reductase) of the nitrogenase enzyme complex, was determined by direct DNA sequencing using both the chemical and chain termination methods. This DNA fragment was then used as an hybridization probe to isolate the homologous gene from clone 'banks' of DNA constructed from two strains of *Rhizobium*:- *Rhizobium trifolii SU329* which fixes nitrogen only in symbiotic association with various species of clover, and a strain of *Rhizobium* (ANU289) capable of nitrogen fixation *in vitro* and in symbiotic association with some species of leguminous and non-leguminous plants.

Comparison of the protein sequences deduced from these three DNA sequences shows a marked conservation of Fe-protein primary structure. Extensive regions of strong conservation (90-95%) are found while other regions show relatively weak conservation (30-35%). In particular, five cysteine residues are retained in highly conserved regions in all three proteins. Since the active centre for Dinitrogenase Reductase is believed to be a 4Fe-4S cluster, these cysteine residues are obvious candidates for ligands to the active centre. The strongly conserved regions are thus most likely to be of special importance to the catalytic properties of these enzymes and suggest that the homology observed is related to function and probably not merely the result of late evolution and dispersal of nif genes. While these three species of bacteria show strong conservation at the amino acid sequence level, they share very little homology (~30%) at the triplet codon level. This implies that while strong, presumably functional, constraints have acted at the protein structure level, the nucleotide sequence of the gene and its mRNA have only been restrained by the coding requirements allowing substantial drift in codon usage.

Plasmid effects on inducible DNA repair in Pseudomonas aeruginosa.

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A system has been developed using Pseudomonas aeruginosa bacteriophage C5 which allows the demonstration of an inducible DNA repair system in this organism. This inducible repair system is dependent on a functional rec^+ gene. The inducible repair system increases phage survival after UV or gamma irradiation as well as markedly increasing the mutation rate. The features of the system, as well as the effects of the plasmid pMG15 on repair, will be presented.

STRUCTURE AND FUNCTION OF THE TYROSINE OPERON OF ESCHERICHIA COLI.

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The tyrosine operon of Escherichia coli contains two structural genes, aroF and tyrA which encode enzymes responsible for the biosynthesis of tyrosine [DAHP synthetase (tyr) and chorismate mutase T - prephenate dehydrogenase, respectively]. Transcription occurs in the order aroF, tyrA and is controlled by the interaction of an adjacent cis-acting regulatory locus (a hypothetical operator) with the protein coded by the tyrR gene (a hypothetical aporepressor) and tyrosine. The tyrosine operon has been cloned from a λtyr transducing phage into the plasmid vector, pMB9. Further cleavage of the cloned DNA containing the tyrosine operon and recloning of smaller fragments into pBR322 indicates that the entire operon lies within a region of about 2700 base pairs (bp). Estimated molecular weights of 40,000 daltons each for the two polypeptide products of the operon, indicate a combined aroF-tyrA translated sequence of about 2000 bp. About 85% of the base sequence of the 2700 bp region containing the tyr operon has now been determined by direct DNA sequencing. This DNA sequence has been correlated with much amino acid sequence of the aroF-coded protein (the latter determined by K. Herrman and J. Schulz, Purdue University) and with the first few amino-terminal amino acids and the last two carboxyl-terminal residues of the tyrA-coded protein (determined by C. Yanofsky, Stanford University). The translation start codon of aroF is preceded by a putative ribosome binding site and further upstream is an initiation site for in vitro mRNA transcription. The precise nucleotide (an adenine residue) at which transcription initiates in vitro has been determined by sequencing the RNA products of transcription of DNA fragments containing the 5' end of the tyr operon. This nucleotide is 51 residues 5' to the aroF translation start codon, i.e., the untranslated "leader" of the tyr operon is 51 nucleotides long. This sequence contains no features which might indicate an attenuation control mechanism for transcription such as occurs in several other amino acid biosynthetic operons e.g., the tryptophan, phenylalanine and histidine operons. The DNA sequence immediately preceding the transcription start site contains the putative promoter of the tyr operon i.e., the DNA sequence signalling recognition, binding and transcription initiation by RNA polymerase. It exhibits many features common to many known E. coli promoter sequences. Following the aroF translation start codon is a sequence of about 1000 bp which exhibits extensive correlation with the amino acid sequence of the protein product including the C-terminal region. Immediately following the C-terminus is an ochre stop codon, 10 untranslated nucleotides and the translation start codon of tyrA. Within the untranslated, intercistronic region is a putative ribosome binding site for tyrA. About 1000 nucleotides 3' to the tyrA start codon is a sequence corresponding to the C-terminal dipeptide of the tyrA-coded protein immediately followed by tandem ochre translation stop codons. This is followed in turn by a sequence of about 34 nucleotides exhibiting features characteristic of many known transcription stop signals.

CLONING THE ACETAMIDASE GENE OF *ASPERGILLUS NIDULANS*

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The *amdS* gene of *A. nidulans* codes for an acetamidase enzyme which is required for utilization of amidase as carbon or nitrogen sources. Extensive genetic studies of the regulation of this enzyme have shown that it is subject to complex control by a number of unlinked regulatory genes acting independently of each other. *Cis* acting regulatory mutations have been mapped in a controlling region adjacent to the *amdS* structural gene. Some of these mutations show specific interactions with mutations in the regulatory genes. DNA containing the *amdS* gene has been cloned in λ vectors by differential hybridization using cDNA probes made from RNA extracted from wildtype and *amdS* deletion strains. Whole genome Southern blot analysis is being used to map the *amdS* gene within the cloned DNA as a preliminary step in analysing the *amdS* gene and its controlling region in detail.

PERTURBATION OF GENE FREQUENCIES IN A CELLAR POPULATION OF *DROSOPHILA MELANOGASTER*

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Previous studies on the *Adh* polymorphism in the *D. melanogaster* cellar population of the "Chateau Tahbilk" vineyard have correlated temperature and gene frequency such that the Adh^S allele is more common at lower temperatures. Changes in gene frequency have been related to the temperature dependent specific activities of the enzymes associated with the *Adh* genotypes.

In the present study *Adh* gene frequencies have been perturbed, over years, to values above and below those normally observed in the cellar population. Phenol oxidase gene frequencies have also been perturbed and have acted as a control for the *Adh* system. Changes in *Adh* gene frequency subsequent to perturbation may be explained by a temperature dependent model relating enzyme activity to the relative fitness of the phenotypes and support the hypothesis of selection acting at the *Adh* locus.

SEX AND VARIABILITY IN THE COMMON BROWN BUTTERFLY

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An analysis of wing-pattern variability in the Australian bufferfly Heteronympha merope merope (Satyrinae) has shown that (1) the genetic component of variation is larger in females than in males for those wing pattern characters which can be compared directly; (2) within populations females are consistently more variable than males; (3) all female characters show significant differences between populations whereas not all male characters do; (4) there is a clinal pattern to the variation in females which is significantly associated with changes in environmental variables whereas males show no obvious pattern of geographic variation. These results are convincing evidence that H. m. merope exhibits reduced male variability and that this phenomenon affects many aspects of variation both within and between populations.

Current theories on the genetic basis and evolutionary significance of reduced male variability in Lepidoptera are discussed. Recent evidence of the absence of dosage compensation provides an underlying mecahnism for the phenomenon. However, the favoured evolutionary theory, sexual selection by female choice is strongly criticized. Alternative sexual selection hypotheses based on habitat separation and male competition, are discussed.

GENETIC DIFFERENCES IN A CACTOPHILIC *DROSOPHILA* FOR FEEDING AND OVIPOSITION PREFERENCES ON CACTUS YEASTS

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Of what consequence is the *Opuntia* (prickly-pear cactus) microbial community to the nutrition of *Drosophila buzzatii*? Do the micro-organisms play a role in the maintenance of some enzyme polymorphisms in this insect? Laboratory experiments show that actively growing bacteria and yeasts differentially affect the nutritional sufficiency of cactus homogenate as measured by developmental time and per cent emergence of the insect. As the myriads of metabolic products of numerous microorganisms could function as substrates for different enzyme forms, we are searching, first, for the yeast preference the fly may have for feeding and oviposition and second, for associations between fly genotype and its feeding and oviposition preference. We are also investigating the distribution of microorganisms within a single rot in space and time as a measure of one facet of environmental heterogeneity. The results of these ongoing investigations will be discussed.

FURTHER STUDIES ON HYBRID DYSGENESIS IN *DROSOPHILA MELANOGASTER*

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Hybrid dysgenesis is a recently discovered complex of aberrations, including sterility, transmission ratio distortion, mutation and male recombination, which affects the progeny of certain crosses of *D. melanogaster*. Dysgenesis is known to be associated with insertion sequences (IS) since dysgenic progeny derive from crosses of males carrying such an IS to females lacking it. The suggestion has been made that the IS causes dysgenesis in a manner analogous to the zygotic induction of phage λ in *E. coli*. In our studies, radiation and chemical treatments known to enhance zygotic induction of λ had some effect on the rate of male recombination and hybrid sterility but the effect is not directly ascribable to induction of an IS. Our studies of the sterility of females from the second generation backcrosses do, however, support the hypothesis.

How an IS might cause male recombination remains obscure. We have made a number of studies aimed at further characterising this aspect of dysgenesis. No effect of dysgenesis on reversion of the Bar locus to wild-type was demonstrable so that dysgenic recombination is probably not unequal. Dysgenesis was shown to cause a slight increase in the rate of sister chromatid exchange and to induce a small amount of crossing over in the fourth chromosome of heterozygous females.

PARASITIC B-CHROMOSOMES : POPULATION AND CYTOGENETICAL STUDIES ON THE AUSTRALIAN BUSHRAT

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Individuals carrying B-chromosomes (sometimes called accessory- or supernumerary-chromosomes) are found in a great many species from most groups of plants and animals. Detailed studies of B-chromosome behavior have, however, been made on only a few species, mainly from the Orthopteran insects or the grainbearing grasses. B-chromosomes in mammals have received little attention, and research has tended to be directed at characterising the B-chromosome cytology, with studies on the population genetics being quite rare.

This report describes the cytology of B-chromosomes in Rattus fuscipes (the Australian bushrat), and the results of a study assessing the comparative survival of B-chromosome-bearing rats in two natural populations.

Laboratory breeding studies of R.fuscipes show that at meiosis, non-disjunction of the Bs occurs at both divisions. Such non-disjunction may not be at random. In females, there is evidence of a "meiotic drive" system. Observations of meiosis in males show that there is an increase in chiasma frequency associated with the presence of B-chromosomes.

Although the association of increased chiasma frequencies with the presence of B-chromosomes in other species has often been cited as an important aspect of the adaptive significance of the Bs, it is difficult to reconcile this view with the observations made in this population study. The results presented here show that a far greater proportion of rats with B-chromosomes are lost from the population before breeding season than rats without B-chromosomes. It is concluded that the existence of B-chromosmes in these populations cannot be explained by a model involving an overall heterotic advantage of B-chromosome individuals, but that it is more consistent with the "parasitic" model of B-chromosome maintenance. The latter model involves a balance between the apparent accumulation of B-chromosomes in progeny from B-carrying female parents with the loss of individuals carrying Bs from the population before they have the opportunity to breed.

HORMONAL REGULATION OF α-AMYLASE mRNA LEVELS IN BARLEY ALEURONE LAYERS

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Gibberellic acid (GA_3) induces α-amylase synthesis in barley aleurone layers. PolyA$^+$ RNA from GA_3-treated layers (which synthesizes α-amylase *in vitro*) was cloned into pBR322. Several colonies were obtained which hybridized with +GA cDNA but not with -GA cDNA. A plasmid from one such colony was shown by hybrid-release translation and immunochromatography of the translation products to code for α-amylase. This plasmid (pHV23) was used to probe Northern filters of +GA_3 RNA. Hybridization to an RNA species of approx. 1600 nucleotides was observed representing amylase mRNA. Since variation is known to exist at the polypeptide level for α-amylase, any single amylase clone used as a probe may not detect all amylase mRNA species. The induction kinetics of the pHV23-related amylase mRNA following GA_3 treatment revealed a 40-fold increase in abundance between zero and 18 hours. RNA from layers treated for 24 hr with a mixture of GA_3 (1μM) and abscisic acid (ABA, 25 μM) (conditions under which there is very little α-amylase synthesis) was deficient in this amylase mRNA relative to layers treated with only GA_3 (3.4-fold and 48-fold stimulation over control respectively). Hence this particular α-amylase is apparently regulated by GA_3 and ABA at the level of mRNA abundance.

MATING SYSTEM & CROSS-COMPATIBILITY OF RACES OF EPACRIS IMPRESSA

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The mating system, and cross-compatibility of races of Epacris impressa Labill., were examined by a series of selfings, outcrosses between plants of the same race from the same and different populations and outcrosses between plants of different races. The percentage of pollinations that gave capsules and the number of seeds present per capsule for these four types of crosses, and whether the seeds germinated were scored. The results indicate that E. impressa is an outcrossing species with a self-incompatibility mechanism to keep selfing at a low level, that the races cross freely, and that the hybrid seed is viable. The means over all races for the above four types of crosses were 8.7 (selfing), 62.3, 62.5 and 65.5 for the percentage of pollinations that were successful and 8.4 (selfing), 43.5, 41.2 and 39.0 for the number of seeds per capsule.

A limited number of crosses between Epacris impressa and other Epacrid species have also been made.

SPREADING MAIZE SYNAPTONEMAL COMPLEXES FOR ELECTRON MICROSCOPY.

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A technique has been developed which allows the spreading of the synaptonemal complexes (SCs) of maize pachytene microsporocytes for direct examination in the electron microscope (EM). All steps prior to EM examination are carried out on plastic-coated glass microscope slides, so that procedures can be monitored by light microscope phase optics. After phosphotungstic acid or ammoniacal silver staining the SCs and kinetochores of all 10 bivalents are readily recognised in the EM. Using relative lengths and arm ratios, SC karyotpyes can be prepared which agree well with published determinations. The method has been used to prepare spreads of two inversion heterozygotes and two reciprocal translocation heterozygotes. In all cases the breakpoints of the re-arrangements which were determined from SC spreads were in agreement with light microscopic and genetic estimations. The spreading technique also allows investigation of variability in synapsis, and the degree of non-homologous association in early pachytene.

PROPERTIES OF HALDANE'S MODEL OF ASSORTATIVE MATING WITH DOMINANCE

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The frequencies at generation t of genotypes gg, gG, and GG are denoted by $f_0(t)$, $f_1(t)$, and $f_2(t)$ respectively. The mating pair frequencies in canonical form are

$$f_{ij}(t) = f_i(t).f_j(t)\{1 + \rho(t)x_i(t)x_j(t)\}. \qquad (1)$$

Haldane's mating frequencies are obtained from (1) by the substitution

$$\rho(t) = \rho^*(t) = 1 - \theta(t)/\{f_0(t)\{1 - f_0(t)\}\}, \text{ where}$$

$$\theta(t) = \left[\{1 + 4\Lambda f_0(t)\{1 - f_0(t)\}\}^{\frac{1}{2}} - 1\right]/(2\Lambda), \text{ for constant } \Lambda$$

and $x_1(t)=x_2(t)= \{f_0(t)/\{1-f_0(t)\}\}^{\frac{1}{2}}$, $x_0(t)= -\{x_1(t)\}^{-1}$. (2)

In O'Donald's system a fixed proportion α of phenotypes mate with like phenotype and the remainder mate at random. The frequencies are obtained in canonical form (1) by the substitution

$$\rho(t) = \rho \equiv \rho' = \alpha \text{ and } \{x_i(t)\} \text{ as in (2).}$$

In equilibrium the frequency of genotype gG is given by O'Donald as

$$f_1 = p^2(\alpha-1) + p\{(1-\alpha)\{p^2(1-\alpha)+4q\}\}^{\frac{1}{2}},$$

where $q = f_0 + \frac{1}{2}f_1$ and $p = 1-q$. Using the corresponding f_0, the same mating, and therefore the same offspring frequencies, can be obtained in equilibrium under Haldane's scheme by setting Λ to be

$$\Lambda = \alpha/\{f_0(1 - f_0)(1 - \alpha)^2\} ,$$

for then $\theta(t) = \theta = f_0(1-f_0)(1-\alpha)$ and $\rho^*(t)=\rho^*=\alpha=\rho'$.

The rate of approach to equilibrium is of the same order in both. If the genotypic frequencies in equilibrium are expressed as $f_0 = q^2 + \lambda pq$, etc., ρ is related to q and λ by

$$\rho = \lambda\{1 + q(1-\lambda)\}/(q+\lambda p),$$

and this identity, together with (2), define all such mating systems in equilibrium.

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FRA (X)(q27) IN MENTALLY RETARDED FEMALES

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A fragile site on the long arm of the X-chromosome in man has been closely associated with some forms of X-linked mental retardation. Affected males are retarded and usually have enlarged testes and carrier females are often normal but sometimes described as "dull" and more recently even mildly retarded.

A pedigree showing the fragile site at Xq27 in a severely retarded females and in less retarded carriers will be displayed. Four moderately retarded males are in the pedigree but only two of these show macro-orchidism and other features suggestive of the effects of the fra (X)(q27) are also variable.

The pedigree is of particular interest because the fra (X)(q23) site is also present and it is detectable at normal frequencies in the two oldest females. In these two females the fra (X)(q27) cannot be detected although probably present.

It is concluded that pedigrees showing mentally retarded females and probable X-linkage should be included in studies of the fra (X)(q27).

Report on Boden Conference "Molecular Genetics and Plant Improvement"
February 4-6 1981

"How can plant molecular biology and the methodology of tissue culture research contribute to Australian plant breeding?" was the question discussed at the 2nd Boden Conference, sponsored by the Australian Academy of Science, held at Thredbo from 4-6 February this year. The meeting provided a unique opportunity for contact between plant breeders, molecular biologists and tissue culture geneticists. Professor J. Shepard of Kansas State University was the guest speaker at the meeting. He described the increased phenotypic variability which he has achieved in potato by using a tissue culture cycle. He produces protoplasts from mesophyll cells, cultures them and subsequently regenerates plants. The resultant lines show wide variation for many characters which remain stable in subsequent vegetative propagation. These lines are already in the process of field testing and should result in the production of improved cultivars.

Other speakers discussed the range of plant species in which usable variation has been recovered after regeneration of plants from cell cultures. Colchicine can be used to induce a similar effect in sorghum seedlings and it is possible that this could be an alternative to tissue culture for species which cannot be cultured and regenerated. Although little is known about the molecular basis for the increased variability in plants following a tissue culture cycle, this methodology currently promises to produce variation useful to breeders of vegetatively propagated species and is expected soon to be applicable to the improving of cereal and woody plants.

With a heavy emphasis towards wheat breeding, plant breeders provided an overview of the strategies and priorities of their various breeding programmes. The economic losses due to plant diseases were stressed as was the consequent high priority of breeding for disease resistance.

Current knowledge of plant molecular biology was another topic of the conference. The nature of plant genes and the regulation of their expression was discussed. The first plant genes to be well-characterized, the 5S ribosomal genes of wheat and the phaseolin gene of bean, were discussed in detail. Some examples of successfully cloned messenger RNAs coding for proteins were described: the small subunit of RuBP carboxylase and its induction by light, alcohol dehydrogenase and its synthesis under anaerobic conditions,
α-amylase and the effect of plant hormones on its synthesis, and genes expressed specifically in meiosis. In at least the first three cases, control of gene expression was at the level of transcription; the specific messenger RNA level increasing many fold when the gene was switched on.

The transfer of desirable plant genes is the aim of both plant breeders and molecular biologists and potential vectors for such gene transfer were discussed. Possible vectors include viruses, the tumour inducing (Ti) plasmid from Agrobacterium, or simply naked DNA. The Ti plasmid appears to be a most promising vector since it enters the cells of a wide range of dicotyledonous plant species. Previously, its tumorgenicity had mitigated against its use as a vector but now lines containing unstable tumor inducing genes have been constructed.

Whatever method is used to provide DNA for transformation it must be taken up by the recipient plant cells. It has been routinely considered that protoplast cells in tissue culture will be used to take up DNA but pollen was discussed as an alternative system. Pollen is known to take up DNA readily and its use avoids many of the technical limitations of tissue culture. No matter how the DNA enters the cell the necessity of having a good selection system for detecting cells containing the desired genes was stressed. A number of possibilities using a mutant cell line and coupling the desired characters to the wild type genes were considered.

Despite the range of topics covered many other problems were not discussed. The possibility of obtaining expression of introduced genes in plant cells, the difficulty of regulating introduced genes, the question of whether such genes could function in regions of a chromosome other than their rightful position are some of the major problems remaining.

In a summary session plant breeders suggested examples of genes which they would like to modify or transfer across existing sexual barriers. These included cases where the products of single genes are involved and clones are available; important characters such as waterlogging resistance (alcohol dehydrogenase), brewing quality and sprouting resistance (α-amylase), photosynthetic efficiency (RuBP carboxylase), oil content (fatty acid desaturases), and seed protein quality. More complex multi-locus situations were also considered; for example the transfer of nitrogen fixation genes, or the conversion of C_4 plants into C_3 plants. Instances where the gene products involved are not currently known were also mentioned e.g. pathogen resistance genes,

male fertility genes, genes that influence the extent of meiotic recombination and genes controlling the extent of outbreeding.

This meeting provided a unique opportunity for contact between the different plant scientists. Fundamental research into the nature and control of plant genes deepens the understanding of plant breeders in their task; molecular biologists, at the same time, found that definition of the problems of plant breeding helps in the search for relevant systems.

All plant scientists are excited by the potential of new techniques for the improvement of agriculture; the Boden Conference at Thredbo, February 1981 lived up to this expectation.

Dr. E. Dennis
Dr. W. Gerlach

2ND BODEN CONFERENCE : "Molecular Genetics and Plant Improvement"

Thredbo, 4-6 February 1981

List of Participants

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Dr. E. Williams, University of Melbourne, Melbourne.

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