

GSA 2001

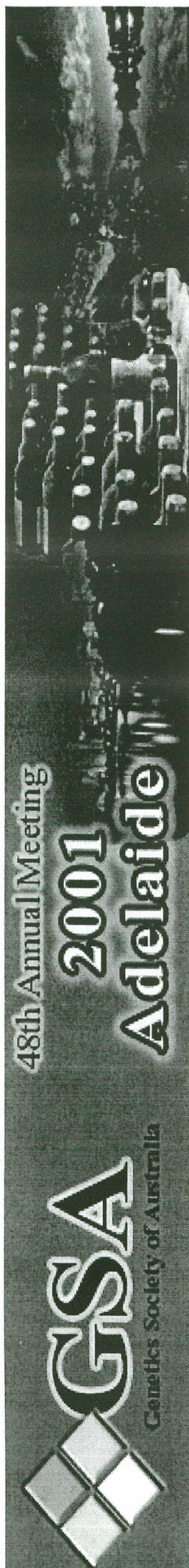
Genetics Society of
Australia
2001 Conference

48th Annual Meeting

ADELAIDE
SOUTH AUSTRALIA

3rd – 7th July 2001

ISSN 1329-2420





48th Annual Meeting

2001
Adelaide



Genetics Society of Australia - 48th Annual Meeting

Adelaide, 3rd – 7th July 2001

Organising Committee:

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** Sustaining members mounting trade displays*

Student Prizes:

Oral presentations: XIX International Congress of Genetics, Melbourne, 2003

Poster prizes: Qiagen Pty Ltd

Special Thanks:

Special thanks to Wyndham Estate for providing wines for the wine tasting.

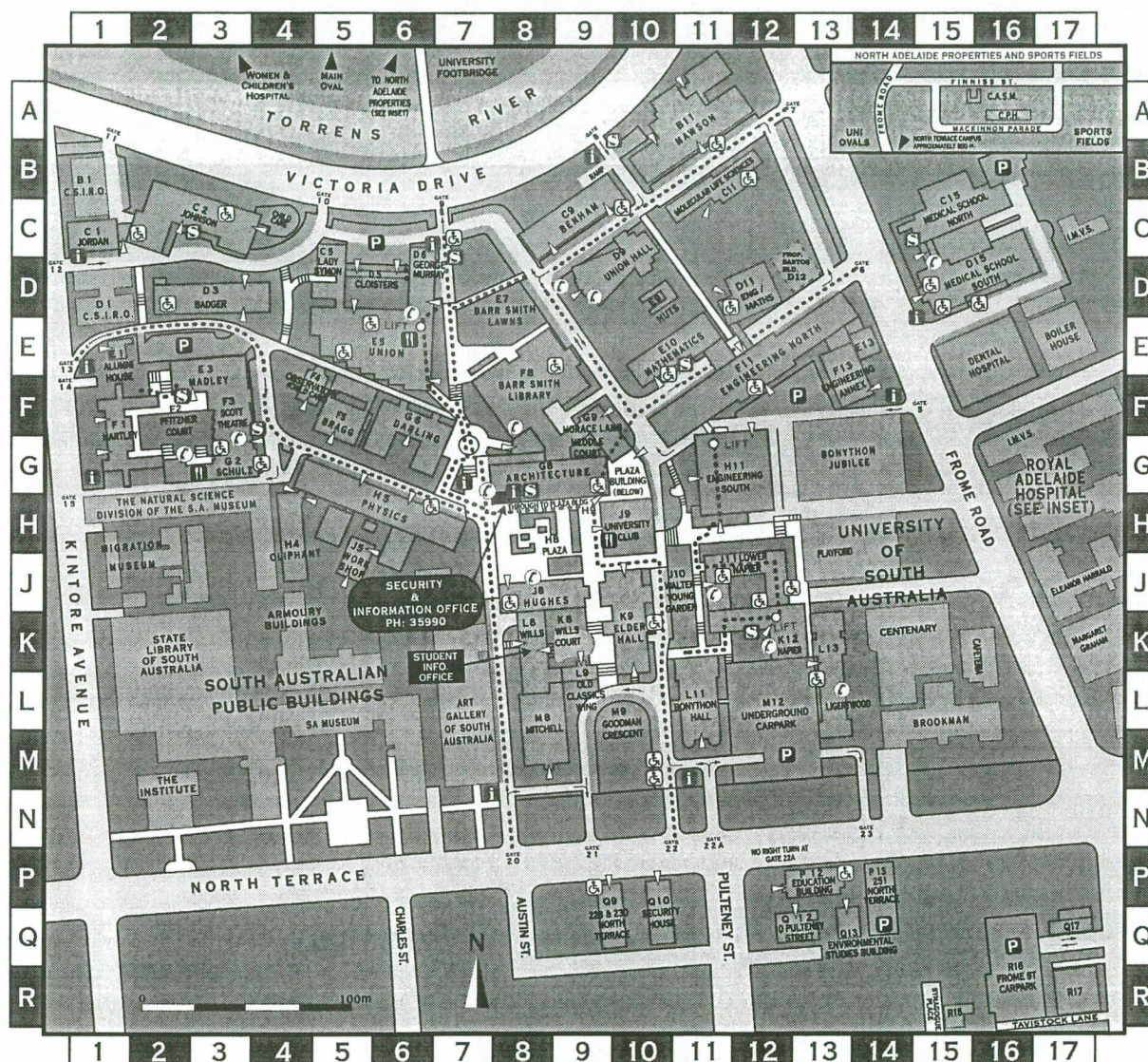


2001

Adelaide

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NORTH TERRACE CAMPUS



☎ TELEPHONES ⓘ INFORMATION BOARDS ■ THE UNIVERSITY OF ADELAIDE BLDGS. P MAJOR PARKING ■ CAR PARKS (PERMIT ONLY) 🍽️ FOOD ▲ PRINCIPAL ENTRANCES S SECURITY CALL POINT - - - - - SAFE WALKWAYS

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Conference Venues:

C 11 = Molecular Life Sciences Bldg

B 11 = Mawson Building

C 9 = Benham Building

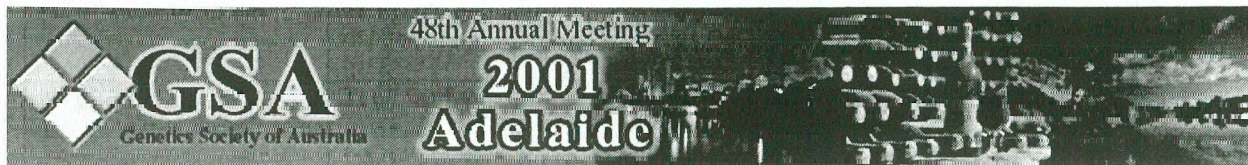
Other places of interest:

E 5 = Union Building (Food, coffee)

Shopping = Rundle Mall ***

Food = Rundle Street (City), ***
O'Connell St (North Adelaide) ***

*** Please refer to City Map.



Programme

TUESDAY, 3 rd July 2001		
4.00 pm – 7.30 pm	Registration	1 st Floor, Molecular Life Sciences Bldg.
7.30 pm – 8.30 pm	B. Weir (North Carolina State University, USA) Measuring Human Relatedness (p. 14)	Fisher Lecture, Mawson Lecture Theatre (Chair: R. Hope)
8.30 pm	Mixer	

WEDNESDAY, 4 th July 2001		
9.00 am – 9.10 am	Welcome	Keynote Address, Mawson Lecture Theatre
9.10 am – 10.10 am	M. Fuller (Stanford University, USA) Regulation of Stem Cell Self-Renewal versus Differentiation (p. 6)	Keynote Address, Mawson Lecture Theatre (Chair: G. Hime)
10.10 am – 10.50 am	Tea / Coffee	1 st Floor, Molecular Life Sciences Bldg.
10.50 am – 11.10 am 11.10 am – 11.30 am 11.30 am – 11.50 am 11.50 am – 12.10 pm 12.10 pm – 12.30 pm	CONCURRENT SESSIONS I <u>Developmental genetics and neurogenetics</u> R. Todd (University of Melbourne) The <i>Penicillium marneffei</i> TUP1 homologue represses both asexual development and yeast morphogenesis to allow vegetative filamentous growth. (p. 84) N. Siddall (University of Melbourne) Mutations in <i>lozenge (Iz)</i> permit ectopic patterned cell death in the developing <i>Drosophila</i> eye. (p. 72) P. Batterham (University of Melbourne) Is the asymmetry <i>Modifier</i> an allele of the <i>Notch</i> orthologue in the Australian sheep blowfly, <i>Lucilia cuprina</i> ? (p. 5) G. Hime (University of Melbourne) Analysing oncogene function in <i>Drosophila</i> (p. 40) K. Snowden (Hort Research, Auckland) Control Of Axillary Branching In <i>Petunia</i> (p. 75)	
	<u>Genome Analysis</u> B. Weir (North Carolina State University) A classical approach to association mapping (p. 91) D. Heckel (University of Melbourne) Molecular basis of insect resistance to <i>Bacillus thuringiensis</i> toxins (p. 39) C. Robin (University of Melbourne) Dissecting a quantitative trait at nucleotide resolution (p.60) D. Shearman (University of Sydney) Towards biological control of tephritid fruit fly pests – genetic transformation with sex-specific genes (p. 70) Y. Parsons (LaTrobe University) Mapping quantitative trait loci (QTL) affecting male courtship song in Hawaiian crickets (p. 60)	
	Benham Lecture Theatre (Chair: R. Saint)	
	Mawson Lecture Theatre (Chair: C. Bottema)	
12.30 pm - 2.00 pm	Lunch	

Programme

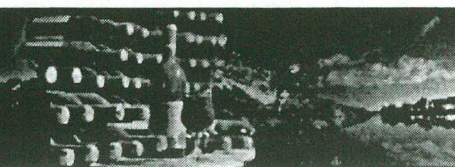
WEDNESDAY, 4th July 2001		
2.00 pm - 2.40 pm	M. Lavin (QIMR) Role of ATM in maintaining genome integrity (p. 8)	Keynote addresses, Mawson Lecture Theatre (Chair: P. Sykes)
2.40 pm - 3.20 pm	M. Tanaka (Emory University, USA) Old topics for new millenia: the evolution of mutation rates re-visited (p. 12)	
3.20 pm - 4.00 pm	C. Warr (Yale University) Odorant receptor distribution and olfactory coding in <i>Drosophila</i> (p. 13)	
4.00 pm - 5.30 pm	Tea / Coffee / Poster Session	1 st Floor, Mol. Life Sciences Bldg

THURSDAY, 5 th July 2001		
9.00 am – 10.00 am	M. Scott (Stanford University, USA) Inducible Antagonists of Hedgehog and Wnt Signaling (p. 10)	Keynote addresses, Mawson Lecture Theatre (Chair: R. Saint)
10.00 am – 10.40 am	D. Miller (James Cook University) Deep Evo-Devo: zootype genes and zootype precursor genes in the cnidarian <i>Acropora millepora</i> (p. 9)	
10.40 am – 11.20 am	Tea / Coffee	1 st Floor, Mol. Life Sciences Bldg
CONCURRENT SESSIONS II		
<u>Recombination/Chromosome rearrangements / repair:</u>		
11.20 am – 11.40 am	M. Dowton (University of Wollongong) Gene rearrangements as phylogenetic characters – evidence that intramitochondrial recombination shapes mitochondrial genome organization (p. 28)	Benham Lecture Theatre (Chair: M. Lavin)
11.40 am – 12.00 pm	J. Sved (University of Sydney) A test for sister-strand recombination induced by P elements in a ring chromosome of <i>Drosophila melanogaster</i> (p. 78)	
12.00 pm – 12.20 pm	P. Sykes (Flinders University) Reduction of somatic intrachromosomal recombination below spontaneous frequency in the pKZ1 recombination mutagenesis model in response to DNA damaging agents and a mismatch repair deficiency (p. 79)	
12.20 pm – 12.40 pm	A. Hooker (Flinders University) The effect of etoposide on somatic intrachromosomal recombination: a comparison in <i>in vivo</i> and <i>in vitro</i> pKZ1 murine models (p. 42)	
<u>Systematic / Population (Thursday 2x4 sessions am and pm, Saturday 1x4 sessions)</u>		
11.20 am – 11.40 am	A. Schultheis (Griffith University) Gene flow, dispersal, and nested clade analysis among populations of the stonefly <i>Peltoperla tarteri</i> in the southern Appalachians, Virginia, USA (p. 68)	Mawson Lecture Theatre (Chair: S. Cooper)
11.40 am – 12.00 pm	L. Broadhurst (Conservation and Land Management, WA) Systematics of broombush – The <i>Melaleuca uncinata</i> complex (Myrtaceae). (p. 20)	
12.00 pm – 12.20 pm	J. MacKenzie (James Cook University) Are Colour Morphs of Corals Genetically Distinct? (p. 53)	
12.20 pm – 12.40 pm	M. Guzik (James Cook University) Evolutionary history of juvenile life history strategies in the benthic octopuses (Cephalopoda: Octopodidae). (p. 36)	
12.40 pm - 2.00 pm	Lunch	



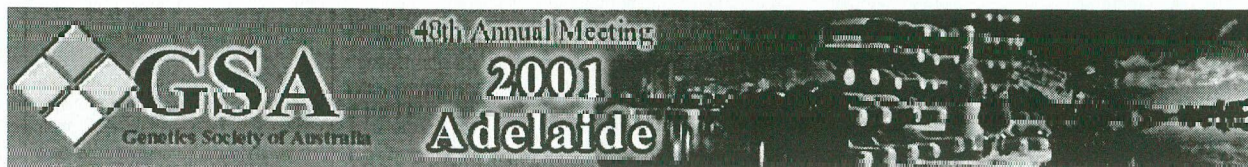
Programme

THURSDAY, 5 th July 2001		
	CONCURRENT SESSIONS III	
	<u>Behavioural ecological genetics</u>	Benham Lecture Theatre (Chair: B. Oldroyd)
2.00 pm – 2.20 pm	K. Palmer (University of Sydney) Red queens in honeybee colonies: How parasites could drive the evolution of extreme multiple mating (p. 59)	
2.20 pm – 2.40 pm	M. Sanetra (James Cook University) Colony genetic structure in the primitive Australian ant <i>Nothomyrmecia macrops</i> . (p. 66)	
2.40 pm – 3.00 pm	A. Corrie (La Trobe University) Life Cycle of Grape Phylloxera, <i>Daktulospairia vitifoliae</i> , in Australia (p. 26)	
3.00 pm – 3.20 pm	E. Cameron (University of Sydney) The Genetic Structure of Colony and Drone Aggregations in Stingless Bees (p. 22)	
	<u>Systematic/Population</u>	Mawson Lecture Theatre (Chair: C. Leach)
2.00 pm – 2.20 pm	M. Eldridge (Macquarie University) Source population of dispersing rock-wallabies (<i>Petrogale lateralis</i>) identified by assignment tests on multilocus genotypic data (p. 29)	
2.20 pm – 2.40 pm	A. Pietsch (Adelaide University) Individual-based Simulation in Population Genetics: Past, Present and Future (p. 61)	
2.40 pm – 3.00 pm	A. Wilton (University of NSW) Dingoes: what levels of introgression can be detected? (p. 94)	
3.00 pm – 3.20 pm	A. Young (CSIRO Plant Industry) The Effects of Genetic Self-incompatibility on the Population Viability of Plants with Varied Life Histories (p. 96)	
3.20 pm – 4.20 pm	Tea / Coffee / Poster Session	1 st Floor, Mol. Life Sciences Bldg.
4.20 pm – 5.00 pm	T. Speed (Walter and Eliza Hall Institute) On some microarray experiments exploring the mouse olfactory systyem (p. 11)	Keynote addresses, Mawson Lecture Theatre (Chair: D. Kortschak)
5.00 pm – 5.40 pm	C. Claudianos (Imperial College, UK) Gene Mining Plasmodium: A new family of scavenger receptor proteins PxSR-1 essential for development of malaria (p. 4)	
7.00 pm	GSA Dinner	Adelaide Oval



Programme

FRIDAY, 6 th July 2001		
9.00 am – 10.00 am	R. Butlin (University of Leeds, UK) Barriers to gene exchange in hybrid zones (p. 2)	Keynote addresses, Mawson Lecture Theatre (Chair: D. Kortschak)
10.00 am – 10.40 am	A. Hoffmann (LaTrobe University) Laboratory and field analysis of clinal variation in two <i>Drosophila</i> species. (p. 7)	
10.40 am – 11.40 am	Tea / Coffee / Poster Session	1 st Floor, Mol. Life Sciences Bldg.
CONCURRENT SESSIONS IV		
<u>Conservation Genetics</u>		
11.40 am - 12.00 pm	R. Frankham (Macquarie University) Is population fitness correlated with genetic diversity? (p. 33)	Benham Lecture Theatre (Chair: J. Timmis)
12.00 pm – 12.20 pm	N. Murray (LaTrobe University) Gene Flow is not Dispersal: lessons from Penguins. (p. 57)	
12.20 pm – 12.40 pm	E. Burns (University of NSW) Genetic diversity and gene flow among Southern NSW catchment green and golden bell frog (<i>Litoria aurea</i>) populations (p. 21)	
12.40 pm - 1.00 pm	D. Coates (Western Australian Herbarium) Population genetic structure and the mating system of the rare ghost wattle <i>acacia sciophanes</i> and its common congener <i>acacia anfractuosa</i> (p.24)	
<u>Speciation/adaptation</u>		
11.40 am - 12.00 pm	J. McKenzie (University of Melbourne) The Evolution of Insecticide Resistance. Chance, Selection and Response (p. 54)	Mawson Lecture Theatre (Chair: A. Hoffmann)
12.00 pm – 12.20 pm	C. Milton (University of Melbourne) Impact of impaired Hsp90 on bristle and wing asymmetry in <i>Drosophila melanogaster</i> (p. 55)	
12.20 pm – 12.40 pm	N. Tunstall (Monash University) Clinal variation in the <i>period</i> gene of <i>Drosophila melanogaster</i> may facilitate behavioural adaptation to photoperiod in different climatic regions (p. 87)	
12.40 pm - 1.00 pm	A. Anderson (Monash University) A gene for all climates: the <i>hsp-omega</i> polymorphism of <i>Drosophila melanogaster</i> associates with both heat and cold resistance. (p. 15)	
1.00 pm - 2.10 pm	Lunch	
CONCURRENT SESSIONS V		
<u>Evolutionary Genetics</u>		
2.10 pm – 2.30 pm	J. Graves (ANU) The rise and fall of the mammalian sex determining gene <i>SRY</i> (p. 35)	Mawson Lecture Theatre (Chair: R. Hope)
2.30 pm – 2.50 pm	R. Crozier (James Cook University) A putative transferrin gene from a termite upregulated in response to fungal infection. (p. 27)	
2.50 pm – 3.10 pm	J. Timmis (Adelaide University) Transposition of chloroplast DNA to the nucleus (p. 83)	
3.10 pm – 3.30 pm	R. Newcomb (HortResearch, NZ) Radiations of genes within fruit tree genomes: comparing apples with apples (p. 58)	



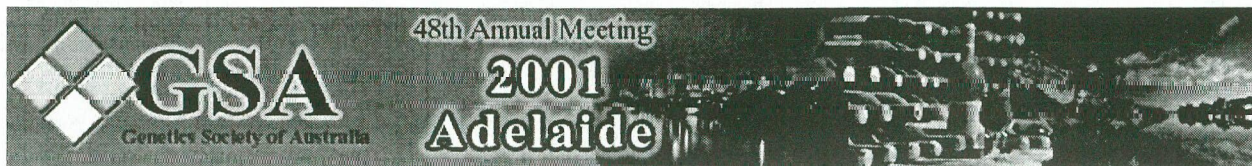
Programme

FRIDAY, 6 th July 2001		
	CONCURRENT SESSIONS V continued	
	Conservation Genetics	Benham Lecture Theatre (Chair: R. Frankham)
2.10 pm – 2.30 pm	D. Tikel (DPI Queensland) Mitochondrial DNA as an informative marker for the conservation of Murray-Darling Basin (Australia) fish. (p. 82)	
2.30 pm – 2.50 pm	M. Elphinstone (Southern Cross University) Conservation genetics of the Eastern Bristlebird, <i>Dasyornis brachypterus</i> , using multiple mitochondrial and nuclear gene sequences. (p. 30)	
2.50 pm – 3.10 pm	S. Krauss (Botanic Garden, WA) Rapid genetic decline in a translocated population of the rare and endangered plant <i>Grevillea scapigera</i> (Proteaceae) (p. 50)	
3.10 pm – 3.30 pm	O. Schmidt (Adelaide University) Two distinct reproductive strategies are correlated with an ovarian phenotype in co-existing parthenogenetic strains of a parasitic wasp (p. 67)	
3.30 pm – 4.00 pm	Tea / Coffee / Poster Session	1 st Floor, Mol. Life Sciences Bldg.
4.00 pm – 4.40 pm	R. Fischer (Max-Planck-Inst. for Terrestrial Microbiology, Germany) Sexual development of <i>Aspergillus nidulans</i> : Catalase-peroxidase is highly induced through the APSES transcription factor STUA. (p. 5)	Keynote Addresses Mawson Lecture Theatre (Chair: J. Kelly)
4.40 pm – 5.20 pm	A. Andrianopoulos (University of Melbourne) Control of dimorphic switching in the human pathogen <i>Penicillium marneffei</i> . (p. 1)	
5.30 pm – 6.00 pm	GSA AGM	

Programme

SATURDAY, 7th July 2001

CONCURRENT SESSIONS VI		
	<u>Systematic/Population</u>	Mawson Lecture Theatre (Chair: A. Wilton)
9.00 am - 9.20 am	M. Ziino (Walter and Eliza Hall Institute) Polymorphisms of Human Microsatellite Markers in Non-Human Primate Populations (p. 97)	
9.20 am - 9.40 am	J. Holman (Griffith University) Molecular variation across a morphological cline in <i>Eucalyptus</i> (p. 41)	
9.40 am - 10.00 am	S. Gilchrist (University of Sydney) Population structure in an economic pest species: microsatellites and the Queensland fruit fly, <i>Bactrocera tryoni</i> . (p. 34)	
10.00 am - 10.20 am	B. Innes (CSIRO Marine Research) Genetic differentiation between Tasmanian cultured Atlantic salmon (<i>Salmo salar</i> L.) and their ancestral Canadian population using archived scale samples. (p. 45)	
	<u>Mammalian genetic analysis</u>	
9.00 am - 9.20 am	I. Hughes (University of Queensland) Analysis of Sequence Variation in p53 and H and K-Ras Genes in Canine Haemangiosarcoma Cell Lines. (p. 43)	
9.20 am - 9.40 am	S. Latham (Flinders University) Limitation in the use of archival plasma-derived DNA for whole genome analysis (p. 51)	
9.40 am - 10.00 am	C. Hyun (University of Queensland) The Effect Of Pentobarbitone On Genetic Analysis by RT-PCR (p.44)	
10.00 am - 10.20 am	G. Webb (Adelaide University) Is the t(1;29) Centric Fusion a Relic from the Pre-domestication Population of <i>Bos taurus</i> ? (p.90)	
10.20 am - 11.00 am	Tea / Coffee	1 st Floor, Mol. Life Sciences Bldg.
11.00 am - 12.00 pm	<u>M.J.D. White Address:</u> D. Catcheside (Flinders University) Genetics of Genetics (p. 3)	Mawson Lecture Theatre (Chair: J. McKenzie)
12.00 pm - 2.00 pm	Lunch	Tandanya Cultural Centre



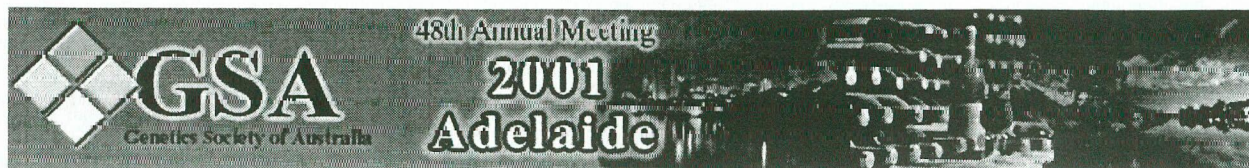
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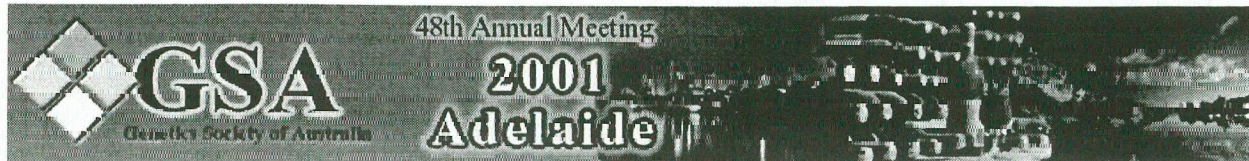


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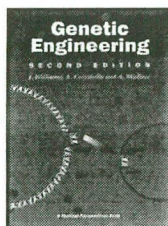
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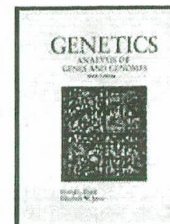


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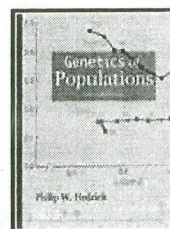
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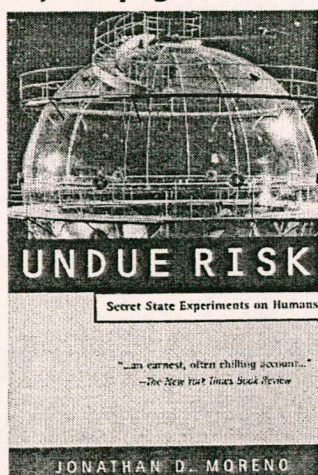
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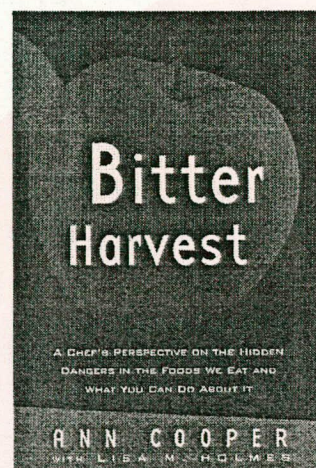
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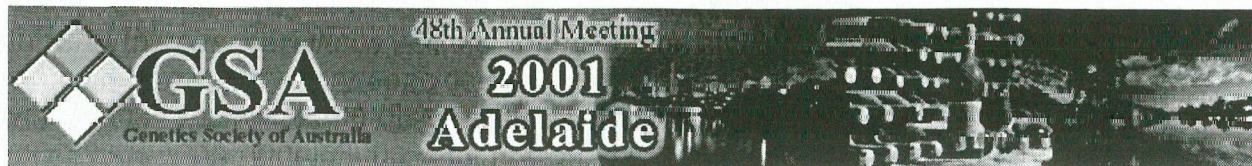
Control of dimorphic switching in the human fungal pathogen *Penicillium marneffei*.

Andrianopoulos, A.

*Department of Genetics, University of Melbourne, Parkville, Victoria, 3010.
Australia.*

Penicillium marneffei like many other pathogenic species of fungi is dimorphic, capable of switching between unicellular yeast-like growth and multicellular filamentous growth. Dimorphic switching in *P. marneffei* is triggered by temperature. At 25°C *P. marneffei* exhibits true filamentous growth producing hyphae consisting of cells which grow apically and divide by septation. Under the appropriate condition, hyphal cells can undergo an asexual developmental program to produce spores borne on complex structures called conidiophores. At 37°C *P. marneffei* undergoes a dimorphic switch to produce uninucleate yeast cells which divide by fission. These yeast cells are incapable of undergoing the asexual developmental program exhibited by hyphal cells. Despite the apparent morphological differences in these two developmental programs, there are significant similarities at the cellular level.

We are interested in understanding the molecular mechanisms which control dimorphic switching using *P. marneffei*. We have targetted a number of genes which control either dimorphic switching or asexual development in other fungi and examined their role in *P. marneffei*, which exhibits both of these programs. We have shown that these two developmental programs, in addition to exhibiting morphological similarities, also share common molecular components.



Barriers to gene exchange in hybrid zones

Roger Butlin

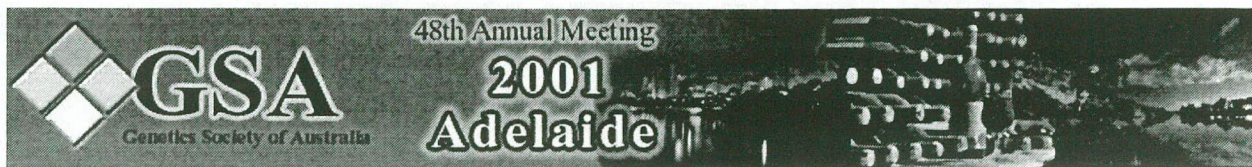
School of Biology, University of Leeds, UK

Secondary hybrid zones provide 'snap-shots' of the process of divergence between allopatric populations. They show how genetic and phenotypic divergence translates into reproductive isolation and might allow genetic dissection of the traits that underlie speciation. Two hybrid zones in the grasshopper genus *Chorthippus* have contrasting patterns of interaction even though the species are closely related and probably have similar levels of genetic divergence between the hybridising populations.

In *Chorthippus parallelus*, clines for all traits studied fit closely to the expected sigmoid form. They vary considerably in width and also in position, suggesting weak selection and low linkage disequilibrium. Studies of the patterns of sexual isolation in crosses among populations within subspecies surprisingly suggest that much of the differentiation observed across the hybrid zone may have arisen rapidly during expansion from refugial populations, rather than slowly during long periods of allopatry. Although F1 hybrid males are sterile, no sterility is observed in the field. Mapping of clines for sterility loci suggests that this is due to displacement of clines for two sets of interacting loci. Although sterility in interpopulation crosses is mainly explained by refuge of origin, some evidence for reduced fertility in UK x mainland Europe crosses suggests that it can also evolve rapidly.

A recently described hybrid zone between *Chorthippus brunneus* and *C. jacobsi* is very different in character. Rather than a simple clinal interaction, these species form a mosaic hybrid zone with parental, intermediate and bimodal populations all present near the zone centre. Unlike the subspecies of *C. parallelus*, these species have diverged in ecological requirements and phenology. They are more divergent in song but show a similar level of assortative mating in laboratory trials. It seems likely that interactions between these three barriers to interbreeding result in the variable outcomes of contact seen in the field.

These two examples will be used to illustrate the contribution that studies of hybrid zones can make to understanding the evolutionary genetics of speciation.

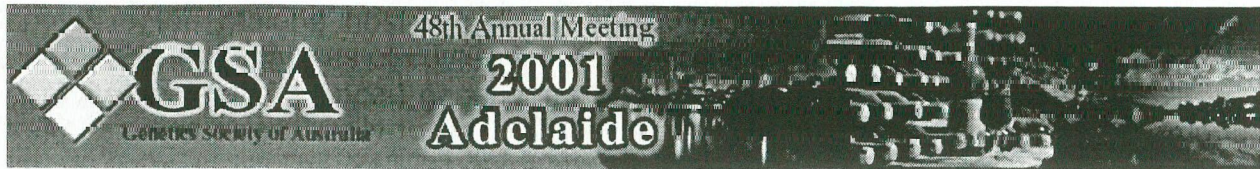


Genetics of Genetics.

David EA Catcheside

School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, Australia

Beginning thirty six years ago, DG Catcheside and his students discovered a system of polymorphic genes that determine the amount of recombination in specific sections of chromosomes of *Neurospora crassa*. These promised the existence of exquisite differential controls on the rate of evolution of subsets of genes within the *Neurospora* genome. At Flinders, my students and I set out to understand how these controls work at the molecular level. In the process we found we had tools that allowed dissection of meiotic recombination events at higher resolution than had been achieved with other model systems. These have provided new insights into the events that exchange sequence information between homologous chromosomes and have enabled us to devise a system for shuffling sequences cloned adjacent to a recombination hotspot that allows directed evolution of chosen genes *in vivo*. There is evidence that local controls of recombination also exist in other multicellular eukaryotes and that these systems may regulate the speed of evolution of specific genes not only by controlling the rate of sequence shuffling but also by determining the local rate of spontaneous mutation.



Gene Mining Plasmodium: A new family of scavenger receptor proteins PxSR-1 essential for development of malaria

Charles Claudianos¹, Holly Trueman¹, Johannes Dessens¹, Jacqui Mendoza¹,
Meiji Arai¹, Robert Sinden¹.

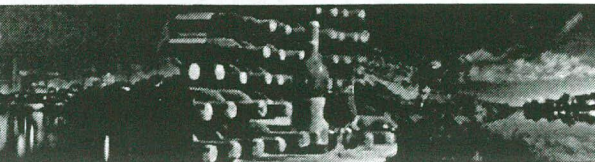
¹Imperial College of Science Technology and Medicine., London, UK.

The human malaria parasite *Plasmodium falciparum* genome project is nearing completion. A number of other *Plasmodium* species are also being analysed (genomic, EST, GSS) including the rodent malaria *P.yoelii*. Sequencing of the human genome and malaria mosquito *Anopheles gambiae* is well advanced and will complete the malaria host-parasite genomic picture. Together, these data offer unprecedented analyses of content, patterning, and evolution of genes within a host-parasite context. Advanced genetic, molecular and bioinformatics technologies are being applied directionally to drive discovery of new biological processes and drug targets in malaria. The PxSR-1 gene family of the malaria parasite *Plasmodium* was identified from first principles using gene-mining techniques. *P. falciparum* genome project was searched for sequences whose predicted proteins had high homologies to human and insect receptor or receptor modulating domains useful for development of antiparasitic targets. The isolation and characterisation of conserved sequences from three *Plasmodium* species including the human malaria indicate PxSR-1 is a unique family of secreted proteins containing multiple domains (LCCL, lipoxxygenase, SRCR and pentaxin) associated with binding and modulating cell surface proteins putatively involved in host immunity. The single gene knockout disruption in *P. berghei* results in *PbSR-1* null mutants that do not produce sporozoites and consequently are dead-end parasites in the midgut of the mosquito.



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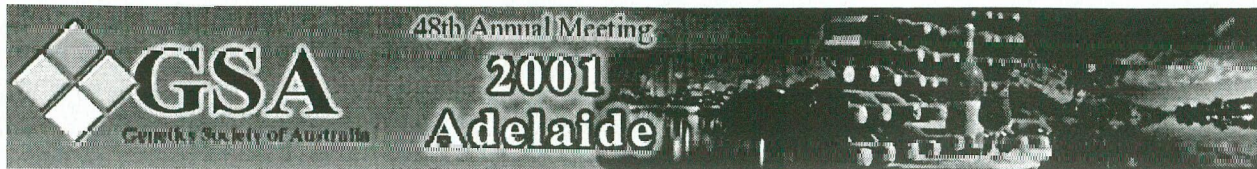
Sexual development of *Aspergillus nidulans*: Catalase-peroxidase is highly induced through the APSES transcription factor STUA

Reinhard Fischer, Mario Scherer and Huijun Wei

Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., D-35043 Marburg, Germany.
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Sexual development in *Aspergillus nidulans* is an interesting morphogenetic process, which is only poorly understood. We set out to study differentially expressed genes to use them as tools for the isolation of central regulators for development. We isolated laccase II, which was described to be specifically expressed in early developmental structures, such as Hülle cells and primordia. The corresponding primary protein sequence revealed that it is a heme-containing catalase-peroxidase, CPEA, rather than a laccase. A CPEA-GFP fusion protein localized in sexual tissues. The expression pattern revealed an early induction upon carbon starvation and during sexual development. The tissue-specific expression was dependent on the transcription factor STUA.

In a non-targeted approach, differentially expressed genes were isolated using the subtractive suppressive hybridisation method (Clontech). One of the identified genes was α -1,3-glucanase, which probably plays a central role in energy supply during sexual differentiation. Similarly, the gene is specifically induced early during development in Hülle cells. Experiments to unravel the regulatory circuits are on the way.



Regulation of Stem Cell Self-Renewal versus Differentiation

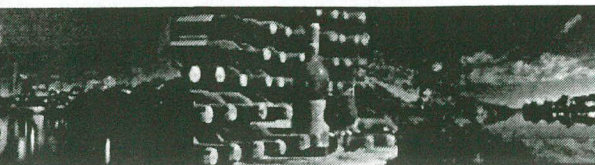
Margaret T. Fuller, Leanne Jones, Salli Tazuke, Yukiko Yamashita,
Cordula Schulz, Madolyn Rogers, and Amy Kiger.

*Departments of Developmental Biology and Genetics,
Stanford University School of Medicine, Stanford, CA 94305, USA.*

Many highly differentiated but short-lived cell-types, including blood, skin, and sperm, are produced throughout life from adult stem cells. The central characteristic of adult stem cells is their remarkable, long-term capacity to divide as relatively undifferentiated precursors while also producing daughter cells that initiate differentiation. The choice of fate that daughters of stem cell divisions make must be highly regulated to replenish the stem cell population throughout the life of the individual, yet rein in the high proliferative capacity of stem cells and ensure continued production of differentiating cells. Understanding the mechanisms that regulate stem cell specification and the choice between stem cell self-renewal and differentiation is crucial for realizing the potential of stem cells for regenerative medicine.

We are using the *Drosophila* male germ line as a powerful genetic system to identify both the cell autonomous determinants and the extrinsic cell-cell interactions that govern stem cell behavior. One of the great advantages of this system is that stem cells can be studied *in situ*, in the context of their normal support cells. Our results indicate that signals from surrounding somatic support cells specify asymmetric division of male germ line stem cells by inducing one daughter cell to self-renew stem cell identity while directing the other daughter cell to differentiate. We have discovered that JAK-STAT pathway signaling is required for male germ line stem cell self-renewal: loss of function JAK-STAT pathway mutants cause stem cell loss, while over-expression of the activating ligand *unpaired* results in massive stem cell over-proliferation. We have found that the activating ligand *unpaired* is normally expressed in the testis specifically in a small population of somatic support cells adjacent to the male germ line stem cells. Thus somatic cells of the apical hub provide a "niche" that specifies stem cell self-renewal by the daughter germ cell that retains contact with the hub. We have also discovered that a separate population of somatic support cells plays a guardian role to rein in stem cell self renewal capacity by insuring that daughter cells displaced away from the stem cell niche initiate differentiation. The ability of the somatic guardian cells to encapsulate germ cells and drive them to differentiate requires wild type function of the EGFR pathway in somatic cells.

In addition, we have identified several new candidates for genes encoding extrinsic or intrinsic factors that act to specify stem cell self-renewal or differentiation in genetic screens for mutations that cause either over-proliferation of early male germ cells at the expense of differentiation, or loss of stem cells due to failure of establishment or self-renewal. For example, wild type function of *early germ cell overgrowth (ego)*, which encodes a predicted transcriptional co-activator, is required for early male germ cells to initiate differentiation instead of continuing proliferation. Wild type function of *zero population growth*, which encodes a gap junction protein expressed in germ cells, is required for differentiation of early germ cells in both males and females. Genetic dissection of stem cell behavior thus offers great promise for elucidating both intrinsic mechanisms that regulate stem cell self-renewal and differentiation *in vivo* and the role of the support cell niche.



Laboratory and field analysis of clinal variation in two *Drosophila* species

Ary Hoffmann¹, Rebecca Hallas¹, Paul Mitrovski¹, Melissa Carew¹, Andrea Magiafoglou¹, Michele Schiffer¹, Andrew Weeks², Steve McKechnie²

¹ CESAR, La Trobe University, ² CESAR, Monash University

To understand climatic adaptation in *Drosophila* we have embarked on a research program that spans across the organismal and molecular levels, linking approaches based on mutagenesis, selection experiments, population comparisons, and interspecific studies. One aim is to dissect clinal variation in eastern Australia in *D. melanogaster* the endemic *D. serrata*. This includes the characterization of clines at the quantitative and molecular levels, linking of clinal traits with specific markers, and assessing the intensity and patterns of selection on traits under field conditions.

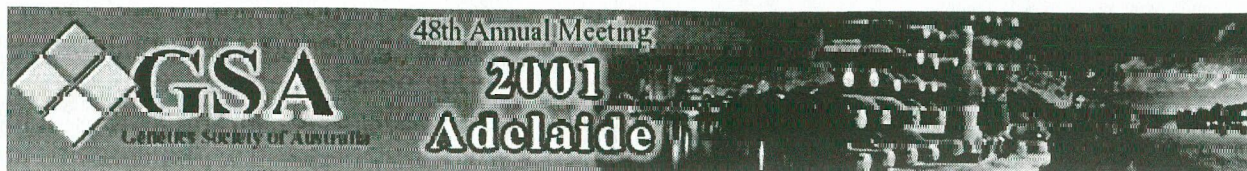
In *D. melanogaster*, we characterized variation among isofemale lines from populations to examine variation in quantitative traits. Clinal variation for resistance to starvation and desiccation was weak or absent despite highly significant line effects. For adult cold resistance as scored by mortality, there was local differentiation and strain variation but no geographic variation. However, adult chill coma resistance showed a linear cline. For size (thorax length) which shows a well-known linear cline, geographic differentiation was high despite some overlap among strains. Crosses indicated that these results were not confounded by inbreeding effects.

In *D. serrata*, there was a non-linear cline for body weight but no clinal variation for desiccation resistance and starvation resistance. As in *D. melanogaster* chill coma recovery showed a linear cline. Unfortunately, clinal patterns in *D. serrata* are complicated by seasonal patterns: a detailed study of part of the cline for size and life history traits before and after winter indicated significant shifts in patterns and non-linear associations. Seasonally-dependent shifts in cold resistance were also evident and support findings from previous years.

We used field cages to test for local adaptation and Wolbachia effects in *D. melanogaster*. In the winter infected flies from temperate and tropical sites had a lower fecundity in tropical north Queensland, whilst in temperate southern Victoria Wolbachia effects depended on the nuclear population background and infected Melbourne flies were more fecund. In Victoria, a local population had a higher late-life fecundity than a tropical population from Queensland.

To further test geographic adaptation, this experiment was repeated with *D. melanogaster* populations initiated from seven sites. Fecundity and survival were monitored in cages over 5 months. Total fecundity showed a curvilinear relationship with latitude. Adults from temperate locations survived winter conditions better than those from subtropical populations but not tropical ones. There was a linear cline in the timing of egg production; temperate populations produced eggs later than populations from lower latitudes. This cline is likely to be adaptive because egg-to-adult viability experiments indicated that only eggs laid in spring developed successfully to the adult stage.

In *D. serrata*, microsatellites were developed to test for shifts in population structure among seasons and investigate geographic patterns in neutral markers for comparison with quantitative traits. In *D. melanogaster*, attempts were made to link clines in several genes to these adaptive shifts among populations.



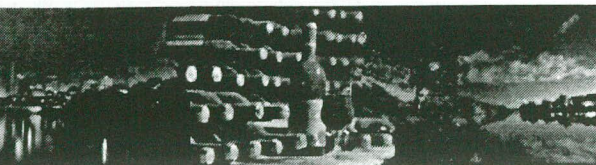
Role of ATM in maintaining genome integrity

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Patients with the human genetic disorder ataxia-telangiectasia (A-T) show adverse and severe responses to radiotherapy and this hypersensitivity is also evident in A-T cells in culture. The molecular basis for increased sensitivity to ionizing radiation has not been established but failure to repair all of the double strand breaks in DNA accounts at least in part for this sensitivity. Identification of a series of substrates for ATM kinase, the product of the defective gene in A-T, has greatly assisted in elucidating the signalling events downstream from ATM in radiation signal transduction. Some of these substrates are themselves products of genes defective in other genetic diseases, characterized by genome instability and cancer predisposition. These include NB-SI, defective in Nijmegen Breakage Syndrome; BRCA1, breast cancer susceptibility gene and BLM defective in Bloom's Syndrome. Thus ATM plays an early upstream role in response to radiation-induced DNA damage. In the absence of ATM one of the consequences of this genomic instability is a predisposition to develop tumours and there is evidence that carriers of the A-T gene defect also have a propensity to develop cancer. Expression of mutant ATM protein containing missense mutations in some A-T carriers may account for the cancer risk due to a dominant interfering effect of the mutant protein.



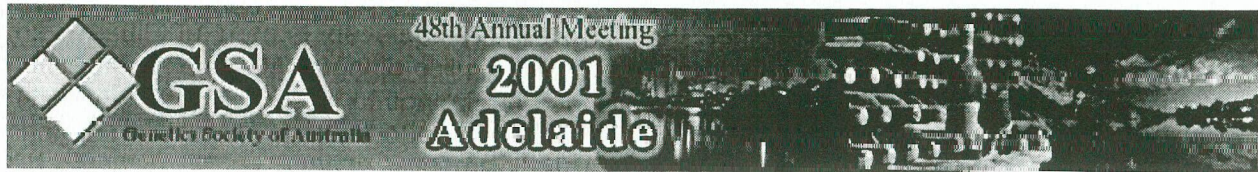
Deep Evo-Devo: zootype genes and zootype precursor genes in the cnidarian *Acropora millepora*

David J Miller¹, John Reece-Hoyes^{1,2}, Danielle de Jong¹, Serge Plaza³, Melanie Morris¹, Ingo Scholten¹, Walter J Gehring³, David C Hayward² and Eldon E Ball²

¹ James Cook University, ² Australian National University, ³ University of Basel

The zootype is a set of regulatory genes thought to be common to all bilateral animals; the original zootype proposal identified a Hox cluster, *evx*, *otx* and *emx*, and has subsequently been elaborated to include *Pax-6* and the *nkx*, *gsx*, and *msx* system. Genes corresponding to most, but not all, of the zootype genes have been identified in cnidarians, the closest outgroup of the bilateral animals. The key absentees are the Hox genes. In some cases, clear cnidarian orthologs of zootype genes have been identified – for example, the cnidarian *cnox2* genes are thought to be orthologs of the *gsx* homeobox class, and *emx* homologs are known from cnidarians. In the *Acropora* planula larva *emx* expression is restricted to a subset of cells, putative neurons, at the aboral end, which is anterior relative to the direction of swimming. This is the end, however, which will form the base of the settled polyp, raising interesting question as to the relation of oral/aboral to anterior/posterior at the time of metamorphosis. In other cases, it is probably more accurate to view cnidarian genes as being derived from precursors of gene classes recognised in bilateral animals. Thus, common features of cnidarian and bilaterian genes were presumably present in the common ancestor. The *Acropora PaxC* gene is an example of the latter type – we believe that it represents a *Pax-6* precursor.

PaxC is expressed in a subset of presumed neurons during larval development of *Acropora*, but is unable to induce ectopic eyes when expressed in *Drosophila* imaginal discs. However, transgenic expression of chimeric proteins containing the *PaxC* paired domain and the *eyeless* transcription activation domain induced slightly reduced, but otherwise morphologically normal, ectopic eyes. These findings indicate that *PaxC* is able to bind *eyeless* targets in vivo, and imply that the failure of *PaxC* to initiate eye formation results from the absence of a transcription activation domain. The in vivo data are also supported by in vitro DNA-binding assays, in which the *PaxC* paired domain was shown to bind to a known *eyeless* target, a footprinted sequence within the *so10* sub-fragment of the *sine oculis* enhancer, and *Pax-6* consensus sites. Whilst these results support the hypothesis that *PaxC* represents a *Pax-6* precursor gene, it is clear that many aspects of the evolution of specificity in the Pax family remain to be clarified.



Inducible Antagonists of Hedgehog and Wnt signaling

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Animal development requires that cells respond appropriately to localized secreted signals. One way in which the signals are controlled is by inducible antagonists. The Hedgehog (Hh) signal, for example, induces transcription of the membrane protein Patched (Ptc), the function of which is to antagonize Hh both by binding and sequestering it and by negatively regulating the transcription of genes that can be induced by Hh. When Hh signal is not properly restrained, as in people or mice lacking one of their copies of the *ptc1* gene, cancer may result. In normal development we find that Sonic hedgehog signal is a powerful mitogen for cerebellar granule cell precursors. Inadequate Ptc function evidently allows this mitogenic effect to go out of control, probably in concert with other genetic or environmental changes. We have made mouse models of skin and cerebellar cancer by manipulating the Hh pathway. They provide evidence that reduced Ptc function allows cells to remain in a mitotic state inappropriately. In order to understand Hh signal transduction more fully, we have studied pathway components in cultured cells and in vivo to learn how protein movements inside cells are linked to reception of the signal.

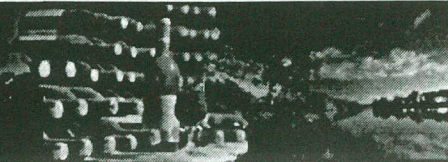
Wnt signals play crucial roles in development and neoplasia. We show that *naked cuticle* (*nkd*), a *Drosophila* segment polarity gene, encodes a novel inducible antagonist for the Wnt signal Wingless (Wg). In fly embryos and imaginal discs *nkd* transcription is inducible by Wg. In embryos, decreased *nkd* function has an effect similar to excess Wg; reduction of postembryonic *nkd* function is without apparent consequence. Conversely, Nkd overproduction results in phenotypes resembling Wg/Wnt loss of function. Using ectopic expression we find that Nkd affects, in a cell-autonomous manner, a transduction step between the Wnt signaling components Dishevelled (Dsh) and Zeste white-3 kinase (Zw3). Zw3 is essential for repressing Wg target gene transcription in the absence of a Wg signal, and the role of Wg is to relieve this inhibition. Our double mutant analysis shows that, in contrast to Zw3, Nkd acts when the Wg pathway is active to restrain signal transduction.

nkd encodes a novel protein with a single EF-hand most similar to the recoverin family of calcium-binding myristoyl switch proteins. Nkd may therefore link ion fluxes to the regulation of Wnt signal potency or duration. Yeast two hybrid and in vitro experiments indicate that Nkd directly binds to the basic-PDZ region of Dsh. Specially timed Nkd misexpression is capable of abolishing Dsh function in a distinct signaling pathway that controls planar cell polarity. Our results suggest that Nkd acts directly through Dsh to limit Wg activity, and thus determines how efficiently Wnt signals stabilize Armadillo (Arm)/ β -catenin and activate downstream genes. We have identified two mammalian relatives of Nkd and we are currently analyzing their functions. Signal-inducible feedback antagonists like *nkd* may restrain the effects of Wnt proteins in development and disease.



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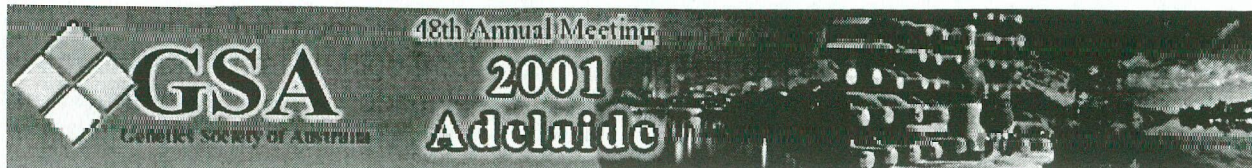


On some microarray experiments exploring the mouse olfactory system

Terry Speed

Walter & Eliza Hall Institute of Medical Research

My Berkeley colleagues studying the mouse olfactory system are interested in identifying genes exhibiting gradients, regionalization or localization across the mouse olfactory epithelium and bulb. With this in mind we jointly designed and carried out some cDNA microarray experiments. In this talk I will discuss the design principles and the analysis of these experiments. The experiments were successful in that we found and subsequently were able to confirm some genes showing the desired patterns. The collaborators are John Ngai, Dave Lin and their students, and my student Yee Hwa (Jean) Yang.

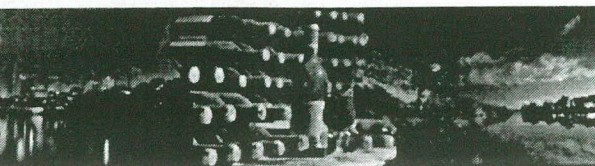


Old topics for new millenia: the evolution of mutation rates revisited

Mark Tanaka¹, Carl Bergstrom², Bruce Levin¹

¹ Dept of Biology, Emory University; ² Dept of Zoology, University of Washington

Abstract. Empirical researchers have repeatedly uncovered high frequencies of mutator genes - genes that increase the genomic mutation rate - in both natural and experimental bacterial populations. This is somewhat surprising; although a number of recent theoretical analyses have shown the advantages of elevated mutation rates, other factors favour non-mutator strains. In particular, when mutators are rare compared to non-mutators, novel beneficial mutations will be overwhelmingly likely to arise first in the much larger non-mutator subpopulation and thus mutator alleles seem unlikely to reach high frequencies in the first place. How can we reconcile this argument with the high observed frequencies of mutator alleles in certain bacterial populations? How do mutators ever get off the ground? To answer these questions, we model the dynamics and timing of mutator spread. We show that, subsequent to the generation of a beneficial mutation in the non-mutator subpopulation, there will be a relatively long "lag" period during which the mutator subpopulation has the opportunity to produce a beneficial mutation and thereby avoid declining in frequency. We present both analytical and numerical analyses of a stochastic model of this system. We find that because of the lag period, there is a non-trivial probability that a mutator will reach a substantial frequency during the course of generation and fixation of a novel beneficial allele.



Odorant Receptor Distribution and Olfactory Coding in *Drosophila*

Warr, C.^{1,2}, Dobritsa, A.¹, van der Goes van Naters, W.¹ and Carlson, J.R.¹

¹Molecular, Cellular and Developmental Biology Dept., Yale University, New Haven, CT, USA

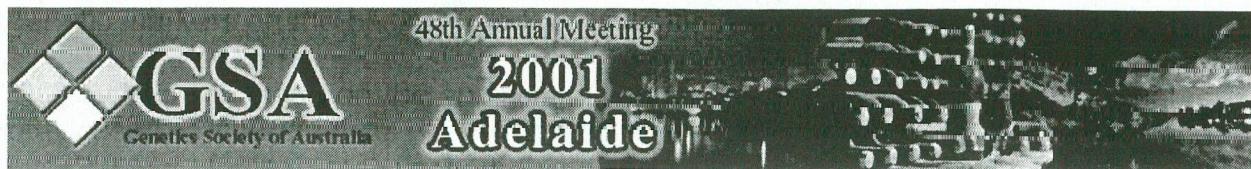
²Present address: Department of Biological Sciences, Monash University, Clayton, VIC.

Although insects have proven to be valuable models for exploring the function, organization, and development of the olfactory system, the receptor molecules which interact with odorants had not previously been identified in any insect. We developed a novel search algorithm, used it to search the *Drosophila* genomic sequence database, and identified a large multigene family (currently 61 members) encoding putative G protein-coupled odorant receptors. The family is highly divergent from previously identified proteins, including odorant receptors from other organisms.

Nearly all the DOR (*Drosophila* Odorant Receptor) genes are expressed in one or both of the olfactory organs: the third antennal segment and the maxillary palp. In addition, individual genes are expressed in subsets of olfactory receptor neurons (ORNs), and different genes are expressed in different subsets of ORNs, as expected for odorant receptors.

Odorant receptors should localise to the dendrites of ORNs, so we are determining the subcellular localisation of DOR proteins using antibodies. An antibody raised against a particular DOR protein reacts with a single band on Western blots of antennal protein extracts. Immunofluorescence labelling shows that this DOR protein is localised within a subset of olfactory sensilla, exactly as would be expected for an odorant receptor protein expressed in the dendrites. To confirm this dendritic localisation we have generated transgenic flies expressing GFP and myc tagged DOR proteins under the control of DOR gene promoters, and shown that at least one C terminally myc tagged DOR protein does in fact localise to the dendrites.

A question critical to the understanding of olfactory coding is that of the number of odorant receptors expressed in a given ORN. To address this, we are linking expression of individual DOR genes with functional types of ORNs, for which we know of at least 29 in *Drosophila*. To do this we have created DOR gene promoter-GAL4 lines, which when crossed to a UAS-mcd8:GFP reporter line (mcd8:GFP is a membrane localised form of GFP) show GFP expression in a subset of ORNs corresponding to those in which the endogenous DOR gene is expressed. GFP is also produced in a matching set of olfactory sensilla, enabling us to perform electrophysiological recordings from these sensilla in the live fly and determine which class they are. Individual sensilla house two to four ORNs. We are using several genetic techniques to then determine precisely which of these ORNs expresses the particular receptor. We are making promoter-GAL4 lines for all the DOR genes expressed in the maxillary palp, which contains only 6 functional types of ORN, to enable us to construct a complete map of odour coding across this organ.



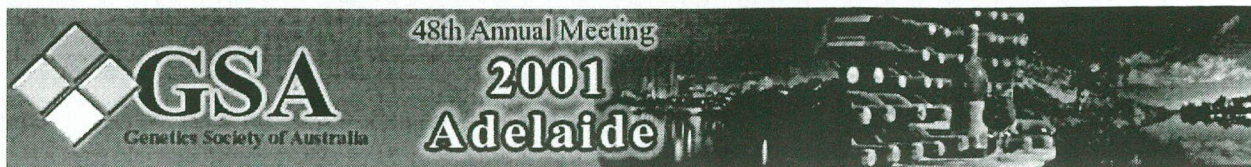
Measuring Human Relatedness

Bruce S. Weir

North Carolina State University

Individuals are said to be related when they share alleles that are identical by descent, and the concept of relatedness is central to population and quantitative genetics. Although relatives generally have recent common ancestors, there is also a low level of relatedness in the general population because of shared evolutionary history. The implications of relatedness are recognized in marriage laws, and are exploited in determinations of parentage or identification of remains. The resemblance between quantitative trait values for relatives is described in terms of probabilities of identity by descent, and human disease genes are located relative to genetic markers on the basis of marker similarities between affected relatives.

This lecture will focus on the applications of the coancestry parameter θ that is often used to characterize relatedness. Particular attention will be paid to allowing evolutionary forces to affect θ and so influence forensic and paternity calculations as well as affected relative linkage tests. The history of human populations is often reconstructed on the basis of θ values for pairs of populations, and the effects of allowing different populations to have different θ values will be described. Classical methods for estimating θ will be reviewed, and newer methods outlined. Results based on data collected by Australian forensic agencies will provide an Australian perspective.

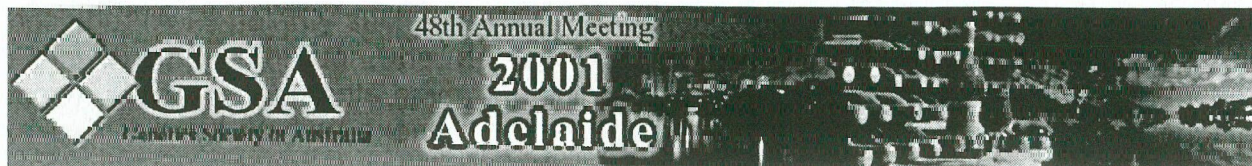


A gene for all climates: the *hsr-omega* polymorphism of *Drosophila melanogaster* associates with both heat and cold resistance.

Alisha R. Anderson, Janelle E. Collinge, Mark Kellet, and Stephen W. McKechnie

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Allelic variation in the *hsr-omega* gene has previously been implicated in the determination of heat resistance variation in *D. melanogaster*. Here we report a polymorphism that shows strong latitudinal association among samples taken along the climatically diverse eastern coast of Australia. Controlling for latitudinal variation indicated that the frequency of the allele found at high frequency in the cooler regions negatively associated with the average maximum temperature of the hottest month. Among an independent set of derived laboratory strains, manipulated to thoroughly mix the genetic backgrounds of tropical and temperate populations, the previously reported association with heat resistance variation was repeated and a new association with cold resistance was detected. Cold resistance segregated strongly with the cool region *hsr-omega* allele, supporting the hypothesis of a functional role for *hsr-omega* in the determination of natural cold resistance variation. A diallele cross indicated additive effects of *hsr-omega* variation on this ecologically pertinent measure of cold resistance. A cold tolerance test on recently collected field strains of *D. melanogaster*, applied after culture under end-of-warm-season conditions (18°C, 10hrs light:14 hrs dark photoperiod), indicated that tropical strains were more susceptible to short exposures of cold stress than strains from temperate regions. These data suggest that the *hsr-omega* polymorphism marks a region on the right arm of chromosome 3 that is important for thermal adaptation to both temperature extremes in this species.



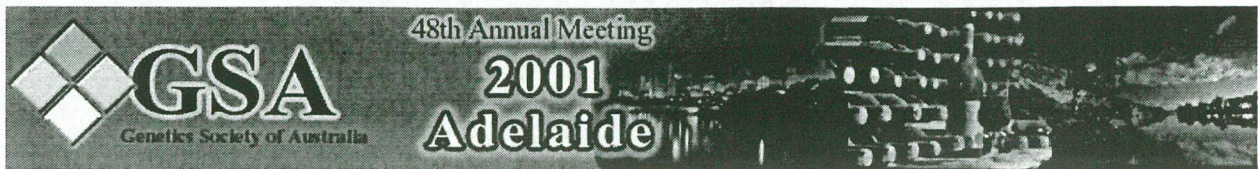
The role of Eph A/ephrin-A guidance factors in avian peripheral nervous system patterning.

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The vertebrate peripheral nervous system (PNS) in the trunk is characterised by repeating metameric units of tissue (or somites) which coordinate the growth of PNS components during development. Within each somite there exists a distinct anterior-posterior (A-P) polarity such that PNS components - motor axons and neural crest cells - only migrate through the anterior-half to their peripheral targets. Nerve trunk manipulation experiments have demonstrated that the somite contains intrinsic factors responsible for guiding axons and neural crest cells through the anterior-half somite. Posteriorly expressed molecules create an inhibitory environment for outgrowth of motor and sensory axons and neural crest cells whilst the anterior-half is permissive.

One family of molecules that have been implicated as guidance molecules in this system are the Eph receptor tyrosine kinases and their ligands, ephrins. The Eph/ephrin family are membrane bound receptors and ligands whose interactions activate intracellular signalling cascades, ultimately affecting cell-cell contact, morphogenesis, and movement. EphB2 and B3 are distributed on motor axons and neural crest cells in both mouse and chick trunk whilst their ligands ephrin-B1 and -B2 are located in the posterior-half sclerotome, suggestive of a repulsive guidance role. It has been shown in the avian system that inhibition of B-subclass interactions causes aberrant migration of neural crest cells. However, perturbation of this interaction does not affect motor axon outgrowth. We hypothesised that the Eph A/ephrin-A subclass may also be acting to guide motor axons through anterior-half somite either as local attractants or repulsive cues. *In Situ* hybridisation and immuno-histochemical analysis has revealed that ephrin-A5 and EphA4 are present at low levels in the anterior-half somite at Stages 19-21, suggesting a role for this receptor/ligand interaction in PNS patterning.



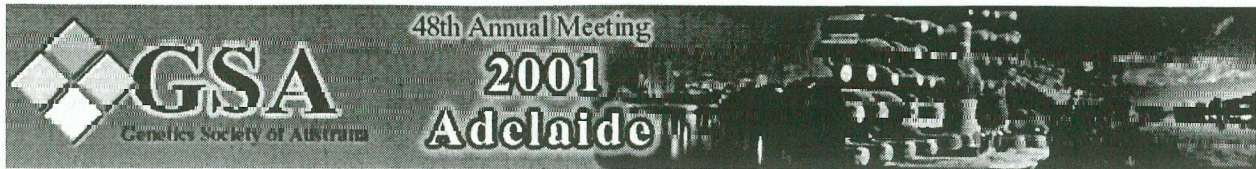
DNA markers for the delineation of southern hemisphere abalone (*Haliotis*) species for forensic applications.

Jason Bartlett¹, Nick G. Elliott¹, Brad S. Evans¹, Neville A. Sweijd² and Peter Cook²

¹CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia, ² Department of Zoology, University of Cape Town, Private Bag, Rondebosch, 7701, Cape Town, South Africa.

Abalone poaching, and fraudulent substitution of abalone products, are crimes that are increasingly prevalent in South Africa, as well as Australia the USA and other countries that have abalone resources. This high value product is the target of organised crime syndicates, and legislation governing its exploitation has not always kept abreast of developments on the ground. In South Africa and Australia, several measures have been put in place to combat these crimes since their severity is threatening both the legitimate industry and the sustainability of the resource. One such measure is the development of suitable forensic markers with which to close legal loopholes and secure convictions in court.

We present the results of a collaborative project between the CSIRO in Australia and the University of Cape Town in South Africa which aimed at developing robust genetic markers to distinguish 11 commercially exploited southern hemisphere abalone species. Several mitochondrial genes and the lysin gene (nuclear) were used to establish a PCR-RFLP profile that will consistently delineate abalone samples to species level. The South African Police Forensic Laboratories are currently validating this technique for routine application to abalone poaching cases. Research is continuing to standardize the technique so that dried, canned and abalone mucous can be tested. Also, this technique will be expanded to incorporate all commercially exploited abalone species world-wide.



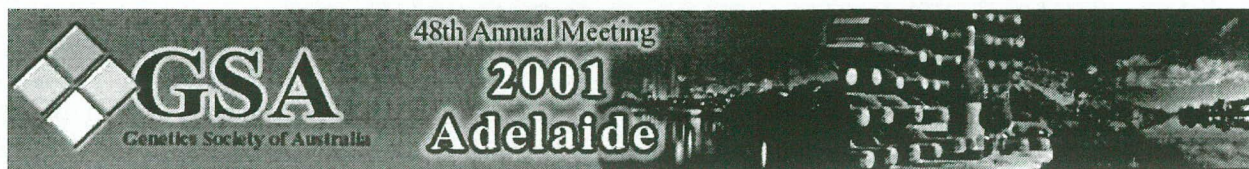
Is the asymmetry *Modifier* an allele of the *Notch* orthologue in the Australian sheep blowfly, *Lucilia cuprina*?

K. Freebairn, J. Fair, J.A.McKenzie, and P.Batterham

CESAR - Centre for Environmental Stress and Adaptation Research,
Department of Genetics, University of Melbourne, Parkville, Victoria 3052

Diazinon resistance in Australian sheep blowfly, *Lucilia cuprina*, is mediated by the *Rop-1* gene, that encodes the carboxylesterase E3. Resistant flies have increased levels of bristle asymmetry and, in the absence of the insecticide, decreased fitness in comparison with susceptible flies. This suggests that the allelic substitution at the *Rop-1* locus disrupted the co-adapted genetic background and imposed developmental stress reflected as asymmetry.

A mutation, *Modifier* (*M*), at a second locus reverses the deleterious impact associated with the *Rop-1* mutations, normalizing the fitness and asymmetry. It has been proposed that *M* is an allele of the *Scalloped wings* (*Scl*) gene. Previous work in our laboratory has demonstrated that the *Scl* gene is the *L. cuprina* homologue of the *D. melanogaster Notch* gene that plays a major role in bristle development. We have analysed molecular variation at the *Scl* locus in isogenic *Modifier* and non-*Modifier* lines. Levels of haplotypic variation are similar in both types of lines, indicating that the *Modifier* may not be an allele of *Scl*. In a subsequent experiment the *Modifier* was precisely mapped with respect to two flanking phenotypic markers and three molecular markers within *Scl*. *Modifier* maps 1.3 map units from *Scl*. In molecular terms we estimate the gene to be approximately 200 kb 3' to *Scl*. Our approaches to the cloning of *Modifier* will be discussed.



Cloning and characterisation of *acrB* from *Aspergillus nidulans*- a role in carbon catabolite repression?

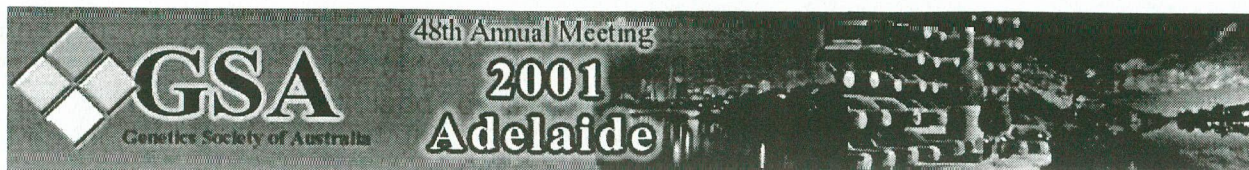
Natasha Boase, Robin Lockington, Lousie Rodbourn, Joan Kelly

Department of Molecular Biosciences, Adelaide University

Carbon catabolite repression is the regulatory process by which filamentous fungi, such as *Aspergillus nidulans*, utilise the most favourable carbon source available, while simultaneously repressing the genes that utilise less favourable carbon sources. A number of genes have been identified in *A. nidulans* that are involved with this repression mechanism, such as *creA*, *creB* and *creC*.

acrB was initially identified by a mutant phenotype of resistance to malachite green and acriflavine. In addition to this, we provide evidence here that the *acrB2* mutation also suppresses aspects of the phenotypes of *creB* and *creC* mutant alleles. This implies that *acrB* may also be involved in the process of carbon catabolite repression.

Recently, we have cloned the *acrB* gene via complementation analysis, involving a chromosome walk from the *creB* gene, which was known to be closely linked to *acrB* on chromosome II. Preliminary characterisation of *acrB* has included sequence analysis, revealing that the *acrB* gene encodes a putative transmembrane protein, with a close homologue present in *Neurospora crassa* but interestingly, not in *Saccharomyces cerevisiae*. The molecular nature of the mutations have been identified in three mutant alleles, and they all lead to truncated AcrB protein, with one of these mutations resulting in a disrupted splice site.

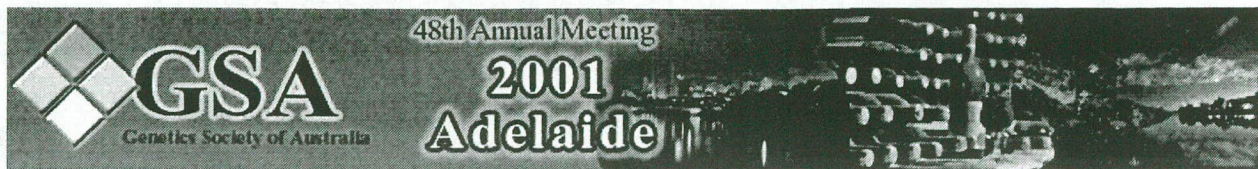


Systematics of broombush – The *Melaleuca uncinata* complex (Myrtaceae)

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¹ Department of Conservation and Land Management, Perth Western Australia

Broombush (*Melaleuca uncinata*, Myrtaceae) is a dominant component of many mallee-shrublands communities across much of southern Australia. It is morphologically diverse occurring in a range of ecological habitats. Within the complex two species are formally recognised - *M. concreta* and *M. uncinata* – however, a series of distinct morphs also exist. Several of these morphs have sympatric distributions suggesting they may represent discrete biological species. Nuclear RFLPs were used to examine systematic relationships between 40 populations of the species/morphs distributed across the southwest land division of Western Australia. Differences in allele frequencies distinguished the two described species and two new taxa from the remaining populations. Within the unresolved populations there was some congruence between morphological and genetic characteristics.



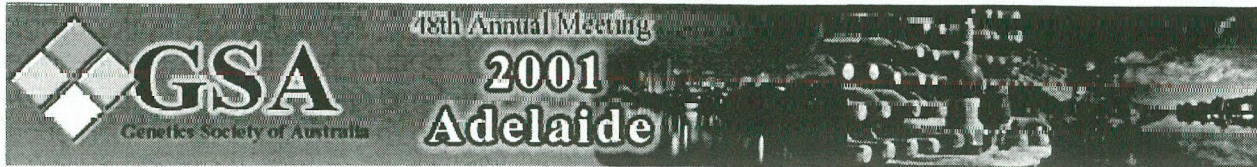
Genetic diversity and gene flow among Southern NSW catchment green and golden bell frog (*Litoria aurea*) populations

Emma L Burns¹, Bronwyn A Houlden², Arthur White³

¹ School of Biological Sciences University of New South Wales, 2052, Sydney, Australia,

² School of Biological Sciences University of New South Wales, 2052, Sydney, Australia, ³ Biosphere Environmental Consultants, Rockdale, 2216, Sydney, Australia.

The Green and Golden Bell Frog (*Litoria aurea*) was once considered to be one of Australia's common Hylids. However, the species has been in decline for the past 30-40 years, and is now listed as endangered in New South Wales under the Threatened Species Conservation Act, 1995. Although the Green and Golden Bell frog still persists from the far North Coast of NSW to East Gippsland in Victoria, remaining populations are mostly small and fragmented. The viability of existing populations may have been undermined by local population bottlenecks and increased isolation of breeding colonies. It is probable that these events have led to a reduction in genetic variability through inbreeding and genetic drift, with the potential for a loss of evolutionary potential. A panel of *L. aurea*-specific microsatellite markers have been developed to analyse levels of genetic variation within and between populations occurring within the southern catchment of NSW, from Port Kembla to Lake Meroo. Surprisingly, levels of allelic diversity ($A = 6.5$ to 7.5) and heterozygosity ($H_e = 0.57$ to 0.81) within populations were relatively high. Preliminary results indicate there is genetic differentiation between populations in the southern catchment ($R_{ST} = 0.095$, $P < 0.01$), and an isolation by distance model may apply to the species.



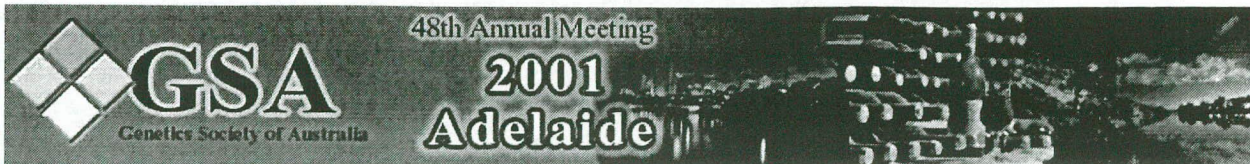
The Genetic Structure of Colony and Drone Aggregations in Stingless Bees

E. Cameron, B. Oldroyd, P Franck

School of Biological Sciences, University of Sydney, NSW 2006

Stingless bee colonies reproduce by swarming. During this process a virgin queen leaves the natal nest together with several hundred workers. Unlike honeybees, swarming workers transfer resources to the new nest over a number of weeks. The dependence of the daughter colony on the resources of the mother colony suggests that mother and daughter colonies should be in close proximity and that this should lead to aggregations of relatives. Colonies of the stingless bee *Trigona collina* showed a highly aggregated spatial distribution. However, microsatellite analysis revealed that colonies within these aggregations are largely unrelated. Thus, a short swarming distance cannot account for the formation of these aggregations. Nor were aggregations due to a limitation in nest sites or food.

Following swarming, a virgin queen will visit a drone congregation area (DCA) and mate with a single drone. In *T. collina* and *T. carbonaria*, drone aggregations form near the entrances to nests. Genetic analysis of these DCAs showed that the drones were not produced in the colonies that they were directly outside. Instead, many colonies from a large area contributed drones to the mating swarm. This may be a mechanism that reduces inbreeding, which, via the production of diploid drones, can have a severely deleterious affect on these species.



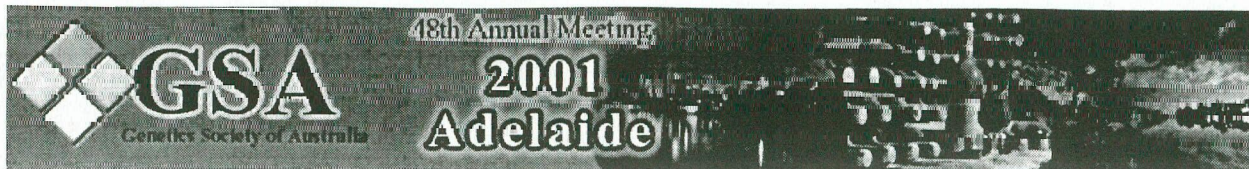
Analysis of gene expression using cDNA microarrays

Julianne Camerotto, Jane Sibbons, Chris Cursaro, and Robert Saint

*Centre for the Molecular Genetics of Development, Department of Molecular Biosciences,
Adelaide University, North Tce, Adelaide, SA, 5005*

The recent turning of the millennium heralds an exciting new era in molecular genetics, providing new insights into the nature of transcriptional regulation and understanding the processes involved in genome-wide expression. The advent of microarray technology, in parallel with the advances made in genomics and bioinformatics, has enabled the development of high-throughput mechanisms for profiling gene expression patterns on a global scale. In a single, typical array experiment thousands of genes can be surveyed; DNA targets (PCR products or oligonucleotides) spotted onto glass slides are simultaneously probed with fluorescently tagged cDNA representations of total RNA pools derived from test and reference samples. Relative transcript abundance is determined by the colour and strength of fluorescent signal generated. The 'fold difference' (ratio of expression in test pool versus reference pool) is used as a quantitative measure of differential expression. The large amounts of data generated from array experiments are 'mined' using computational techniques, in order to extract meaningful information regarding functional relationships among genes under a given set of physiological or developmental conditions. Microarray-based expression technology has a variety of applications, and may be useful for mutational analysis, genetic mapping studies, analysis of regulatory gene defects in disease states, and analysing cellular responses to environmental stimuli.

At present, we are amplifying a subset of the *Drosophila* Gene Collection (DGC) for fabricating a 6000 gene (~50% of the genome) cDNA microarray. The generated DGC array will be subsequently utilized for analysing gene expression during *Drosophila* development.

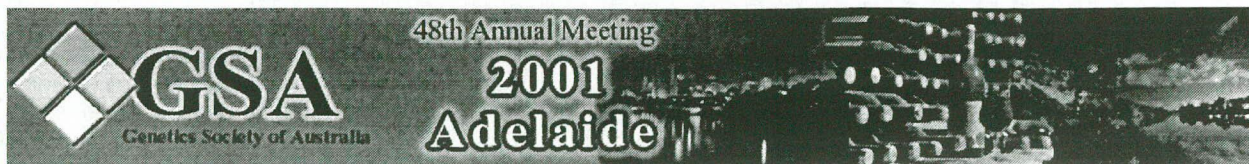


Population genetic structure and the mating system of the rare ghost wattle *acacia sciophanes* and its common congener *acacia anfractuosa*

David J. Coates¹, Genevieve Tischler^{1,2}, Margaret Byrne¹, Marcelle Buist¹ and Jennifer A McComb²

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Comparative studies of genetic variation and the mating system in pairs of closely related rare and common or widespread species have particular merit in the assessment of the genetic and ecological consequences of rarity and the implications such findings may have for the conservation and management of rare and threatened species. *Acacia sciophanes* is an extremely restricted species covering a geographic range of only 4 Km. Its closest relative *A. anfractuosa* has a much wider geographic range of about 200 km. Both species are found in floristically diverse low woodland and scrub heath communities on deep yellow sandy soils. Phylogenetic studies based on RFLP analysis of cp DNA indicate that both species are sister species and form a relatively recent evolutionary lineage. Population estimates of genetic variation based on 14 allozyme loci showed that allelic richness (A), polymorphism (P) and heterozygosity (H_o) were lower and gene diversity (H_e) significantly lower in *A. sciophanes* than *A. anfractuosa*. However, genetic diversity in both species was relatively high compared to estimates for most other long lived woody angiosperms. Mating system analyses indicated that the multilocus outcrossing rate in the Wundowlin *A. sciophanes* population ($t_m = 0.61$) was significantly lower than outcrossing rates for two *A. anfractuosa* populations ($t_m = 0.86, 0.85$) and that the correlated paternity (probability that sibs shared the same father) in the *A. sciophanes* population ($r_p = 0.61$) was higher than either of the two *A. anfractuosa* populations ($r_p = 0.44, 0.36$). Studies on other naturally rare *Acacia* species in south-west Australia indicated similar reductions in genetic variation in the rare and geographically restricted species compared with their more common widespread closely related congeners. These findings are discussed in relation to the evolution and conservation of the south-west flora and particularly in relation to the unusually high proportion of rare and geographically restricted species in that region.



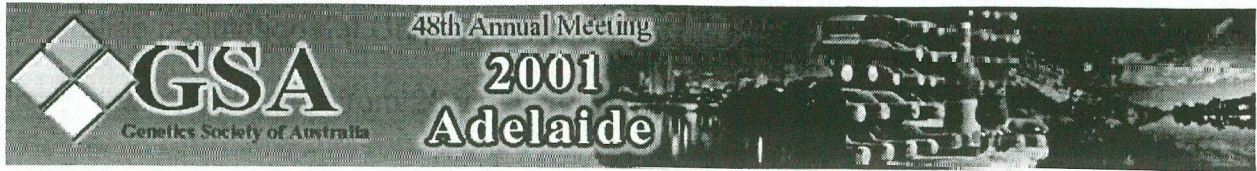
Islands in the Australian desert: evolution of subterranean water beetles in calcrete aquifers

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Recently a diverse subterranean invertebrate fauna was discovered from isolated calcrete (terrestrial limestone) aquifers associated with paleaodrainage channels of the Yilgarn Craton in central Western Australia. Remarkably, each calcrete aquifer, studied to date, contains a unique assemblage of organisms, including water beetles and a variety of crustaceans. The subterranean water beetles were the first to be described and eleven new species were identified, each of which appear to be locally endemic to an individual calcrete aquifer (Watts & Humphreys 1999, 2000).

Here we use a molecular phylogeny, based on mitochondrial DNA sequences, to investigate the origin and evolution of the subterranean water beetles. Our results indicate that there have been multiple origins of subterranean water beetle lineages and that each lineage has been isolated for more than 10 million years, probably since the onset of aridity in Western Australia. These data suggest that there has been long-term isolation of water beetle populations in each calcrete aquifer, implying that each calcrete can be viewed as an 'island in the desert'.



Life Cycle of Grape Phylloxera, *Daktulospairia vitifoliae*, in Australia

Angela Corrie¹, Ross H. Crozier², Robyn van Heeswijck³, Ary Hoffmann¹

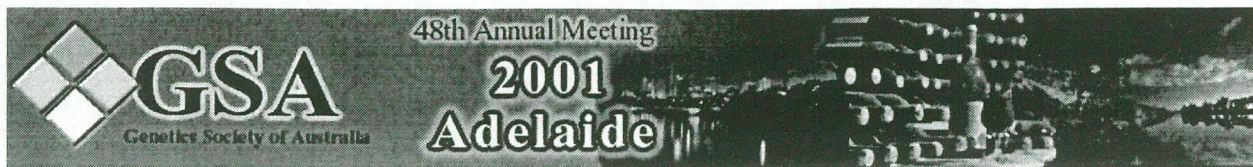
¹Centre for Environmental Stress and Adaptation Research, La Trobe University, Bundoora, Vic. 3086.

²Department of Zoology and Tropical Ecology, James Cook University, Townsville, QLD

4811. ³Department of Horticulture, Viticulture and Oenology, University of Adelaide, PMB 1 Glen Osmond, SA 5064.

The Grape Phylloxera, *Daktulosphaira vitifoliae*, is a viticultural pest that has devastated vineyards world wide, yet little is known about this insect's biology. An understanding of the life cycle is critical to the successful management of this pest. Through the use of four polymorphic microsatellite loci we have elucidated the mode of reproduction of Australian populations of Grape Phylloxera.

Insects were collected from 28 vineyards located in Victoria and New South Wales. Our results showed the majority of vineyards were infested by clonal lineages of Grape Phylloxera that inhabit the root system. There was little support for the traditionally described holocyclic life cycle for this species. Clonal diversity was limited in all but one of the vineyard regions suggesting that multiple founders may have contributed to the diversity within this region. However the non-random distribution of clonal lineages within this same region indicates other factors are also involved in clone distribution. We are currently assessing the association between vine host genotype and Grape Phylloxera clones.

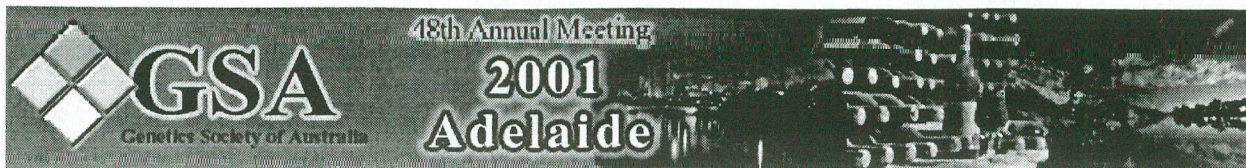


A putative transferrin gene from a termite upregulated in response to fungal infection.

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¹ James Cook University, Townsville, Queensland 4811

Social insects have unusual susceptibility to pathogen attack, in that they make up a major part of animal biomass in total, and live in dense aggregations. Within this overall picture, social insects vary considerably in exposure to pathogens in terms of the microhabitat occupied (e.g., arboreal or terrestrial), social environment (highly as against weakly related nestmates) and ecology (large populations /colonies as against small). Although they also display behavioral adaptations to limit exposure to pathogens, considerable variation is expected in polymorphism and evolutionary rate between lineages and perhaps between genes within lineages according to kind of pathogen encountered. Lacking the 'adaptive' system of vertebrates, all insect immune genes are in the 'innate' system lacking true immunological memory.-- Termites have a major ecological and economic influence, and we began our search for immune genes using the large-bodied species *Mastotermes darwiniensis*. We challenged individuals by exposing them without wounding to the insect pathogenic fungus *Metarhizium anisopliae*. We used suppression subtractive hybridization to extract genes preferentially expressed in the challenged termites relative to unchallenged ones. Among the genes retrieved are several putative immune system genes. We have begun characterising a putative transferrin, and confirmed using a Virtual Northern procedure that it is indeed upregulated following exposure, as are transferrins of *Aedes* and *Drosophila*. The sequence bears the closest resemblance among known transferrins to one reported from the cockroach *Blaberus discoidalis*.



Gene rearrangements as phylogenetic characters – evidence that intramitochondrial recombination shapes mitochondrial genome organization

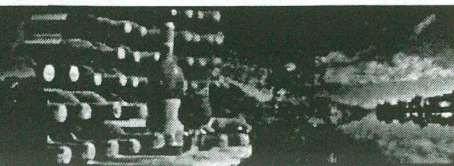
Mark Downton

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The relative positions of genes in the mitochondrial genome has been proposed as a novel, highly reliable phylogenetic character. It has been argued that the huge number of ways of arranging the 37 mitochondrial genes make convergent organizations extremely unlikely (1). However, the underlying rearrangement mechanism may bias the spectrum of possible organizations, and little is known of the rearrangement mechanism. Previously, most gene rearrangements were consistent with a 'duplication/random loss' mechanism, wherein slipped-strand mispairing during replication leads to partial duplication of the mitochondrial genome (2). Subsequently, random loss of one copy of each of the duplicated genes produces rearranged genomes.

Parasitic wasps appear to have experienced many more mitochondrial gene rearrangements than most other animal groups (3). In just one family of wasps, there are at least four different organizational states for the mitochondrial genome. Mapping of these organizational states onto a comprehensive phylogeny for the family (two gene fragments, 96 morphological characters, 74 taxa) indicates that two independent gene inversions and two independent gene translocations have occurred during the evolution of this family, rearrangements considered very rare according to the 'duplication/random loss' model. In particular, this model cannot explain the gene inversions observed. Recent work on human mitochondria suggests that intramitochondrial recombination operates at low levels in healthy patients (4), a process that could produce gene inversions. In this paper, I will present a putative scenario explaining why parasitic wasps might be prone to mitochondrial gene rearrangement (5).

1. Boore, J.L. *et al.* (1995) *Nature* **376**:163-165.
2. Moritz, C., Dowling, T.E. & Brown, W.M. (1987) *Annu. Rev. Ecol. Syst.* **18**:269-292.
3. Downton, M. & Austin, A.D. (1999) *Mol. Biol. Evol.* **16**:298-309.
4. Kajander, O.A. *et al.* (2000) *Hum. Mol. Genet.* **9**:2821-2835.
5. Downton, M. & Campbell, N.J.H. (2001) *TREE* **16**:269-271.



Source population of dispersing rock-wallabies (*Petrogale lateralis*) identified by assignment tests on multilocus genotypic data

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¹ Department of Biological Sciences, Macquarie University, NSW 2109, ² Wildlife Research Centre, Department of Conservation and Land Management, Wanneroo, WA, 6065.

The ability to confidently identify or exclude a population as the source of an individual has numerous powerful applications in molecular ecology. Several alternative assignment methods (frequency, Bayesian, distance) have recently been developed and are yet to be fully evaluated. In this study we tested the efficacy of different assignment methods by using a translocated rock-wallaby (*Petrogale lateralis*) population, of known provenance. Specimens from the translocated population (n=43), its known source population (n = 30) and four other nearby populations (n=19-32) were genotyped for 11 polymorphic microsatellite loci. The results identified the frequency and Bayesian methods as the most consistent and accurate, correctly assigning > 90% of individuals up to a significance threshold of $P = 0.001$. Performance was variable amongst the distance based methods, with the Cavalli-Sforza and Edwards chord distance performing best, while Goldstein *et al.*'s $(\delta\mu)^2$ consistently performed poorly. Using the frequency and Bayesian methods we then attempted to determine the source of rock-wallabies which have recently recolonised an outcrop (Gardners) 8 km from the nearest rock-wallaby population. Results from the frequency and Bayesian assignment tests indicate that the population at Gardners has originated via a recent dispersal event from the eastern end of Mt Caroline.

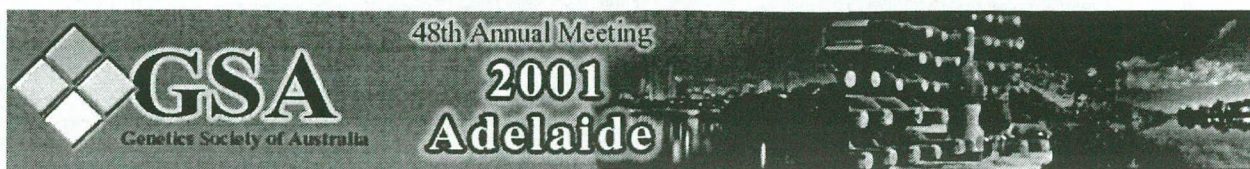


Conservation genetics of the Eastern Bristlebird, *Dasyornis brachypterus*, using multiple mitochondrial and nuclear gene sequences.

Martin S. Elphinstone and Peter R. Baverstock

Centre for Animal Conservation Genetics, Southern Cross University, Lismore, NSW, 2480, Australia

The Eastern Bristlebird, *Dasyornis brachypterus*, has been recorded from Wilson's Promontory in eastern Victoria to the Conondale Range in south-eastern Queensland. Its current distribution can at best be described as patchy, with populations confined to three main regions. In the north they exist in a series of locations between the Conondale Range and Border Ranges National Parks on the Queensland/NSW border. In the central region, Barren Grounds Nature Reserve and Jervis Bay are the major strongholds. To the south, a population can be found in the Nadgee Nature Reserve near the NSW/Victorian border. Some authors have suggested that the species may be comprised of two distinct forms based on morphological, ecological and biogeographical considerations: (i) those in the northern region and (ii) those in the central and southern regions. We have tested the hypothesis that these forms correspond to Evolutionarily Significant Units using molecular genetic data from multiple mitochondrial and nuclear genes. The data clearly demonstrate that this is not the case. Rather, a distinct genetic lineage exists in the southern population and conservation priorities should account for this to best preserve within-species genetic diversity. Statistical power and the pitfalls of type 2 errors when drawing conclusions from limited data will be discussed. In addition, we describe the protocols used for the design and application of new PCR primers for four mitochondrial genes (Control Region, ATPase 8, ATPase6 and NADH 6) and three potentially polymorphic nuclear loci (myoglobin intron, myosin heavy chain 2 intron and the FGR viral oncogene homologue). Although these primers were designed for use in passerine birds, we provide a general protocol for the development and application of a similar suite of markers in any vertebrate taxon.

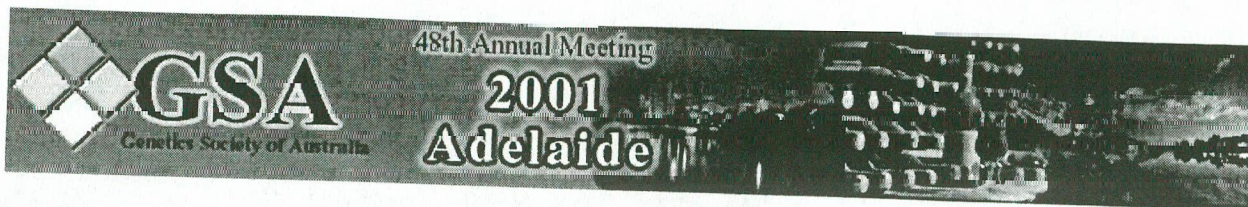


The use of reporter systems to identify genes upstream of *lozenge* in *Drosophila* eye development.

Ashley Farlow, Ben Hogan and Philip Batterham.

Department of Genetics, University of Melbourne, Parkville, Victoria 3052.

The *Drosophila* adult eye is composed of 800 ommatidia each of which is composed of bristle cells, cone cells, pigment cells and eight photoreceptor cells (R1-R8). The eye develops from an unpatterned 'parcel' of epithelial tissue, the eye imaginal disc. An indentation, the morphogenetic furrow, moves across the disc from posterior to anterior during late larval and early pupal development. Behind the furrow cells are recruited to particular fates. The *lozenge* gene encodes a transcription factor of the Runt/AML1 (Acute Myeloid Leukemia) family. The gene is widely expressed in the eye disc and *lz* mutants are defective in the differentiation of cone cells, bristle cells, pigment cells and photoreceptor cells R1, R6 and R7. It has been shown that *lz* exerts its effect by controlling the transcription of genes encoding transcription factors essential for the specification of these cell types. Given the central role of *lz* in eye development, it is important to determine how the *lz* gene itself is regulated. The enhancer controlling *lz* expression in the eye has been localized to the second intron of the gene. We have used two approaches to identify the transcription factors that bind to this enhancer. The first of these has used a cross-species comparison of the eye enhancer sequences. The second approach uses a P element containing the yeast *Gal4* gene. This P element is inserted at the 5' end of the *lz* gene and the *Gal4* gene is faithfully expressed from the *lz* eye enhancer. When this construct is used to drive *Cyclin E* expression a rough eye phenotype is observed in adult flies. This system is being used to identify genes upstream of *lz*.



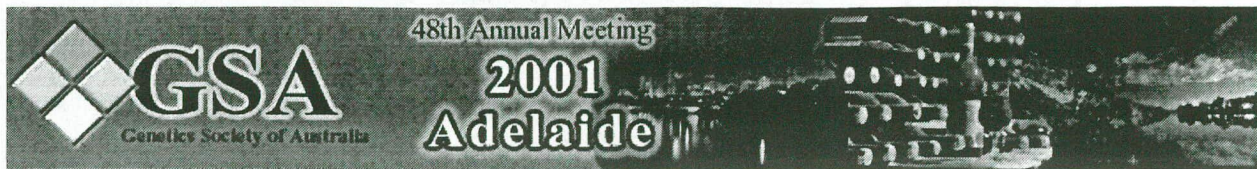
Molecular Evolution of the Internal Transcribed Spacers (ITS1 & ITS2) and Phylogenetic Relationships within the Genus *Brachycome*.

B. Field, C. Leach, J. Timmis, T. Donald, M. Donnon and A. Houben*

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The sequences of the internal transcribed spacer regions (ITS1 and ITS2) within the genes coding for cytoplasmic rRNAs have been determined in 10 members of the higher plant genus *Brachycome*. After microdissection and cloning, the ITS1 and ITS2 region from rDNA clusters specific to the B chromosomes and micro B chromosomes of *B. dichromosomatica* [Houben et al., Chromosoma 106: 513-519 (1997)] have also been determined. The ITS1 sequence of species within the *B. lineariloba* complex [Carter, Telopea 1: 387-393 (1978)] contain a 59 bp tract that is also found in the B and micro B chromosome rDNA and in some other members of the *Asteraceae*, but is absent from all other species in the genus *Brachycome* examined so far. Phylogenetic trees based on these ITS regions reveal possible evolutionary relationships of the A and B chromosomes, and of species within the genus *Brachycome*.

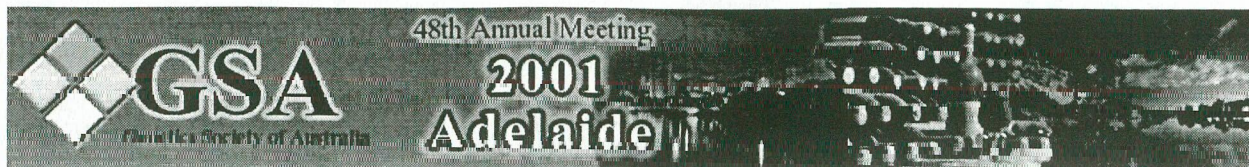


Is population fitness correlated with genetic diversity?

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Genetic diversity is one of the three forms of biodiversity recognised by the IUCN as deserving conservation. The need to conserve genetic diversity within populations is based on two arguments, the necessity of genetic diversity for evolution to occur, and the expected relationship between genetic diversity and population fitness. Since loss of genetic diversity is related to inbreeding, and inbreeding reduces reproductive fitness, a correlation is expected between genetic diversity and population fitness. Population size, which determines rates of inbreeding, is also expected to correlate with fitness. However, other theoretical considerations and empirical observations suggest that the correlation between fitness and genetic diversity may be weak or non-existent. We performed a meta-analysis, using all the data sets we could locate (33) in order to resolve the issue. The mean weighted correlation between measures of genetic diversity, at the population level, and population fitness was 0.40. The correlation is highly significant, and explains 16% of the variation in fitness. This study validates concerns that the loss of genetic diversity has a deleterious effect on population fitness, and supports the IUCN designation of genetic diversity as worthy of conservation. Since most endangered species have lower genetic diversity than related non-endangered species, they probably have lower reproductive fitness.



Population structure in an economic pest species: microsatellites and the Queensland fruit fly, *Bactrocera tryoni*.

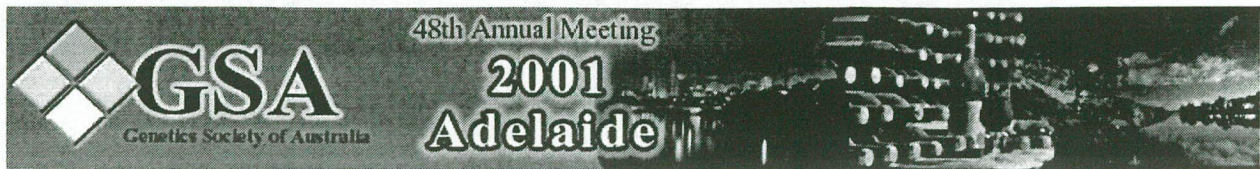
Stuart Gilchrist, Hong Yu, Marianne Frommer and John Sved

Fruit fly Research Centre, School of Biological Sciences A12, University of Sydney, NSW 2006

Originally a native of rainforests, the Queensland fruit fly (Q-fly, *Bactrocera tryoni*) has expanded its range since the mid-19th century, adapting to more variable environments outside Queensland. In particular, it has become the major economic pest of orchards throughout eastern Australia, inflicting damage estimated at over \$100 million dollars each year. Given its significance as an economic pest, surprisingly little is known about the population genetics of Q-fly.

At the Fruit Fly Research Centre, University of Sydney (FFRC), we have developed 30 microsatellites in order to investigate the genetics and population structure of Q-fly. Using these microsatellites, we have shown that there is significant population structuring along the eastern seaboard. Here, at least four main population groups can be distinguished. Given the wide dispersal ability of Q-fly, it was surprising that this structuring is highly stable between years. This suggests that Q-flies may be becoming regionally adapted.

We have also exploited this population structuring to investigate the origins of Q-fly outbreaks in the Fruit Fly Exclusion Zone (FFEZ). Since 1994, State Agriculture departments have attempted to keep this area (which covers major fruit growing areas in the Murrumbidgee Irrigation Area, SA Riverland and the Goulburn Valley) Q-fly-free to facilitate horticultural exports. Despite their efforts, outbreaks of Q-fly occur within the FFEZ each year, with consequent suspension of exports from areas within an 80km radius of the source of each outbreak. Using microsatellites, we are currently attempting to determine which, if any, of the endemic populations are the likely sources of these outbreaks. Preliminary results suggest that some outbreak populations most closely resemble endemic populations bordering the FFEZ. This is contrary to the commonly held view that most outbreaks result from infested fruit imported to the FFEZ from more distant regions (e.g. Queensland). We have used methods based on either simple allele frequency data or rare-allele tests. The use of these methods will be discussed.



The rise and fall of the mammalian sex determining gene *SRY*

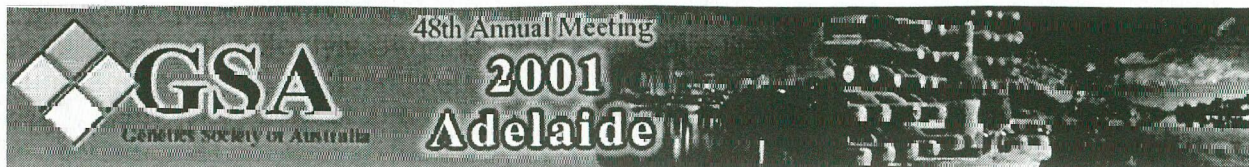
Jennifer A. Marshall Graves

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The human and mouse XX female:XY male system works by way of the male-dominant *SRY* gene on the Y chromosome that triggers testis determination. The closest relative of *SRY*, *SOX3* on the X chromosome, is the likely "father of *SRY*". This gene is expressed largely in the developing central nervous system, so is more likely to be brain-determining than testis-determining. When and how did this gene take on its role in sex determination?

It is assumed that *SRY* was the gene that started the whole process of degradation of the Y chromosome, and that this occurred early in mammalian evolution, 200-300 million years ago. In order to understand how and when *SRY* took over from an ancestral vertebrate sex determining system, we have looked for relatives of *SRY* in non-mammal vertebrates, as well as marsupial and monotreme mammals, which diverged from therian mammals (eutherians and marsupials) early in mammal evolution.

Eutherian ("placental") and marsupial mammals have an *SRY* gene on the Y chromosome, implying that *SRY* is at least 130 million years old. However, there is no evidence of a male-specific *SRY* gene in birds, reptiles or even monotremes, and related genes are on autosomes. This implies that the "father of *SRY*" was autosomal and was not involved in sex determination in the common ancestor of mammals. Another sex determining gene must have pre-dated *SRY* in mammal sex determination – perhaps a Y chromosome gene *ATRY* we recently cloned from marsupials. Nor is *SRY* a permanent fixture in mammals, for two species of mole voles have recently lost the entire Y chromosome, including *SRY*. In this group the *SRY* gene must have been supplanted by an as yet unknown new sex determining mechanism. Thus the *SRY* gene evolved in therian mammals only 130-170 million years ago, and can be degraded and lost like other genes on the fast-disappearing mammalian Y chromosome.



Evolutionary history of juvenile life history strategies in the benthic octopuses (Cephalopoda:Octopodidae).

M.T. Guzik¹, M.D. Norman², R.H. Crozier¹

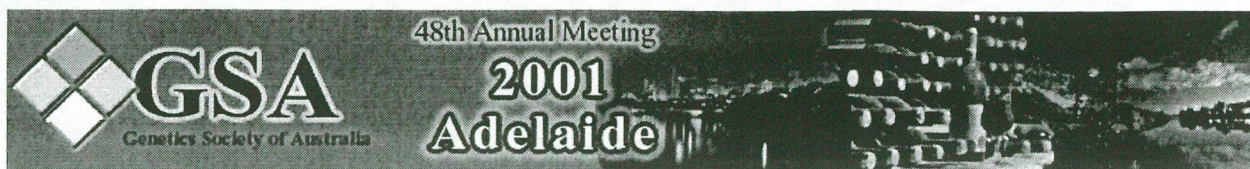
¹ Previous address: Department of Genetics and Human Variation, LaTrobe University, Bundoora, VIC, 3806, Australia.

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The benthic Octopodidae are one of only two cephalopodan families where juvenile life history is polymorphic among species. Juveniles may be planktonic or benthic. Planktonic juveniles develop in the plankton, while benthic juveniles immediately assume an adult lifestyle after hatching. Juvenile lifestyle is closely associated with egg number and size, and is likely to play an important role in species dispersal and distribution.

Phylogenetic relationships of 35 species from the subfamily Octopodinae were reconstructed using sequence data from two mitochondrial genes, cytochrome oxidase subunit III and cytochrome b. We then mapped juvenile life style onto the phylogenetic tree to infer the transitions in these characters. Results revealed a divergence among taxa into two distinct clades. The majority of taxa within the first clade possessed small eggs and planktonic young, whereas the reverse situation was true in the second clade. Furthermore, both clades contained exceptional species that showed the alternative character state. These results suggest that modes of juvenile life history may be evolutionarily labile among the Octopodidae and phylogenetic constraints may have had a minimal influence on these traits during their evolution. It is proposed that ecological factors play a considerable role in the maintenance and variability of juvenile life history strategies.

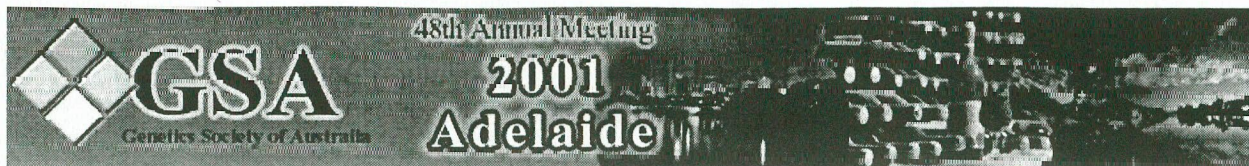


Structural and Functional Analysis of *pebble*, a Gene Required for Cytokinesis

A.Harley¹, L.O'Keefe¹, S.Prokopenko², H.Bellen², and R.Saint¹.

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The *pebble* (*pbl*) gene is required for cytokinesis in *Drosophila*. The Pbl protein contains Dbl Homology (DH), and Pleckstrin Homology (PH) domains, characteristic of GTP exchange factors which activate the Rho family of small GTPases (RhoGEFs). In their active form, these Rho family GTPases mediate reorganisation of the actin cytoskeleton, which is an essential event during cytokinesis. Pbl also contains BRCT (BRCA1 C-Terminal) domains, which are found in a number of proteins involved in DNA repair. The presence of domains within a RhoGEF that normally function in the nucleus is both unique and intriguing. In accordance with a potential nuclear role for Pbl, Pbl contains a nuclear localisation sequence and is observed to localise to the nucleus after its transient cortical and cleavage furrow localisation late in mitosis. Nuclear localisation of Pbl could, however, simply be a means of removing the Pbl RhoGEF from the cytoplasm during interphase. In an attempt to examine the potential nuclear role of Pbl, two complimentary procedures have been employed. Site-directed mutagenesis was used to abolish nuclear localisation of the protein and to delete its BRCT domains, and the effect of these deletions was examined in transgenic flies. Irradiation sensitivity experiments were also performed to examine whether Pbl plays a role in DNA repair processes. Initial experiments have indicated that *pbl* mutants are more sensitive to irradiation than wild type, and the basis of this sensitivity is currently being investigated.



Characterisation Of The Expansin Gene Family In *Gossypium Hirsutum* (Cotton)

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Cotton fibres arise from single epidermal cells of the ovule and initiate as protrusions on the day of flowering (anthesis). Elongation continues for approximately 20 days, resulting in fibres which are 2-3.5 cm in length. As fibres are single cells and their development is synchronous, they make an excellent model system for the analysis of the molecular processes involved in cell growth, the control of which is an important consideration in cotton bioengineering.

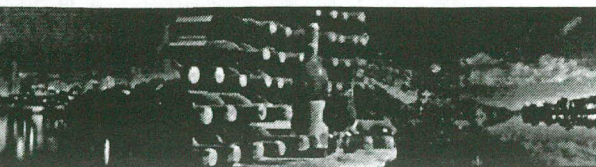
The growth of plant cells requires components of the cell wall to slip relative to each other, thereby enabling cellular expansion. Expansins are a group of proteins tightly associated with the cell wall and are able to facilitate non-reversible extension of the cell wall in a pH dependent manner¹. The exact mechanism by which expansins act is unknown, but it is believed that they disrupt the non-covalent bonds between cellulose microfibrils and hemicellulose matrix polymers, thus allowing the microfibrils to 'slip' relative to each other, while maintaining the structural integrity of the cell wall. Expansins have been characterised in a number of plant species and are encoded by large gene families (up to 25 members in *Arabidopsis*). Family members often show distinct patterns of expression and are generally expressed in tissues undergoing rapid cell expansion¹.

Differential screening of a cotton fibre cDNA library identified a number of fibre-specific cDNAs, one of which encodes an expansin². Using this cDNA as a probe to a genomic Southern indicated that cotton has at least three expansin family members which are closely related to the fibre-specific gene, and up to ten more distantly related expansin genes. Here we describe the characterisation of six cotton expansin genes, which were isolated using a combination of genomic library screening and PCR-based approaches. Sequencing of the six different genes revealed that they encode putative expansin proteins, as defined by a high degree of sequence similarity to known expansins and the presence of specific amino acid signatures¹.

Sequence differences between the genes were used to design gene-specific primers and RT-PCR revealed individual expression patterns of the six different expansin genes within different cotton tissues. Transcripts were detected for all except one expansin gene, and the expansin gene which corresponds to the fibre-specific cDNA was expressed at high levels exclusively in cotton fibres. These results suggest that there is a single expansin gene active during fibre elongation, whilst other expansins contribute to cell growth elsewhere in the cotton plant.

1. Cosgrove, D.J. (2000) *Current Opinion in Plant Biology* **3**: 73-78.

2. Orford, S.J and Timmis J.N. (1998) *Biochimica et Biophysica Acta* **1398**: 342-346.

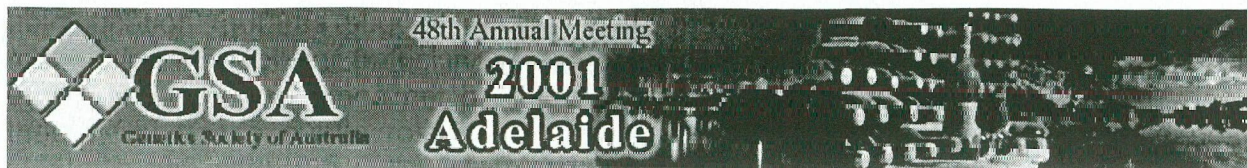


Molecular basis of insect resistance to *Bacillus thuringiensis* toxins

David G. Heckel¹, Fred Gould², and Linda J. Gahan³

¹ University of Melbourne ² North Carolina State University, and ³ Clemson University

Transgenic crops expressing insecticidal toxins from *Bacillus thuringiensis* (Bt) are widely used for pest control. Bt-resistant insect strains have been studied but the molecular basis of resistance has remained elusive. We have made a major step in solving this problem by cloning the first Bt-resistance gene from an insect. The YHD2 strain of *Heliothis virescens* is > 10,000-fold resistant to Cry1Ac toxin, and the *BtR-4* gene accounts for up to 80% of this resistance. We will present our cloning and expression studies that show that the *BtR-4* gene encodes the functional receptor or "toxic target" of the Cry1A class of Bt-toxins in Lepidoptera. We will also review other recent findings in this area, and in the context of this work, discuss the role and relative importance of altered-target-site mutations compared to other Bt-toxin resistance mechanisms.

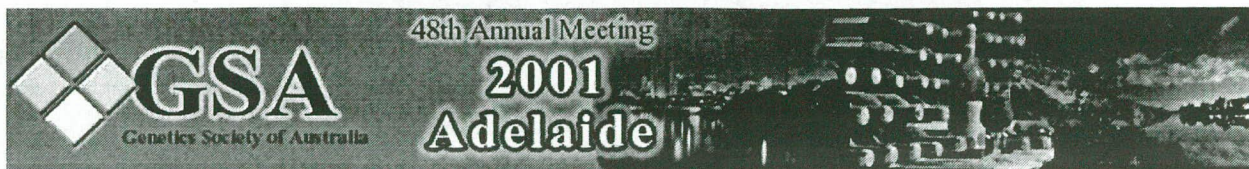


Analysing oncogene function in *Drosophila*

Gary Hime¹, Hannah Robertson, Deborah Gunthorpe.

Dept. of Anatomy and Cell Biology, University of Melbourne, Vic 3010.

The mammalian proto-oncogene product, c-Cbl, has been shown to act as a ubiquitin-protein ligase to down-regulate receptor signalling. The v-Cbl oncoprotein, a truncated form of c-Cbl, is associated with haematopoietic tumours in mice. We have shown that ectopic expression of a *Drosophila* analogue of v-Cbl, Dv-Cbl, acts as a dominant-negative protein *in vivo* and enhances signalling through several receptors. Furthermore, Dv-Cbl interacts genetically with vesicular trafficking mutants as well as ubiquitination mutants suggesting that v-Cbl may affect receptor down regulation by modulating intracellular trafficking. A recently identified *D-cbl* loss of function mutant is embryonic lethal but does not exhibit any cuticular patterning defects. This suggests that D-Cbl has a subtle role in modulating levels of receptor signalling. We have identified two alternate splice products of the *D-cbl* gene with one being restricted to developing neurons of the central nervous system. The D-Cbl protein expressed in neurons is localised along the length of axons. We are currently analysing if the embryonic lethality is due to nervous system defects. Co-expression of Dv-Cbl with activated signalling molecules in the developing fly eye can result in melanotic outgrowths suggesting that Dv-Cbl may act as a co-operative oncogene. This phenotype will be used to develop a screen for mutations that interact with Dv-Cbl to promote tumour formation.



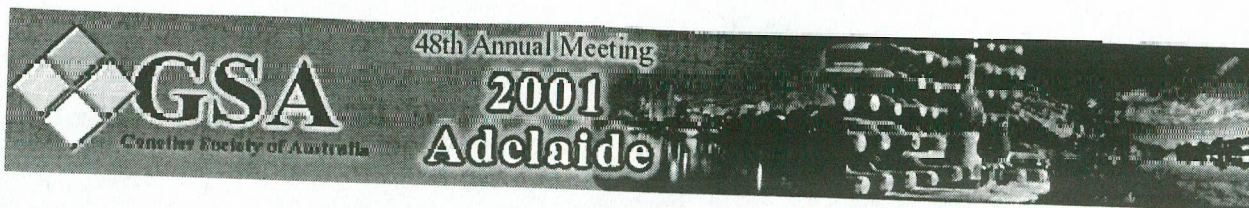
Molecular variation across a morphological cline in *Eucalyptus*

James Holman¹, Jane Hughes¹.

¹Australian School of Environmental Studies, Griffith University, Brisbane, Qld.

The evolutionary importance of hybridisation, as a mechanism that has shaped diversification within *Eucalyptus* is becoming more widely accepted and is being used to explain contemporary patterns in morphological variation. This study examines a morphological cline that exists between two Ironbark species, *E. melanophloia* (Silver-leaved Ironbark) and *E. whitei* (Whites Ironbark). This morphological cline spans an east-west rainfall gradient in central Queensland, with the broader leaved *E. melanophloia* being found in the more mesic eastern regions and the narrower leaved *E. whitei* found in the drier more western regions. It has been suggested that hybridisation is responsible for the presence of this morphological cline, however such patterns of clinal variation may simply be the product of primary differentiation and not hybridisation.

This study integrates both morphometric and genetic analysis to investigate the maintenance of the morphological cline through examining patterns of morphological differentiation, inter-population genetic exchange and the effects of gene flow on the genetic structuring of the plant populations. The morphological analysis indicates that the putative pure species are morphologically distinct, with intermediate morphotypes showing continuous variation between these forms. Analysis of 5 Embra microsatellite loci indicates that there is wide spread inbreeding and little genetic differentiation across the morphological cline. The results from this study therefore indicate that this morphological cline is not the product of hybridisation and furthermore question the taxonomic status of this species complex.

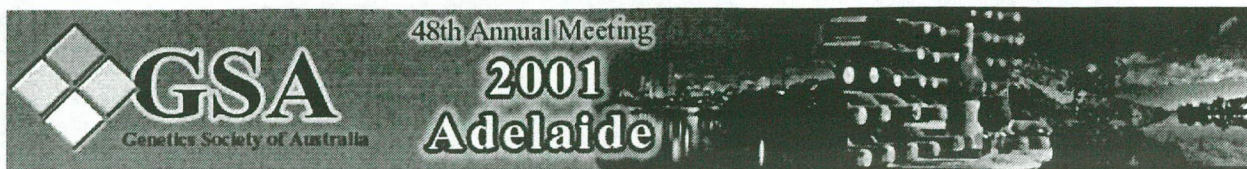


The effect of etoposide on somatic intrachromosomal recombination: a comparison in *in vivo* and *in vitro* pKZ1 murine models

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Department of Haematology and Genetic Pathology, Flinders University and Medical Centre, Bedford Park, South Australia, 5042.

Somatic intrachromosomal recombination (SICR) can result in deletions and inversions of DNA, lesions that are important in carcinogenesis. Our laboratory is using the pKZ1 mouse recombination mutagenesis model to study the role of SICR in mutation and cancer. The pKZ1 construct contains an *E. coli lacZ* gene in inverse orientation to an enhancer-promoter complex. Expression of the *lacZ* gene can only occur if an SICR inversion event occurs within the pKZ1 construct. SICR inversion events are detected by histochemical staining for transgene expression in frozen sections using X-gal. Etoposide (VP-16) is a topoisomerase-II inhibitor that is known to induce aberrant recombination events in human lymphoid cells. Here, we demonstrate the sensitivity of the pKZ1 mutagenesis model to detect changes in SICR inversion frequency in spleen after treatment with VP-16 doses ranging from 0.0005 mg/kg to 50 mg/kg. Four- or 8-month old mice were given a single intraperitoneal injection of VP-16, euthanased 3-days post-treatment, and the spleen analysed for SICR. A significant 1.4X – 3.1X induction of SICR inversion events ($p < 0.0213$) was detected at doses ranging from 0.05-50 mg/kg. However, SICR inversion frequencies after treatment with 0.0005 mg/kg and 0.005 mg/kg VP-16 decreased significantly to 0.67 and 0.43 of the levels observed in control animals respectively ($p < 0.0115$). Our results demonstrate the sensitivity of the pKZ1 model to detect significant mutational changes at a dose of VP-16, 20-fold lower than other *in vivo* mutation assays. The mechanism for a biphasic SICR response to different VP-16 doses *in vivo* is unclear. Recently we generated a pKZ1 mouse hybridoma cell line to study the mechanism of the SICR response to DNA damaging agents *in vitro*. pKZ1 spleen cells were fused to P3653 myeloma cells and single hybridoma colonies expressing the pKZ1 transgene were cloned. The spontaneous SICR inversion frequency in pKZ1 hybridoma A11 is ~ 2 fold higher than observed *in vivo*. A comparison of the SICR response to a range of VP-16 doses *in vivo* and *in vitro* will be presented. We have previously demonstrated the ability to sensitively detect SICR inversions *in vivo* in response to a range of different agents. The development of a cell line that responds to these agents in a similar manner to the *in vivo* situation will help elucidate the mechanism of SICR in response to DNA damaging agents and will provide an alternative cheaper mutagenesis assay.

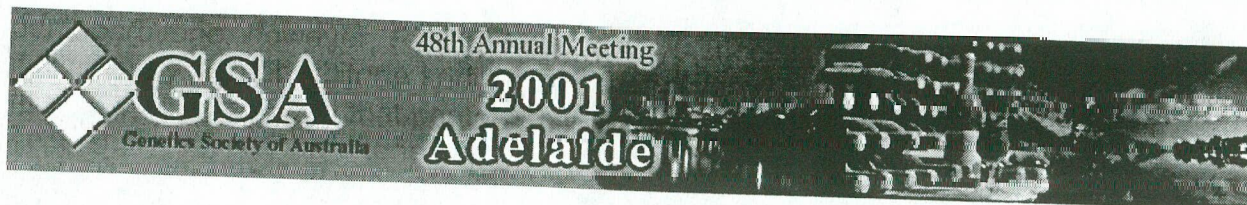


Analysis of Sequence Variation in p53 and H and K-Ras Genes in Canine Haemangiosarcoma Cell Lines.

Chandramouly G., Hughes I., Ploeg R.

School of Veterinary Science, University of Queensland

Haemangiosarcoma is a highly metastatic malignancy arising from vascular endothelial cells that occurs more commonly in dogs (Prevalence 0.3-2%) than in any other species (Oksanen , 1978, *J.Comp.Path.* 88:585). The German Shepherd breed is reported to demonstrate a predisposition to the development of Haemangiosarcoma (Brown et al, 1985, *J.Am.Vet.Med.Ass.* 186:56). Mutations in p53 and H and K-ras genes have been linked to tumour development in human tumours equivalent to haemangiosarcoma. Hence, p53, H-ras, and K-ras were analysed for mutations from tumour cells that were grown in cell-culture. In addition, sequence analysis of normal and affected German shepherd dogs was undertaken to investigate the possible presence of germ-line mutations. In humans, most cancer-linked mutations have been detected in p53 exons 5-8 (Greenblatt *et al*, 1994, *Cancer Res.* 54:4855; and Soussi *et al*, 1990, *Oncogene*, 5:945) and exons 1 and 2 of H and K-ras (Kraegel *et al*, 1992, *Cancer Res.* 52:4724). Hence, these regions of p53, H and K-ras were chosen for mutational analysis in our 12 dogs and 2 tumour cell-lines. The regions were amplified by PCR, the products then cloned and sequenced. On comparison with a published sequence of canine p53 (Medline Acc. No:95323915), we found two G→A transitions at cDNA nucleotides 635 and 771 resulting in Arg→Glu and Glu→Lys amino acid changes respectively. These changes were found consistently in both tumour cell lines and normal dogs and, using the Garnier program (ANGIS), were predicted to result in minimal secondary structural change. Similarly, observed sequences from exon 1 of H and K-ras were compared with published sequences (Medline Acc. No's 92379785 for K-ras and 98161925 for H-ras). No sequence variation was identified in tumour cell lines or dogs for H and K-ras transcripts. These results suggest that p53, H-ras or K-ras are unlikely to be involved in the tumorigenesis of haemangiosarcoma in dogs. Specifically, it can be concluded that no mutations exist in the exons 5,6,7 and 8 of p53 and exon 1 of H and K-ras genes.

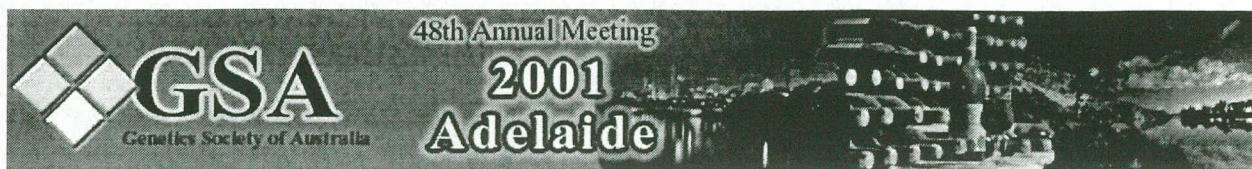


The Effect Of Pentobarbitone On Genetic Analysis by RT-PCR

C. Hyun¹, I. Hughes² & L.J. Filippich¹

Companion Animal Science¹, Immunogenetics²,
School of Veterinary Sciences, The University of Queensland, Brisbane, QLD 4072.

Pentobarbitone sodium (Sodium 5-ethyl-5[1-methylbutyl]-pentobarbitone) is a short acting barbiturate that is commonly used to euthanase animals. As part of our studies into the molecular genetics of copper toxicosis in Bedlington terrier dogs, reverse-transcription (RT) PCR was noted to always fail on RNA samples collected from livers of dogs sacrificed by pentobarbitone injection. When samples were collected without pentobarbitone, however, RT-PCR was always successful. We suspected the possible inhibition by pentobarbitone sodium of either reverse transcriptase or *Taq* polymerase. *In vitro* studies showed that pentobarbitone interference of PCR occurred at $> 4 \mu\text{g}/\mu\text{L}$. To identify if pentobarbitone produced competitive inhibition, each components (*Taq* polymerase, MgCl_2 , dNTP, etc) of the PCR was individually altered. However, inhibition still persisted suggesting multiple PCR components may be affected. Also it was shown that pentobarbitone interference was not dependent on the PCR product size. Simple dilution of pentobarbitone contaminated DNA solutions and the addition of bovine serum albumin (BSA) to the PCR mix overcame pentobarbitone interference. *In vivo*, PCR by pentobarbitone was found to be compounded by high DNA concentration and pentobarbitone contamination. In addition, both high DNA concentration and pentobarbitone contamination could be overcome by dilution and the addition of BSA. Further work is required to quantify pentobarbitone concentration in the liver extracted DNA and RNA samples, before this inhibition effect on PCR can be fully elucidated.



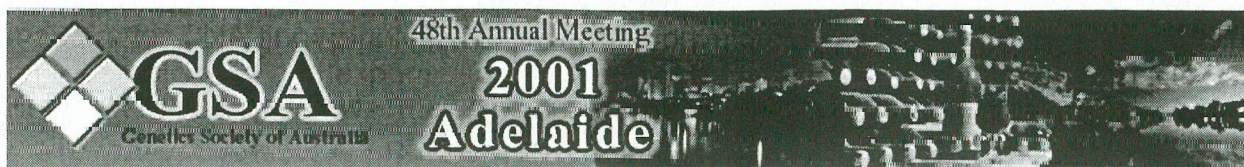
Genetic differentiation between Tasmanian cultured Atlantic salmon (*Salmo salar* L.) and their ancestral Canadian population using archived scale samples.

Bronwyn Innes, Nicholas Elliott and Robert Ward

CSIRO Marine Research, GPO Box 1538 Hobart, Tasmania 7001

Atlantic salmon (*Salmo salar*) were imported to NSW, Australia from the River Philip, Nova Scotia, Canada in the mid-1960s. Anecdotal evidence suggests the Australian population went through a severe bottleneck event during the early years. The Tasmanian salmon industry began in the mid-1980s using ova from the NSW population. The Tasmanian industry produces in excess of 12,000 tonnes of fish annually with a farm gate value of over \$100 million.

A study looking at the genetic variation between the Tasmanian Atlantic Salmon population and its ancestral River Philip population using allozymes and mtDNA indicated no loss of diversity [Ward et al., *Aquaculture* 126, 257-264 (1994)]. This was followed up by a similar study on the same individuals using microsatellite markers [Reilly et al., *Aquaculture* 173, 459-469 (1999)]. The data from this later work suggested a small overall loss of both alleles and heterozygosity in the local population. The Canadian sample used in these studies was collected in 1992 from a small number of broodstock. These individuals may not accurately reflect the genetic composition of the mid-1960s fish used to establish the Australian stocks. Our lab has since obtained archived (dried) scale samples collected from River Philip fish in 1971 and 1972. These scales have been genotyped for 11 microsatellite loci and should provide a better estimate of base-line genetic variation in the progenitor population. Modifications to standard protocols were necessary due to the age of the samples. These modifications and the results of this genetic variation study will be discussed.



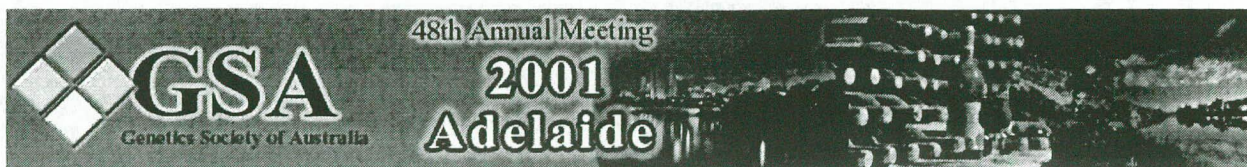
An efficient method for the development of microsatellite markers for large numbers of related species.

Jordan P W ^{1,2}, Goodman A E ², Donnellan S ¹

¹ Evolutionary Biology Unit, South Australian Museum

² School of Biological Sciences, The Flinders University of South Australia

World-wide, the illegal trade in wildlife species is second only to drug trafficking in organised crime activity. High resolution forms of individual identification such as the DNA fingerprinting technologies are needed to assist with the regulation of the legislative and conservation problems caused by the illegal trafficking of native wildlife. Microsatellites, an established form of forensic evidence, are the optimal genetic markers for DNA fingerprinting. However, the development of microsatellites will require considerable time and expense for the very large number of wildlife species effected by illegal activities. Here we trial an approach based on the often observed pattern of evolutionary conservation of microsatellite loci across wide taxonomic spans, to isolate and characterise microsatellite loci from the pythons, a group of snakes that are a prominent target of the "wildlife trade". The eight genera of pythons (26 species) last shared a common ancestor at least 40 million years ago. Microsatellites are usually isolated and characterised from a focal species then screened for both their presence and level of polymorphism on a range of related species often as an afterthought. The approach that we trial here differs in that loci were screened simultaneously for presence and polymorphism on the focal species and related species, the aim being to obtain sufficient polymorphic loci for each species through a single round of isolation. Tetranucleotide microsatellite loci were isolated from the carpet snake (*Morelia spilota*), using magnetic bead enrichment. The loci were screened for microsatellite conservation and polymorphism in the focal species (*M. spilota*) and simultaneously in another 12 species representative of the eight genera of pythons. Of the 21 microsatellite loci isolated, 20 (95%) were polymorphic in the focal species, an average of 18 (86%) and 12 (57%) loci were found to be conserved and polymorphic respectively, in each of the other 12 python species. The high proportion of polymorphic loci obtained for each species will allow the development of DNA fingerprinting markers for all 13 python species surveyed. These results show that this approach to microsatellite development is efficient, and can reduce the costs involved in the isolation and characterisation of polymorphic microsatellite loci in numerous related species. The findings here set a path for future microsatellite isolation/characterisation work and development of DNA fingerprinting identification processes required for the large number of wildlife species under threat from illegal activities.



Carbon Catabolite Repression in *A. nidulans*

Robin Lockington and Joan Kelly

Department of Molecular Biosciences, University of Adelaide, Adelaide, 5005

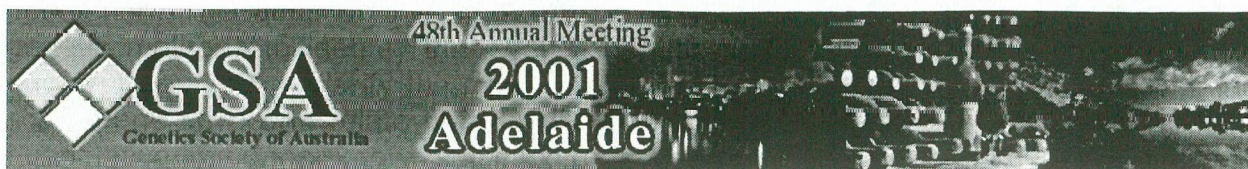
The *creA*, *creB* and *creC* genes were identified in a suppressor screen designed to identify genes involved in the regulatory mechanism of carbon catabolite repression in *Aspergillus nidulans*. The pleiotropic phenotypes conferred by *creB* and *creC* mutations are identical in range and severity, and the phenotypic effects are not additive in strains containing mutations in both *creB* and *creC*, leading us to propose that CreB and CreC are likely to act in the same pathway or in a complex.

The *creA* gene encodes a Cys₂-His₂ DNA binding protein that is required for repression (Dowzer *et al*, *Molec. Cell Biol.*, 11, 5701-5709, 1991).

The *creB* gene encodes a functional member of the novel subfamily of deubiquinating enzymes, defined by the human homologue UBH1, thus implicating ubiquitination in the process of carbon catabolite repression. The best studied role of ubiquitination is to mark proteins for destruction by the proteasome, but in addition it has recently been shown to promote macromolecular assembly and function, and alter protein function, thus playing a regulatory role distinct from protein degradation (Lockington and Kelly, *Molec. Microbiol.*, In Press, 2001).

The *creC* gene encodes a protein which contains five WD40 motifs. The sequence changes in three mutant alleles were found to lead to truncated proteins which lack one or more of the WD40 repeats. The phenotype conferred by these alleles is identical to a *creC* deletion strain, and thus all these alleles represent loss of function alleles. Deletion analysis also showed that at least the most C-terminal WD40 motif is required for function. The CreC protein is highly conserved with *Schizosaccharomyces pombe* YDE3 of unknown function, and human and mouse DMR-N9 which may be associated with myotonic dystrophy (Todd *et al*, *Molec. Gen. Genet.*, 263,561-570, 2000).

We incorporate recent findings from over-expression studies into a working model for the regulation of carbon catabolite repression in *A. nidulans*.



The role of selection in maintaining variation within a single eucalypt population

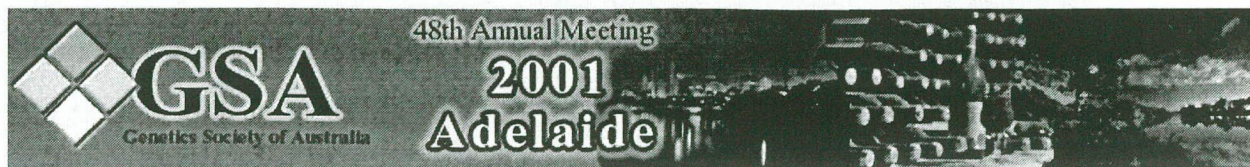
Rachel King¹, Jane Hughes¹ and Brad Potts²

¹Australian School of Environmental Studies, Griffith University, Brisbane, Qld.

²Cooperative Research Centre for Sustainable Production Forestry, School of Plant Science, University of Tasmania, Hobart.

There have been few studies of eucalypt genetic variation at the fine geographic scale, and the relative importance of limited gene flow and microhabitat effects in maintaining genetic substructuring or family groups has not been addressed within a single eucalypt population. The aims of this study are to integrate both molecular and quantitative genetic methods to identify the level of genetic substructuring that exists in a single eucalypt population, clarify the relative influences of pollen and seed dispersal on fine scale gene flow, and relate the identified patterns of gene flow with measures of a quantitative trait under known selection pressure.

A single population of *Corymbia citriodora* subsp. *variegata* near Gympie south-east Queensland was selected for this study. Analysis of 8 microsatellite loci on 130 individual trees from this population will be used to identify the current patterns of fine scale gene flow and the degree of genetic spatial sub-structuring. In conjunction with nDNA microsatellite data, sequencing of the chloroplast JLA+ region will be used to clarify the effect of seed and pollen dispersal on contemporary and historic patterns of gene flow. *Corymbia citriodora* subsp. *variegata* exhibits highly variable levels of susceptibility to the parasitic fungus *Ramularia piterika* which kills young shoots and reduces growth. A seedling trial from seed previously collected from the 130 sampled trees has been established by QFRI and will be used to measure the *Ramularia* susceptibility of families. These susceptibility measures from the F1 seedlings will then be related back to the measures of genetic variation from the 130 parent trees. As disease susceptibility directly affects fitness and, therefore, is influenced by direct selection, it is hoped that comparisons between spatial structuring of this quantitative trait and that found from the neutral nuclear markers (microsatellites) will highlight the relative importance of selection and environmental effects in maintaining variation.



tip/tow, a user configurable multi-platform utility for aligning nucleic acid sequences on the basis of protein alignments.

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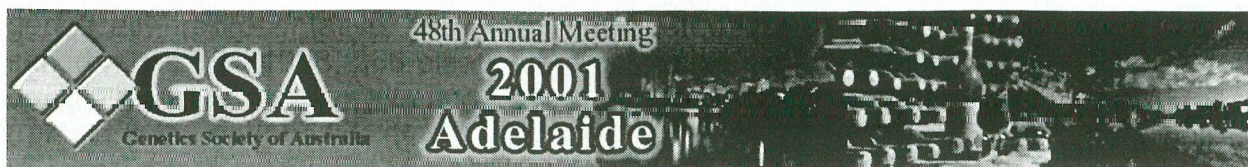
Numerous methods are available for aligning either nucleic acid or protein sequences on the basis of similarity for use in phylogenetic analyses. However, a number of analytical methods require nucleic acids aligned according to the protein sequences they encode, that is by codon. Examples of this are synonymous/non-synonymous substitution analysis (Li *et al.* 1993; Pamilo and Bianchi 1993) and Sawyer's Runs Test for recombination (Sawyer 1989). Few utilities are currently available that automate the conversion of a protein alignment to a nucleotide alignment of the genes coding them, necessitating the manual insertion of gaps into the nucleic acid sequences. Additionally the utilities that do exist do not allow the use of non-standard genetic codes, making these analyses difficult with the genomes of mitochondria and others with divergent translation tables. They also require a certain level of computer training to install and use.

To address the lack of user definable codon tables and the lack of ease of use in the available software, I have developed a utility in the perl programming language to be used with a web interface, providing an easy-to-use system for preparing sequences for the phylogenetic analyses described above. A command line version is also available for the unix environment.

Li *et al.* (1993) *J. Mol. Evol.* **36**:96-99.

Pamilo and Bianchi (1993) *Mol. Biol. Evol.* **10**:271-281.

Sawyer (1989) *Mol. Biol. Evol.* **6**:526-538.

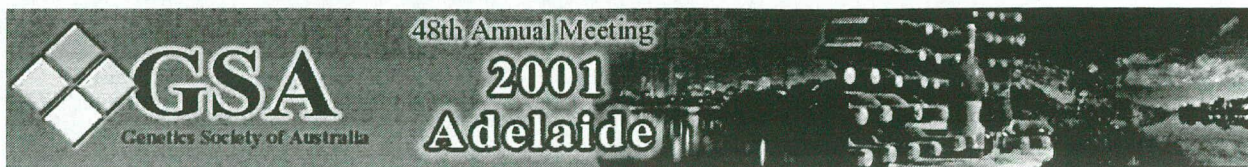


Rapid genetic decline in a translocated population of the rare and endangered plant *Grevillea scapigera* (Proteaceae)

Siegfried L. Krauss, Bob Dixon and Kingsley W. Dixon.

¹ Botanic Gardens and Parks Authority, Kings Park and Botanic Garden, West Perth, WA, 6005.

Translocations into secure sites are rapidly becoming an important tool for conserving rare species in highly vulnerable populations. The Corrigin *Grevillea*, *Grevillea scapigera* is one of the world's rarest plants, occurring in vulnerable roadside remnants in the Western Australian wheatbelt. In 1995, 10 plants were selected to act as genetically representative founders, propagated vegetatively and translocated into secure sites. By 1998, 266 plants were producing copious seed, which were collected, artificially germinated *ex situ* and 161 returned as seedlings in 1999. We used AFLP to (i) assess the genetic fidelity of the clones through the propagation process; (ii) contrast genetic variation and genetic similarities of the parents to the F1's to assess genetic decline, and (iii) assign paternity to the reintroduced seedlings to assess reproductive success of each clone. We found that (i) eight clones, not ten, were present in the translocated population, and that 54% of all plants were a single clone; (ii) the F1's were on average 22% more inbred and 20% less heterozygous than their parents, largely because (iii) 85% of all seeds were the product of only four clones. Ultimately, effective population size of the founding population was approximately two. Our results highlight the difficulty of maintaining genetic fidelity through a species recovery such as this. More generally, rapid genetic decline may be a feature of many small, translocated populations, which may ultimately threaten their long-term survival. Further research is underway to assess the fitness consequences of this genetic decline and to assess genetic decline in the F2's. Strategies to prevent genetic decline in rare species translocations are discussed.



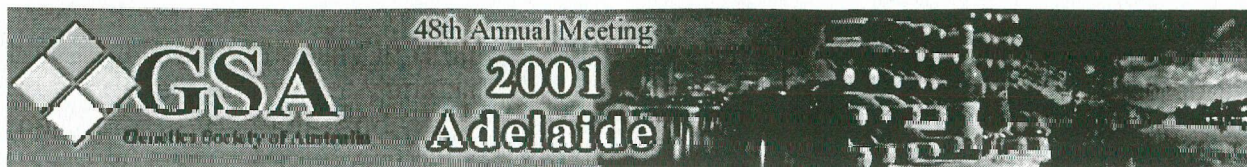
Limitation in the use of archival plasma-derived DNA for whole genome analysis

S. Latham, A. Morley and D. Turner

Department of Haematology & Genetic Pathology, School of Medicine, Flinders University, GPO Box 2100, Adelaide 5001

We have investigated using archival frozen plasma samples as a source of DNA for multi-locus genotyping following whole genome amplification (WGA). The rationale for the study was the availability of plasma samples from a large cohort ($N > 9000$), long follow-up (> 7 years), multi-centre drug trial (LIPID study). The cohort has the potential to provide data on genetic and pharmacogenetic risk factors for both primary and secondary cardiac events. Trace amounts of DNA exist in normal plasma and recovery of DNA from frozen plasma was optimised. WGA was achieved using DOP-PCR and PEP-PCR. Using purified leukocyte DNA the efficiency of WGA was determined by measuring the ratio of amplifiable *ras* alleles following WGA (determined by FRET real-time PCR) to the number of target *ras* alleles in the pre-WGA sample determined by OD₂₆₀. There was a highly significant inverse correlation between input targets and post-WGA yield. The same inverse correlation was observed following WGA of 18 plasma DNA samples. The number of amplifiable genomes present in DNA recovered from plasma, assessed by FRET analysis, was poorly correlated with total DNA, determined by SYBR Green 1™, indicating that plasma DNA is generally degraded. The range of amplifiable *ras* sequence (113bp) was 2%-53% (median 5%) in plasma DNA. In peripheral leukocyte DNA 50% of these sequences are typically amplifiable. For WGA to be useful in genotyping, alleles must be recovered qualitatively and quantitatively. We assessed the recovery of a complex tetranucleotide polymorphic microsatellite locus adjacent to the Ku80 gene. No allele drop-out or microsatellite length artefacts were observed following PCR of this locus using leukocyte DNA above 20 pg. By contrast both allele loss and length artefacts were observed following specific locus PCR using post-WGA DNA as the target. This was observed irrespective of the initial mass of plasma derived DNA in the WGA reaction (range 500 pg - <10 pg).

We conclude that archival plasma derived DNA is commonly sufficiently degraded that allele loss occurs post-WGA and that the combination of degenerate primers and illegitimate annealing of microsatellite sequences facilitates the production of artefactual alleles. We believe it is unsafe to use plasma derived DNA in WGA for genotyping in association studies. More generally our results provide a caution against the use of WGA of microsatellite loci for forensic purposes.



Genetic polymorphisms in methadone drug targets: their association with inter-individual variability in methadone response

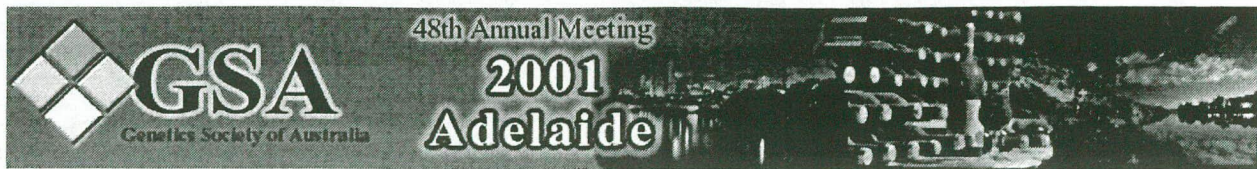
Belinda J. Luciani^{1,2}, Andrew A. Somogyi¹, Timothy C. Cox^{2,3}

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² Department of Molecular Biosciences & ARC Special Research Centre for the Molecular Genetics of Development, University of Adelaide, Adelaide, Australia.

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Inter-individual variability in drug response poses many problems both clinically and in drug development. Variability in methadone response amongst methadone maintenance patients and patients undergoing methadone treatment for chronic pain has been known for several years. Small changes in methadone plasma concentrations have dramatic effects on methadone pharmacodynamics (Dyer et al., J Clin Psychopharmacol 21: 78-84 (2001)) thus factors other than pharmacokinetics contribute to varied methadone response. Evidence suggests that variation in genes that are involved in opioid action such as *OPRM1* which encodes the μ opioid receptor may contribute to this variation in response (Hoeche et al., Hum Mol Genet 9: 2895-2908 (2000)). Variations in *ARRB2*, which encodes β -Arrestin 2 (a protein that is involved in μ receptor trafficking) and *OBCAM*, which encodes opioid-binding cell adhesion molecule (a protein that regulates the μ receptor) and their contribution to varied methadone response have not been investigated to date. Consequently, we will be investigating polymorphisms in *ARRB2*, *OBCAM* as well as *OPRM1* in methadone maintenance patients whose opioid withdrawal symptoms are or are not alleviated with methadone treatment and cancer patients who do or do not experience the analgesic affects of methadone in the hope of identifying an association between them and variation in methadone response.



Are Colour Morphs of Corals Genetically Distinct?

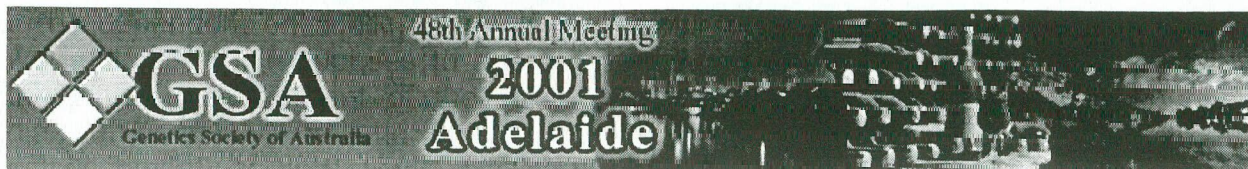
Jason B. MacKenzie^{1,2}, Madeleine van Oppen^{1,2}, Bette Willis¹, David J. Miller².

¹ Marine Biology, James Cook University,

² Biochemistry and Molecular Biology, James Cook University

Modern coral-reef fish assemblages show coordinated morphological diversification in the early Tertiary, suggesting a link between ecology and evolution on coral reefs. In many scleractinian corals (e.g., *Acropora* sp.), morphological characters are generally unreliable indicators of genetic relationships. Alternatively, coral colour is known to influence certain functional relationships, including zooxanthellae fitness and resident fish composition. Recent manipulative experiments demonstrate that selective recruitment of competing species of obligate coral dwelling gobies (*Gobiodon* sp.) on *Acropora nasuta* is highly correlated with coral branch-tip colour. The aim of this study is to establish whether colour morphs of *A. nasuta* represent genetically distinct units.

Genetic variability was investigated in two sympatric colour morphs of *Acropora nasuta* ("brown" vs "blue-tip") across both cross-shelf and latitudinal comparisons of the Great Barrier Reef. We introduce several new population-level DNA markers suitable for molecular ecology studies of the genus *Acropora*, including both microsatellites and single copy nuclear introns. Preliminary results show that at least one locus exhibits significant genetic differentiation between colour morphs. Concordance for divergence in *Acropora nasuta* is discussed from two scales: 1) the patterns of genetic differentiation across loci, and 2) the relative agreement of ecological versus genetic criteria.



The Evolution of Insecticide Resistance. Chance, Selection and Response

John A. McKenzie, Megan Scott, Kylie Diwell

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Melbourne 3010*

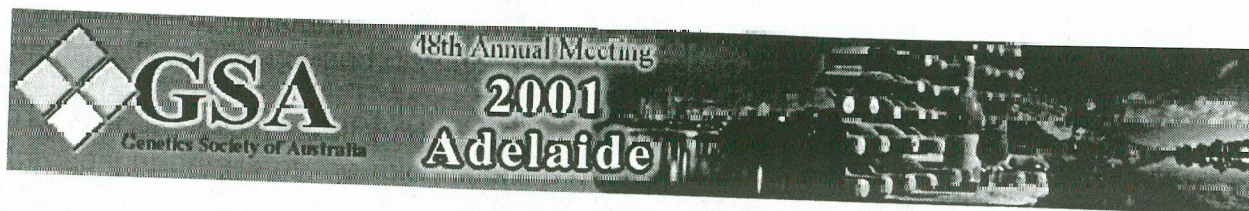
The influences of random genetic drift and selection by the insecticides dieldrin and diazinon on the initial establishment of the resistant allele (*Rdl* or *Rop1*) and subsequent allelic frequency changes within discrete generation population cages of *Lucilia cuprina* were investigated. The probability of the initial establishment of the *Rdl* allele was found to be dependent on allelic frequency and dieldrin concentration. Once the *Rdl* allele was established, responses to selection were concentration-dependent. Conversely, the initial establishment of the *Rop1* allele was independent of allelic frequency, diazinon-concentration and fitness modifier status. However, subsequent to the establishment of the *Rop1* allele, the response to selection was once more concentration-dependent. Responses were influenced by modifier status, particularly at lower concentrations where the resistant phenotype was not at a selective disadvantage in the presence of the modifier. Computer simulations, using fitness sets generated from the population cages, confirmed that the evolution of insecticide resistance is influenced by initial allelic frequency, population size and selection intensity.

Impact of impaired Hsp90 on bristle and wing asymmetry in *Drosophila melanogaster*

Claire C. Milton¹, John A. McKenzie¹, Richard E. Woods², Suzanne L. Rutherford³ and Philip Batterham¹

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Developmental stability, the ability of an organism to buffer disruptions that occur during development, can be measured with bristle asymmetry. The more symmetrical an organism is, the more developmentally stable it is. Mutations in several *Drosophila melanogaster* bristle developmental genes result in an increase in asymmetry for the thoracic bristles. *Hsp83* encodes the heat-shock protein Hsp90, a molecular chaperone whose targets are mainly signal transducers. Hsp90 stabilises these signalling proteins until they are activated through signal transduction. Mutations in *Hsp83* result in a variety of leg, wing, bristle and eye deformities in heterozygotes. Hsp90 is thought to buffer silent genetic variation that affects developmental signalling pathways. When Hsp90 function is impaired this variation is uncovered; if enough variation is expressed the phenotype of the fly is altered. We have shown that mutations in *Hsp83* result in increased asymmetry for the thoracic and vibrissa & carnia bristles. When *Hsp83* mutations are crossed into a standard background, asymmetry for the thoracic bristles increases. Background-dependent bristle asymmetry is observed when these introgressed *Hsp83* mutants are crossed to different genetic backgrounds. When a specific Hsp90 inhibitor is fed to wildtype larvae, the resulting adults exhibit increased thoracic bristle asymmetry. These results indicate that a gene not specifically involved in bristle development has an effect on bristle asymmetry. Measurements of wing asymmetry have been taken for the *Hsp83* mutants and these will also be discussed.



Microsatellite And Microarray Services Provided By The Australian Genome Research Facility

Mitchelson AJ¹, O'Keefe M¹, Ewen-White KR¹, Myers GSA¹, Dubé LM¹, Everest MA¹, Foote SJ^{1,2}, Barlow JW¹.

¹Australian Genome Research Facility and ²Walter and Eliza Hall Institute, PO Royal Melbourne Hospital, Vic. 3050.

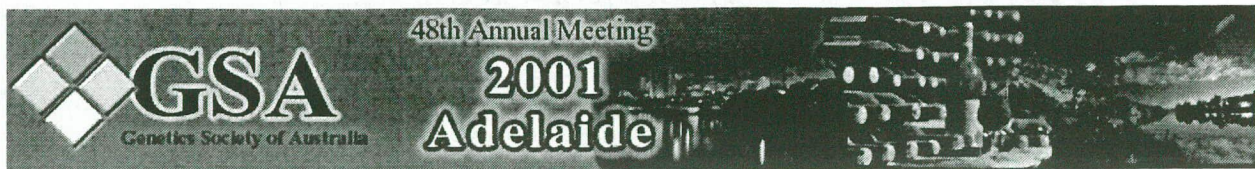
The Australian Genome Research Facility (AGRF) was established by the Federal Government as a Major National Research Facility to provide high-throughput genetic analysis at cost to scientific communities in the Australian and Asia/Pacific regions. The Facility is a resource for sequencing, genotyping, mutation detection, library amplification, storage and distribution and microarrays.

The Facility was established in 1998 and we now report the output parameters and throughput details of the microsatellite analysis service, library storage and distribution and microarray service. Sample processing and accurate handling of large sample numbers is facilitated by a purpose built robotic workstation that consists of: refrigerator, pipetting station, plate sealer, multi-block thermal cyclers, reagent dispensers, independent heating blocks, plate piercer, magnetic settler, automatic plate washer and storage tower. It processes 96- and 384-well plates that are transferred with a xyz robotic arm fitted with a plate gripper. The robotics is controlled by a PC using Windows NT and Polara Robotics software. It currently processes in excess of 18,000 reactions per day for microsatellite analysis and library amplification. Analysis of PCR products is performed exclusively using a bank of eight ABI 377 automated sequencers.

Microsatellite genotyping of human DNA has included 5cM or 10cM genome wide scans for endometriosis, hypertension, schizophrenia, inflammatory bowel disease, pseudohyperaldosteronism and multiple sclerosis. Genotyping of a large variety of non-human mammals and plants has also been performed. In excess of 2 million genotypes have been called since operations began.

Library services include human, mouse and bovine BAC libraries and human, mouse and yeast cDNA libraries. Clones from the BAC libraries are distributed in culture and the cDNA libraries as PCR products. The cDNAs are also printed using a Virtek SDDC2 DNA arraying robot onto microscope slides and distributed as prepared microarrays. Microarray quality is assessed using a Genomic Solutions GeneTAC LS IV four-colour laser microarray reader.

Summary: The AGRF (www.agrf.org.au) is a Major National Research Facility that has been successfully established specifically to provide genomics resources economically to the Australian and Asia-Pacific scientific communities in the form of DNA sequencing, microsatellite analysis, mutation detection, library distribution and microarray production.



Gene Flow is not Dispersal: lessons from Penguins.

Neil D. Murray¹, David M Lambert².

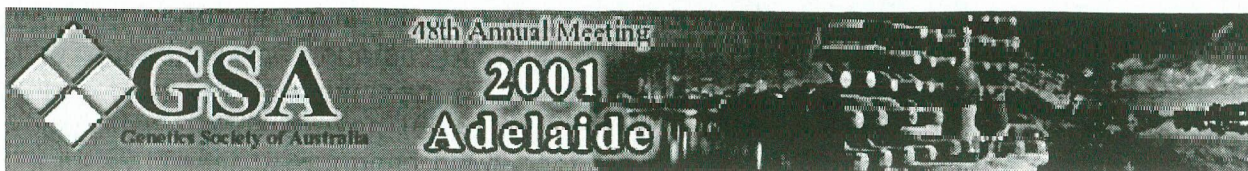
¹ Dept. of Genetics, La Trobe University ² Institute of Molecular Biosciences, Massey University, N. Z..

Models of population structure based on populations of size N , with dispersal (m) amongst them, have revealed the importance of Gene Flow (Nm) in determining the amount of spatial homogeneity in allele frequencies. This relationship has been widely used in ecological contexts where direct field estimates of dispersal are difficult, impossible or expensive. This is commonly the case in marine species, and such estimates are a routine input to Fisheries management. In spite of well-known limitations in their interpretation (historical vs. contemporary processes, model-dependence), the availability of computer packages and data-sets has led to a proliferation of Nm estimates in the literature.

Here we explore estimates of Gene Flow based on our study of microsatellite variation in Adelie Penguins (Amy D. Roeder et al., *Mol. Ecol. In press*). Populations from around the Antarctic continent showed surprising genetic homogeneity in spite of an apparent population structure and low dispersal rate that should pre-dispose to genetic structuring. Estimates of Nm ranged from infinity (FST and RST methods) to 9 (Private allele method). Genotypic assignment tests were also ineffective.

Reasons for this incongruence may lie in ecological uncertainty or in limitations to genetic inference. It is argued that a likely limitation here, and in some other marine species, is that when N is very large Nm is insensitive to variation in m , at least within the errors associated with usual sample sizes. Moreover, it will be argued that ecological circumstances where N and m are inversely related can be easily envisaged, and further reduce the usefulness of gene flow estimates as sensitive analogues of dispersal.

Given that existing methods are used extensively in population management, the development of new approaches in this area of ecological genetics is a practical necessity.

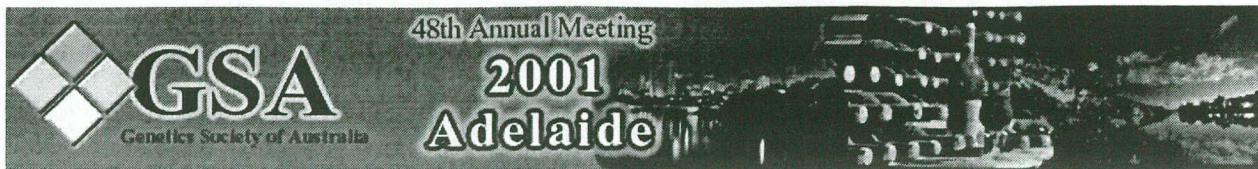


Radiations of genes within fruit tree genomes: comparing apples with apples

Richard Newcomb, Ross Crowhurst, Erik Rikkerink, Bart Janssen, Kimberley Snowden, Lesley Beuning,
Elspeth Macrae, Gavin Ross

*HortResearch, Private Bag 92169 Auckland, New Zealand.
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With the sequence of the Arabidopsis genome complete and partially annotated, there is now an opportunity to compare the radiation of genes within each gene family among different plant species. Are many of the radiations of genes within Arabidopsis typical for plant genomes or is the Arabidopsis genome exceptional? To examine this and other questions, the genomes of *Malus domestica* (apple), *Actinidia* sp. (kiwifruit) and to a lesser extent *Vaccinium* sp. (blueberry) are being sampled for genes using an EST approach. To date over 100,000 ESTs have been sequenced from over 40 cDNA libraries. These sequences have been analyzed in a high throughput bioinformatics analysis system that stores the sequence together with similarity and motif search reports. This data warehouse can then be interrogated through a web portal system using a number of features including keywords, sequence ID numbers, sequences (blast), enzyme hierarchies and motifs. A PCR-based method has also been used in a targeted approach to identify additional candidate resistance genes. Families of genes putatively encoding enzymes, transcription factors and disease resistance genes have been annotated and used to construct phylogenetic trees to compare relationships between genes. This talk will present results from some of these analyses to reveal contrasting patterns of gene radiations within plants.



Red queens in honeybee colonies: How parasites could drive the evolution of extreme multiple mating

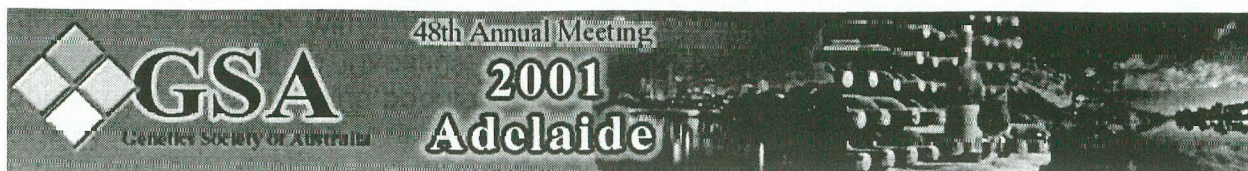
Kellie Palmer, Ben Oldroyd

School of Biological Sciences, University of Sydney, NSW 2006, Australia

Multiple mating (polyandry) by social insect queens is uncommon and unexpected because it lowers intra-colonial relatedness. A rare exception is the honey bee in which queens of some species mate over 100 times (!) on 2-3 brief mating flights. Explanations for the evolution this seemingly mal-adaptive behaviour have been frequently speculated. One hypothesis, often cited as a leading explanation, is that parasite and pathogen pressure selects for multiple mating. That is, a genetically variable worker population is thought to be less likely to succumb to disease than a homogeneous one.

In over a decade since its proposition, this hypothesis has had no experimental support. I tested two essential assumptions of the model. First, I show that there is widespread genetic variance for susceptibility to disease in honeybee populations, and that this is manifest by intra-colonial variance in disease incidence. Second, I show rates of transmission of disease among individual bees is associated with relatedness. Disease spreads more rapidly among groups of related bees than among unrelated bees.

My results give substantial support to the parasite hypothesis, and indicate that queens can increase their fitness by multiple mating, as this can reduce the incidence of diseases among their brood.

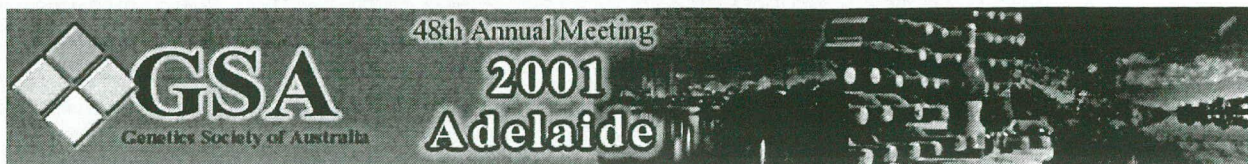


Mapping quantitative trait loci (QTL) affecting male courtship song in Hawaiian crickets

Yvonne Parsons & Kerry Shaw

¹ Dept Genetics, La Trobe University, VIC 3086, ² Dept Biology, University of Maryland, College Park, MD 20742 USA

Male crickets of the genus, *Laupala*, sing to attract females and this behaviour is believed to be important in mate recognition and the maintenance of species status [Shaw, *Evolution* 50, 256 (1996)]. Closely related species of *Laupala* that differ significantly in acoustic phenotype provide an ideal system for genetic analysis of ethological variation within the genus. We have followed a quantitative trait loci (QTL) mapping approach to investigate the genetic basis of this variation in an F2 hybrid population. The mapping population, comprising 72 individuals, was generated from the closely related species, *L. paranigra* and *L. kohalensis*, both of which are endemic to the big island of Hawaii and display species-specific songs. QTL analysis of song phenotype detected several loci from both species with some evidence of non-additive effects. The results will be discussed with reference to testing genomic predictions made by theories of speciation that focus on the evolution of mate recognition systems [Shaw & Parsons, *Amer. Nat.* in press (2001)].



Individual-based Simulation in Population Genetics: Past, Present and Future

Anthony Pietsch¹, Carolyn Leach¹, Steve Cooper², Rory Hope¹

¹ Department of Molecular Biosciences, Adelaide University; ² Evolutionary Biology Unit, South Australian Museum

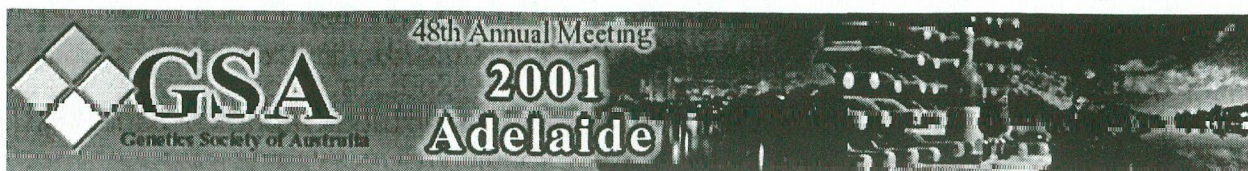
Population models based on the *explicit simulation of individuals* (known to ecologists as 'individual-based models') were first applied to genetical research in 1957. Such models have been used to investigate the response to selection, mutation accumulation, spatial structure and gene flow, speciation, the maintenance of genetic variation and a wide range of other issues.

Individual-based (I-B) models are composed of an integrated hierarchy of computer algorithms, each mediating some elementary process in the biology of a simulated organism (e.g. mating, reproduction, inheritance, recombination, mortality, etc.). The ultimate interest, however, is in global patterns that emerge when these discrete elements are combined and manipulated.

In this paper, I discuss their use and argue that I-B models have been underutilized in modern genetics. Their application to the study of *ecology* started later, but it has steadily progressed and is now quite prominent. In *genetics*, on the other hand, the history of I-B modelling is very different. There was a rapid proliferation in the 1960s, but an initial wave of enthusiasm eventually gave way to disenchantment and rejection. Only over the last ten or fifteen years have such models slowly regained acceptance.

There are various reasons for this relative lack of progress. Individual-based modelling is paradigmatically different from the two main approaches in classical population genetics, lying somewhere in between theoretical algebraic techniques and empirical experimentation. Despite this, there has been remarkably little philosophical discussion and the area has not been reviewed for more than 30 years. In most cases, I-B techniques are not even given a distinguishing label. They are usually called 'Monte Carlo simulation', 'computer models' or the like – terms which do not distinguish them from other types of stochastic model. This ongoing identity crisis has in turn contributed to a lack of cross-fertilization between those using I-B models in different areas of research.

Notwithstanding some significant achievements already, the full potential of individual-based modelling in genetics is yet to be realized. In particular, I-B methods are the most promising means of bridging the considerable gulf that currently exists between the population sciences of genetics and ecology.

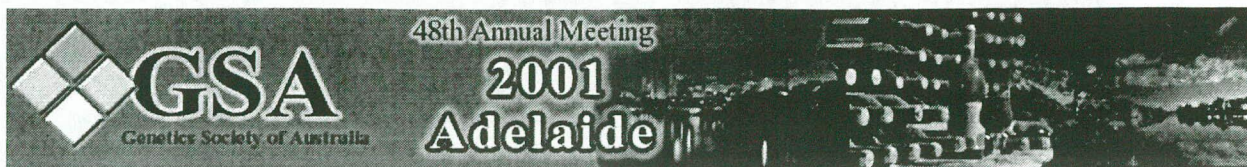


Physical map construction and transcript analysis of chromosome 16q24.3: A region of frequent loss of heterozygosity in sporadic breast cancer

Jason Powell^{1,4}, Gabriel Kremmidiotis^{1,2}, Scott Whitmore³, Chatri Settasatian¹, Joanna Crawford^{1,2}, Grant Sutherland^{1,2,4}, and David Callen^{1,4}.

¹ Centre for Medical Genetics, Department of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, South Australia, Australia, ² Department of Paediatrics, University of Adelaide South Australia, Australia, ³ Bionomics Ltd, ⁴ Department of Genetics, University of Adelaide, South Australia, Australia.

A breast cancer tumour suppressor gene has been localized to chromosome 16q24.3 by loss of heterozygosity (LOH) studies of breast tumour DNA. A detailed physical map has been constructed at 16q24.3, which encompasses this region of restricted LOH. This contig extends approximately 3 Mb from the telomere of 16q and consists of cosmids, BACs and PACs. The clones were aligned with *Not1* restriction and pulse field mapping. A minimum tiling path was identified and these clones were subsequently subjected to large scale sequence analysis. A total of 50 known genes and an additional 50 transcripts have been identified to date. Currently these genes are being analysed for their involvement in breast carcinogenesis.

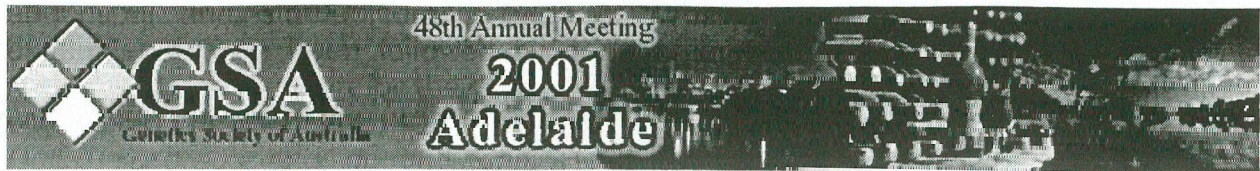


Diversification Of Heterologous DNA In *Neurospora*

J.P. Rasmussen¹, P.J. Yeadon¹, F.J. Bowring¹, E. Cambereri², E. Kato², W.D. Stuart² and D.E.A. Catcheside¹.

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Recombination hotspots active in meiosis provide a means of diversifying pairs of DNA sequences differing at multiple sites. We have constructed plasmids that permit targeted transfection of heterologous genes such that they are located between *his-3* and the *cog* hotspot in *Neurospora crassa*. This positioning enables enrichment for sequences which experience exchanges in the heterologous DNA during meiosis by selecting progeny that are recombinant at *his-3*. We have used this system to shuffle human immunoglobulin kappa chain sequences and also sequences from other fungi. Our system provides a novel method for accelerated evolution of genes in which new gene variants are expressed and the products secreted from cells without further manipulation.

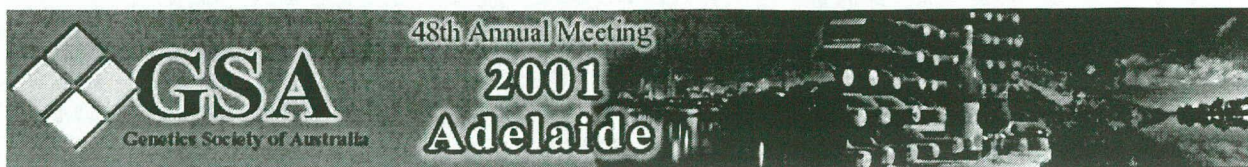


Dissecting a quantitative trait at nucleotide resolution

Charles Robin^{1,2}, Trudy Mackay³, Chuck Langley²

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The genetic component in variance of phenotypic traits is often dispersed among many loci. Although much research has been done on the statistical models for allele effects and interactions in polygenic traits, there are few systems where the actual nucleotide polymorphisms causing the genetic variation have been characterized as is done for monogenic traits. We have been studying naturally occurring variation in the number of abdominal and sternopleural bristles of *Drosophila melanogaster*. QTL mapping experiments have been followed by genetic complementation studies that implicate candidate genes known for their role in peripheral nervous system development (Long et al. 1996 Genetics 144:1497). These genes include *hairy*, *Notch*, *extramacrochaetae*, *Delta*, *scabrous* and *daughterless*. Here we describe the unusual distribution of nucleotide variation across the *hairy* gene and present an association study which identifies a complex insertion/deletion polymorphism that is strongly associated with bristle number variation. We also compare polymorphism to divergence in an attempt to gain some insights into the forces maintaining molecular variation at this locus in natural populations.

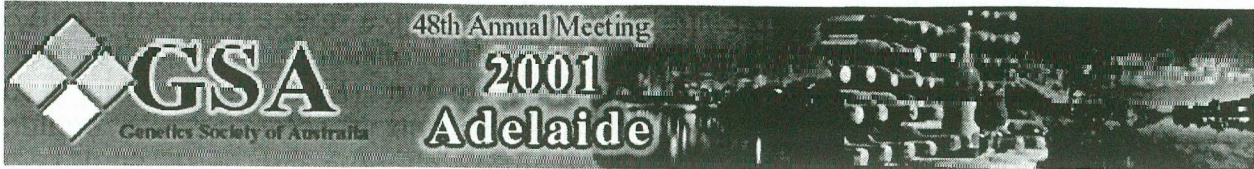


Analysis of Decapentaplegic (Dpp) and its signal transduction cascade in the cnidarian *Acropora millepora*

Gabrielle Samuel and Robert Saint

Centre for the Molecular Genetics of Development, Department of Molecular Biosciences,
Adelaide University

Members of the TGF β -superfamily of signalling molecules are widespread in metazoans, but the evolutionary roots of its particular subclasses are poorly defined. For example, it is well established that the DPP/BMP class is implicated in neural patterning, dorsal-ventral patterning and limb development, however, how and when these functions first arose is less understood. The primitive metazoan *Acropora millepora* provides an ideal system in which the origins of these pathways can be investigated. Here we show that *Acropora millepora* Dpp is a functional homologue of the *Drosophila melanogaster* Dpp. Further, we report the presence of several components of a DPP/BMP-specific signal transduction cascade, including a putative type I receptor and two putative receptor-activated Smads. These results indicate that DPP/BMP signalling predates limb development and raises the possibility that *dpp* plays a role in axis formation in simple non-bilateral animals.

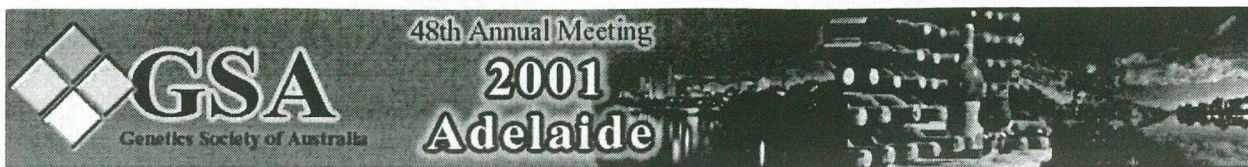


Colony genetic structure in the primitive Australian ant *Nothomyrmecia macrops*

Matthias Sanetra^{1,2}, Ross H. Crozier^{1,2}

¹ James Cook University, Department of Zoology and Tropical Ecology, Townsville 4811, QLD ² La Trobe University, Department of Genetics and Evolution, Bundoora 3083, VIC

The Australian endemic ant *Nothomyrmecia macrops* is considered a 'living-fossil' on the basis of morphological and behavioural evidence [Taylor, Science 201, 979-985 (1978)]. We investigated the genetic structure of colonies to determine possible deviations from a simple family structure. An average of 18.8 individuals from each of 32 colonies, and sperm extracted from 34 foraging queens, were genotyped using five highly variable microsatellite markers. We found that queens were typically singly (65%) or doubly mated (30%), but triple mating (5%) also occurred. The mean effective number of male mates for queens was 1.37, and nestmate workers were related by $b = 0.61 \pm 0.03$. Thus, the average relatedness level remains significantly above 0.5 where altruistic helping behaviour is favoured according to Hamilton's rule [Hamilton, J. Theor. Biol. 7, 1-52 (1964)]. We can therefore conclude that facultative polyandry has not been a general constraint in the early evolution of ant eusociality. Furthermore, we found evidence that the single queen can be replaced by one of her sexual daughters. Through a continuous process of new queen recruitment colonies may become potentially immortal. Intra-colony relatedness is affected such that workers raise nieces and nephews ($r = 0.375$) after queen replacement. Workers can produce males from unfertilized eggs in the absence of a queen. Readoption of closely related queens is an interesting alternative reproductive strategy, which has been previously unknown in *Nothomyrmecia* and in primitive ants in general.



Two distinct reproductive strategies are correlated with an ovarian phenotype in co-existing parthenogenetic strains of a parasitic wasp

Otto Schmidt¹, Markus Beck², Annette Reinecke³, Ulrich Theopold⁴

¹ Department of Applied and Molecular Ecology, Adelaide University, Glen Osmond, South Australia ² Department of Entomology, University of Wisconsin, Madison, WI (USA) ³ University of Hohenheim, Germany ⁴ Stockholm University, Stockholm, Sweden

The question whether different organisms are able to compete for the same resource is of fundamental importance to evolutionary biology. Sympatric co-existence of similar species on a single resource has long been claimed to be unstable. However, indirect evidence suggests that parasitic wasps exhibit evolutionarily stable mixtures of life-history strategies (van Alphen and Visser (1990) *Annu. Rev. Entomol.* **35**, 59-79). Here we describe genetically distinct strains of a parthenogenetic wasp *Venturia canescens* (Hellers et al., *Insect Mol. Biol.* **5**, 239-249) with different ovarian phenotypes that affect egg numbers in oviducts (Beck et al., *Insect Biochem. Mol. Biol.* **29**, 453-460). Wasp females with large egg load search for caterpillars and deposit eggs immediately after host encounter, whereas females with fewer eggs delay parasitism. Since the outcome of interlarval competition within super-parasitised caterpillars depends on the age distribution of competing larvae (Marris and Casperd, (1996) *Behavior. Ecol. & Sociobiol.* **39**, 61-69), the two egg deposition strategies may co-exist under conditions that favour superparasitism.

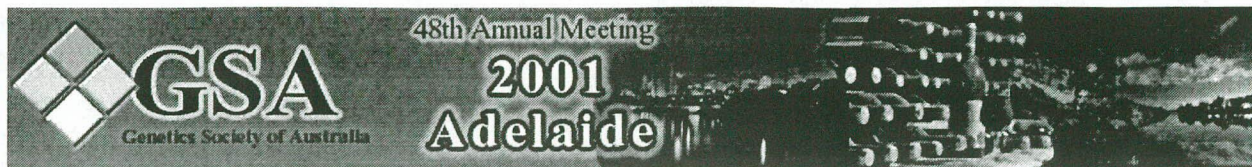


Gene flow, dispersal, and nested clade analysis among populations of the stonefly *Peltoperla tarteri* in the southern Appalachians, Virginia, USA

Alicia S. Schultheis¹, Lee A. Weigt², and Albert C. Hendricks³

¹ Australian School of Environmental Studies, Griffith University, Nathan, QLD 4111, Australia, ² Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA 24061-0477, ³ Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA, 24061-0406

We examined gene flow, dispersal and phylogeography among populations of the stonefly *Peltoperla tarteri* (Plecoptera: Peltoperlidae) in the Southern Appalachians. A 454 bp portion of the mitochondrial control region was sequenced from a minimum of 20 individuals from eight populations. The extent and likely mechanism for dispersal was determined by comparing levels of population genetic differentiation within drainages to that among drainages. There was no consistent relationship between genetic and geographic distance; and genetic differentiation was high within drainages ($\Phi_{ST} = 0.10$, $p < 0.001$), but not among drainages ($\Phi_{DT} = 0.07$, NS). Nested clade analysis indicates that historical range expansion coupled with contemporary gene flow explains the present day pattern of genetic variation in *P. tarteri*. In order for historical processes to have such a strong influence on present day genetic structure, both larval and adult dispersal must be restricted. However, our genetic data suggest that larval dispersal is more frequent than adult dispersal.

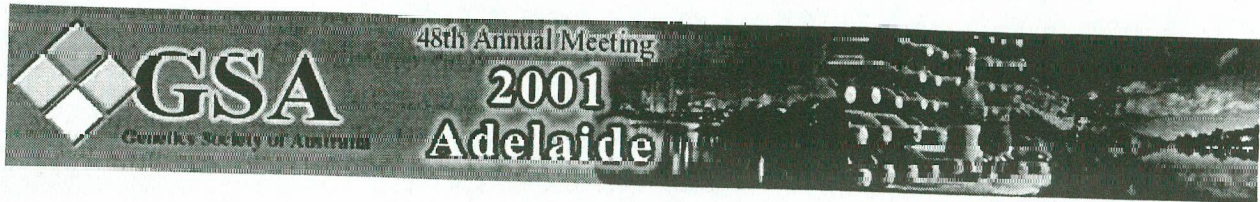


***Drosophila* gene *dead ringer* is essential for embryonic gliogenesis.**

Tetyana Shandala, Robert Saint

Centre for the Molecular Genetics of Development, Adelaide University

Development of longitudinal glia (LG), which are essential components of the central nervous system, is controlled by a genetic regulatory hierarchy. *Drosophila* embryonic LG differentiation is initiated by the Glial cells missing (Gcm) transcription factor, which subsequently activates its downstream targets reversed polarity (repo), pointed (pnt) and locomotion defects (loco). We report here the involvement of the *Drosophila* gene, *dead ringer* (dri), in development of functional LG cells. DRI (an ARID domain DNA binding protein) is expressed in LG cells downstream of gcm. Embryos homozygous for an amorphic dri allele exhibit axon defasciculation, indicating a role for dri in longitudinal glial cell function. Enhancement of axon defasciculation in dri, pnt double mutants suggests a level of redundancy in the genetic control of LG function.

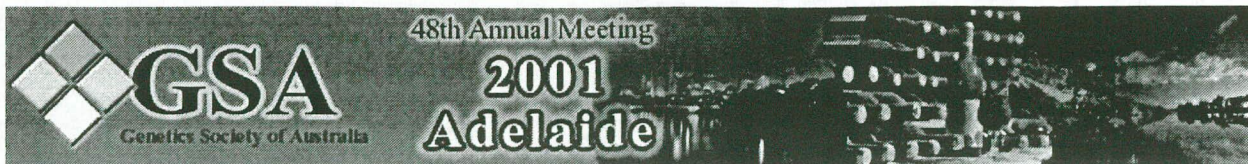


Towards biological control of tephritid fruit fly pests – genetic transformation with sex-specific genes

Deborah C. A. Shearman¹, Susan D. McCombs², Alfred M. Handler³ and Marianne Frommer¹.

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Biological control measures, for example, the sterile insect technique (SIT), are being investigated as a future means of control of fruit flies. Ideally, the SIT release strains should consist of males only, the females having been eliminated from the brood early in development. Candidate genes for the production of such "genetic-sexing" strains are those genes which are expressed in a sex-specific manner, such as those of the sex determination pathway, or genes involved in reproduction, such as *Yolk protein* genes. The *doublesex* (*dsx*) gene in *D. melanogaster* is the last gene in the somatic sexual differentiation pathway and the first bifunctional gene: both male- and female-specific protein products are produced by differential splicing of the *dsx* pre-mRNA. Female-specific splicing of *dsx* pre-mRNA is activated by the binding of the *transformer* (*tra*) and *transformer-2* (*tra-2*) gene products to regulatory elements within the *dsx* repeat element (*dsxRE*), which lies within the non-coding region of exon 4. Male-specific splicing is the default process, as a functional male-specific DSX is produced in the absence of functional *tra* gene product. A homologue of the *dsx* gene is present and expressed in a sex-specific manner in *Bactrocera tryoni* (Shearman and Frommer, 1998) and by using this data as well as data from *D. melanogaster* homologues of the *dsx* gene were sought in a number of tephritid species. Portions of *dsx* have been cloned from three other tephritid insect species. Analysis of the 3' non-coding region of the putative female transcripts from all four tephritid species has identified elements homologous to those regulatory elements found in *dsxRE* element in exon 4 of the *dsx* gene of *D. melanogaster*. The DSX proteins have been shown to differentially regulate the expression of the *Yolk protein* (*Yp*) genes in the fat body of adult *D. melanogaster* flies. The sex-specific expression of the *Yp* genes (*Yp1* and *Yp2*) also makes these good candidate genes for the production of single-sex SIT strains. Homologues of these genes and the associated control regions have also been sought in tephritid species and portions of each of these genes have been cloned and sequenced.



The X-linked Opitz Syndrome protein tethers the rapamycin-sensitive regulatory subunit, Alpha 4, to microtubules and identifies Alpha 4 as a candidate gene for FG syndrome.

Kieran M. Short¹, Blair Hopwood¹, Julie Zou¹ and Timothy C. Cox^{1,2}

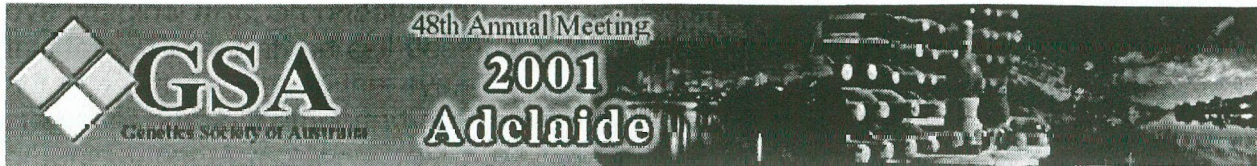
¹ Department of Molecular Biosciences and ARC Special Research Centre for the Molecular Genetics of Development, University of Adelaide, North Terrace, Adelaide 5005.

² South Australian Clinical Genetics Service, Women's & Children's Hospital, North Adelaide, South Australia, Australia 5006

The clinical phenotype of patients with Opitz Syndrome (OS) is highly variable but includes facial dysmorphism, clefts of the lip, palate, larynx, trachea and oesophagus, abnormalities of cardiac septation and defects of the anus and genitalia. Intriguingly, there are both X-linked and autosomal forms of the disorder, with the primary autosomal OS locus mapping to 22q11.2, the same interval deleted in the CATCH22 group of disorders that collectively represent one of the most common genetic causes of birth defects.

Our group has cloned the X-linked OS gene, called *MID1*, whose protein product contains multiple protein-protein interaction motifs. In support of a role for these motifs, *MID1* has been shown, both *in vitro* and *in vivo*, to form part of large multi-protein complexes that co-localise with tubulin. To date, all characterised OS mutations have been shown to disrupt this normal association with the microtubule network.

We have now identified one of the factors with which *MID1* interacts as Alpha 4, a regulatory subunit of protein phosphatase 2A. The binding of Alpha 4 to PP2A is known to be sensitive to the macrocyclic immunosuppressive antibiotic, rapamycin, and controls a variety of processes including cell cycle progression. Interestingly, the Alpha 4 gene is located at Xq13 within the region defined for FG syndrome, a disorder showing some clinical overlap with that of OS. Alpha 4 therefore presents as an excellent candidate for the underlying cause of FG syndrome or related malformations. The possibility that perturbed Alpha4 function underlies the complex OS phenotype is also discussed.



Mutations in *lozenge* (*lz*) permit ectopic patterned cell death in the developing *Drosophila* eye.

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¹Dept. of Genetics, University of Melbourne, Parkville, Victoria 3052;

²Dept. of Biological Sciences, Carnegie Mellon University, Pittsburgh, P.A

The adult compound eye of *Drosophila* is composed of a highly structured array of cells organised into approximately 800 hexagonal facets, or ommatidia. Each of these contains 8 photoreceptor neurons, plus a collection of supporting cells that include cone and pigment cells and a mechanosensory hair nerve group. The eye develops from the eye imaginal disc, an unpatterned epithelial cell sheet set aside early in development for this fate. Programmed cell death is a key component of the normal development of the adult visual system in the eye. Very low levels of cell death occur during the early stages of normal compound eye development in larvae. This death does not exhibit an organised pattern.

The *lozenge* (*lz*) gene encodes a transcription factor of the Runt/AML1 (Acute Myeloid Leukemia) family. *lz* mutants exhibit defects in the differentiation of several cell types in the eye, suggesting that *Lz* is a key transcription factor regulating genes involved in cell specification. However, *lz* mutants also exhibit an elevated level of patterned cell death in 3rd instar larval eye imaginal discs. Evidence indicating that mutations in *lz* directly cause cell death will be presented. We propose that *Lz* functions to repress the cell death pathway during larval eye development.

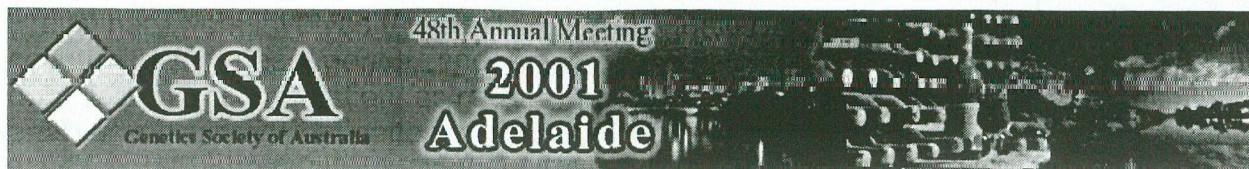


An investigation of a later developmental role for the *Drosophila melanogaster* gene *pebble*

Masha Smallhorn¹ and Robert Saint¹

¹ Centre for the Molecular Genetics of Development, Adelaide University

In *Drosophila melanogaster*, the Rho family of proteins and their regulators have been found to play a role in numerous developmental processes such as oogenesis, dorsal closure, cellularisation and neurogenesis. *pebble* (*pbl*), a putative Rho guanine nucleotide exchange factor (RhoGEF), is known to be required for cytokinesis. Early cytokinetic defects prevent an examination of any later developmental roles for *pbl*. We are taking a number of different experimental approaches to addressing this possibility. In one approach, we are comparing *string* (*stg*) mutant embryos with *stg/pbl* double mutant embryos. Cells in *stg* mutant embryos arrest at G2 phase of cycle 14 and never progress into the division in which the *pbl* mutant phenotype is first evident. If *pbl* is playing a non-cytokinetic role, it may manifest itself by differences in the phenotypes of the single and double mutant embryos. A second approach involves the expression of dominant negative forms of Pbl in the nervous system later in development, to see if any phenotypes arise. The third approach involves the use of RNA interference to lower or eliminate any *pbl* transcripts in cells later in development. Progress using these various approaches will be presented.

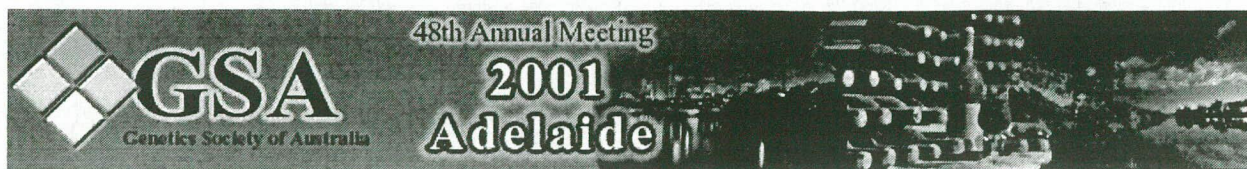


Preliminary analysis of members of the RGK family of G-proteins in *Drosophila melanogaster*

Peter Smibert and Robert Saint

Centre for the Molecular Genetics of Development, Department of Molecular Biosciences, Adelaide University

We report the identification and preliminary analysis of three potential G-proteins of the RGK family. CG15069, CG9811 and a fusion of predicted genes CG15663 and CG15664 were identified through database analysis of the *Drosophila* genome focusing on the highly conserved COOH-terminus of RGK members in other systems. Preliminary work involves the creation of transgenic *Drosophila* lines expressing dsRNAi constructs for these genes, and the production of constructs for gene targeting by homologous recombination, as recently described by Rong and Golic, 2000. Preliminary analysis of expression patterns of these genes through *in situ* hybridisation is under way. A full length cDNA clone of CG9811 has been obtained and overexpression studies are also currently in progress.



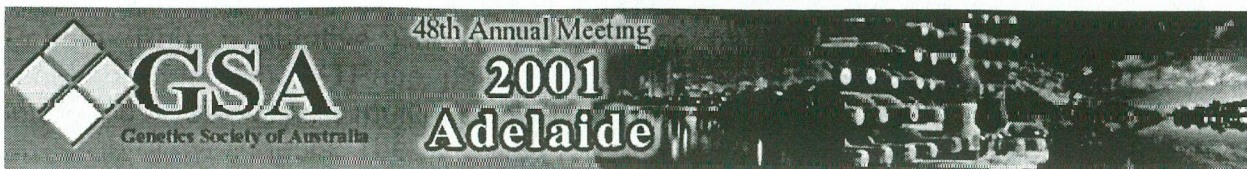
Control Of Axillary Branching In Petunia

Snowden K.C.¹, Janssen B.J.¹, Napoli C.A.²

¹ HortResearch, Private Bag 92169, Auckland, New Zealand. ² Dept. Plant Sciences, University of Arizona, Tucson, AZ 85721 USA.

A mutation approach has been used to study the genetic control of axillary branch development in *Petunia hybrida*. Mutations at three *Dad* (*Decreased apical dominance*) loci act primarily to increase the growth of axillary meristems into branches during vegetative development. This leads to a highly branched phenotype for *dad1* and *dad2* mutants and a moderately branched phenotype for *dad3* [Napoli and Ruehle, J. Hered. 87: 371-377 (1996), Napoli et al., Curr. Top. Dev. Biol. 44: 127-169 (1999)]. Graft analyses of the *dad* mutants with wild-type plants suggest that a factor that promotes branching is produced in *dad1* and *dad3* mutant roots and is transported to the shoot system [Napoli, Plant Physiol. 111: 27-37 (1996)]. Wild-type rootstocks are sufficient to restore near wild-type branching to *dad1* and *dad3* scions, but not to *dad2* scions. Growth of mutant *dad1* roots above the graft union results in branch development. *dad* mutant rootstock however, does not affect the branching of wild-type scions. To analyse possible interactions between the *Dad1* and *Dad3* gene products, the double mutant was isolated. Additional graft analyses have been performed between *dad1* and *dad3*, and results from this work will be discussed.

To continue the genetic analysis of the *dad1* mutation, mutant seeds were mutagenised and M2 seeds screened for a new mutation that would suppress the highly branched phenotype of *dad1*. One such mutant, *Sbr* (for *Suppressor of branching*) was isolated. Analysis of the *Sbr* mutation will be presented, including graft analysis to determine whether *Sbr dad1* roots can change the branching phenotype of *dad1* scions.



Analysis of an interaction between DRacGAP and Pebble in *D. melanogaster*

Greg Somers and Robert Saint.

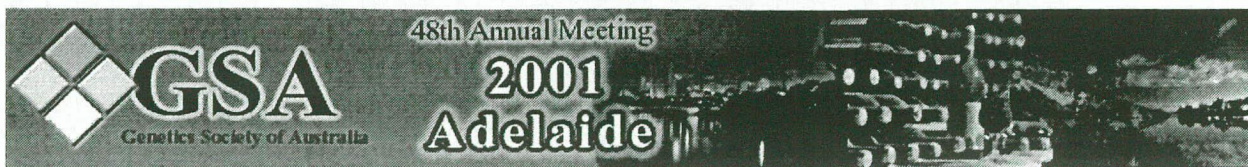
Center for the Molecular Genetics of Development, Department of Molecular Biosciences, Adelaide University.

During cell division, cytokinesis, the constriction of one cell to form two, is initiated late in mitosis. This constriction requires large scale reorganisation of the actin cytoskeleton and activation of non-muscle myosin motor activity. These events are controlled by a number of factors including the Rho family of small GTPases.

Pebble (Pbl), a putative Rho GTP exchange factor (RhoGEF), acts as a positive regulator of the Rho GTPase. pbl is required for cytokinesis. pbl mutants are embryonic lethal, possessing large multinucleate cells formed as a result of rounds of mitosis in the absence of cytokinesis. The activated form of Rho signals cytokinesis, possibly through downstream actin reorganisation effectors as Diaphanous and PI-3-kinase.

Along with tandem Dbl homology and Pleckstrin homology domains characteristic of RhoGEFs, Pbl also has tandem N-terminal BRCT domains. The function of these BRCT domains was explored by looking for protein interactors using a Yeast-2-Hybrid system. DRacGAP, a Rho family guanine nucleotide activating protein, was identified as a strong interactor.

Antibodies raised to DRacGAP reveal a dynamic subcellular localisation with striking similarities to the localisation of Pebble. We propose a model in which the Pebble-DRacGAP complex modifies the actin cytoskeletal network to promote cytokinesis.

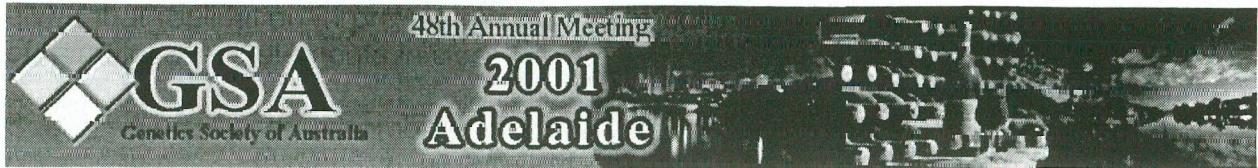


A gene by any other name...

S.C. Spargo¹, R.M. Hope¹.

¹*Department of Molecular Biosciences, Adelaide University.*

During the course of molecular biological research, genes and proteins have variously been named based upon function, molecular weight, homology to other proteins or sequences, interactions with other proteins or sequences, order of discovery and whim. While it could be argued that it is the right of the researcher to name genes and proteins as they see fit, this can lead to problems when gene families are considered, especially gene families conserved across a number of species. Two cases of conserved gene families are examined, the zona pellucida genes and the globin genes; in each case problems and confusions which arise as a result of the current nomenclature are identified. We suggest that, in general, the nomenclature system used for genes, like the system used for species, should be based on phylogeny, taking into account orthologous / paralogous relationships. We propose a clear, unified system of gene nomenclature and apply it to the two illustrative examples.



A test for sister-strand recombination induced by P elements in a ring chromosome of *Drosophila melanogaster*

John Sved, Xiumei Liang

School of Biological Sciences, University of Sydney, NSW 2006

P elements in *D. melanogaster* generate a low level of germ-line recombination. The level of recombination is substantially increased when the P element is present in homozygous rather than heterozygous condition. This led us to query whether there might be high levels of sister-strand recombination induced by P elements, especially given that replication in pre-meiotic germ-line cells is expected to generate the situation of homologous P elements in close proximity to each other.

Such sister-strand recombination is not easily detected in structurally normal chromosomes. However sister-strand recombination within a ring chromosome should lead to detectable consequences via the production of inviable di-centric ring chromosomes.

We used normal female recombination to introduce a P{CaSpeR} element onto a ring-X chromosome. Males from three lines produced by this procedure were backcrossed to normal and attached-X females. We scored for deviations in the sex ratio amongst progeny groups produced by males aged up to four weeks, but found little deviation from expectation. This experiment was somewhat complicated by the potential loss of P elements in aged males. We tested for this by repeating the experiment in a white background, but again with negative results.

We will discuss the significance of these results in the light of the Hybrid Element Insertion model for recombination.

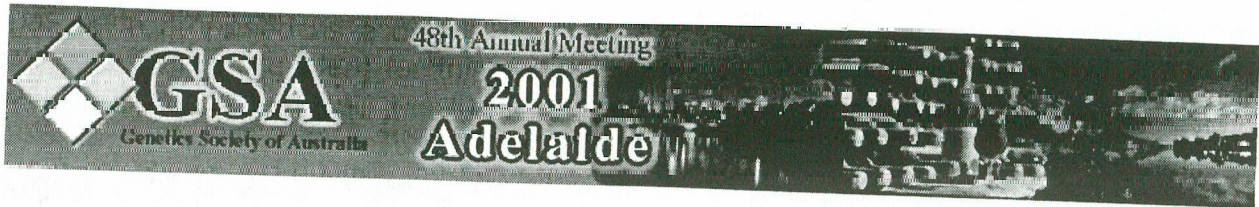


Reduction of somatic intrachromosomal recombination below spontaneous frequency in the pKZ1 recombination mutagenesis model in response to DNA damaging agents and a mismatch repair deficiency

P.J. Sykes, A.M. Hooker, D.R. Turner, B.D. McCallum and A.A. Morley.

Department of Haematology and Genetic Pathology, Flinders University and Medical Centre, Bedford Park, South Australia 5042.

Somatic intrachromosomal recombination (SICR) can lead to mutations such as deletions and inversions. The pKZ1 recombination mutagenesis model contains an *E.Coli lacZ* transgene in inverse orientation to a promoter-enhancer complex. If an inversion event occurs in the transgene then the *lacZ* gene is brought into correct transcriptional orientation and the *lacZ* gene product (β -galactosidase) can be detected in frozen tissues using a chromogenic substrate for the enzyme. Inversions can also be detected by PCR. The spontaneous frequency of SICR inversion events in spleen tissue is $1-2 \times 10^{-4}$. We have previously observed a significant induction in SICR inversions in response to a range of DNA damaging agents which have widely different genotoxic mechanisms. pKZ1 mice were exposed to a single intraperitoneal dose of etoposide, acetamide or crocidolite asbestos, or multiple whole body doses of 900 MHz radiofrequency (RF). Three days post-treatment the spleen (or liver mesothelium in the case of asbestos) was removed and tissues analysed for inversion events. A significant 1.4-3.1 fold induction of SICR was observed for the higher doses of etoposide (0.05, 0.5, and 50mg/kg) but SICR inversion frequencies after treatment with 0.0005 and 0.005 mg/kg etoposide decreased significantly to 0.67 and 0.42 of the levels observed in control animals respectively. A significant reduction below spontaneous frequency was also observed when animals were treated with 109 mg/ml acetamide (0.6X), 200 μ g/animal acute and chronic (3 months) exposure to crocidolite asbestos (0.1X) or exposed to 25 daily 30 minute exposures of 4W/kg RF (0.4X). We have also observed a reduction below spontaneous SICR frequency in spleen in pKZ1/*msh2*^{-/-} mice. This latter result suggests that *msh2* may play a role in recombination repair as well as mis-match repair. The high spontaneous frequency of SICR in spleen of pKZ1 mice may facilitate the detection of agents which reduce mutation frequency below spontaneous compared to other mutation assays. The significance of a reduction below spontaneous inversion frequency in treated animals is unclear. Given the known mutational effect of *msh2* deficiency and some of the DNA damaging agents above, it is unlikely that a reduction in SICR below spontaneous is anti-mutagenic.



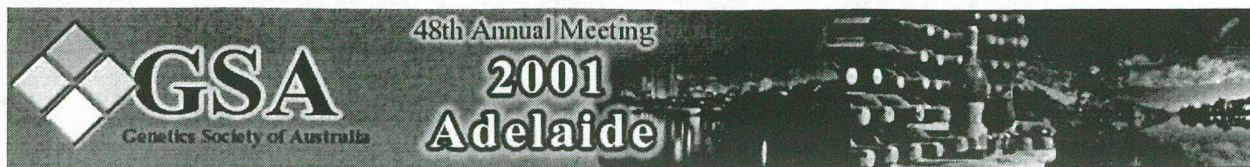
Genotypic and environmental influence on the asymmetry phenotype in *Arabidopsis thaliana*

A. Tan-Kristanto¹, R.E. Woods² and P. Batterham¹

^{1,2} CESAR - Centre for Environmental Stress and Adaptation Research,
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² Latrobe University, Bundoora, Victoria 3083

Asymmetry is used to measure developmental stability, defined as the ability of an organism to buffer disturbances that occur during development. Therefore asymmetry has been proposed as a useful biomonitoring tool for the detection of environmental stress. Plants are ideal for this purpose because they cannot avoid stress through behavioural modification.

Both genotypic and environmental factors can cause asymmetry. Many field studies fail to partition out the relative contributions of these factors to asymmetry. This laboratory-based study is using the model plant, *Arabidopsis thaliana*, to examine the impact of cadmium stress on asymmetry. A range of characters will be analysed in cadmium resistant and sensitive genotypes grown on a range of cadmium concentrations.

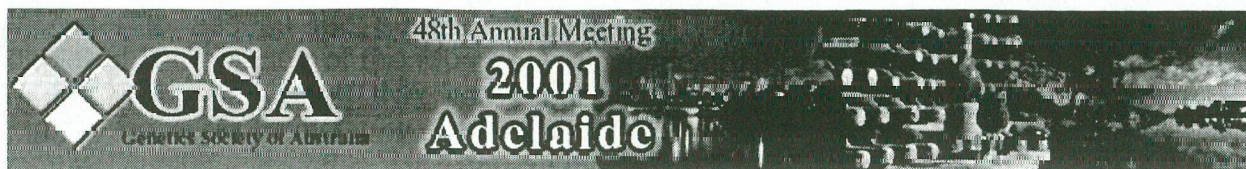


Desiccation and Starvation Resistance in *D. melanogaster*: Chromosome Location using Deficiency Kits

Marina Telonis, Melissa Carew and Ary Hoffmann

CESAR, La Trobe University Bundoora Victoria

Quantitative variation in *D. melanogaster* for desiccation and starvation resistance has been studied extensively, providing insights into the genetic mechanisms underlying these stress responses. However, it is still unclear which genes are responsible for the variation in these traits, and in this study we have sought to investigate relevant chromosomal areas using the deficiency mapping technique. The Bloomington deficiency kit is comprised of 194 stocks containing deficiencies, in total providing 75% coverage of the *Drosophila* genome. The kit contains some overlapping deficiencies that are especially useful for mapping potential stress response regions. We subjected 141 deficiency lines to desiccation and starvation stresses, and found significant variation among the lines for these traits. We observed only strains significantly more resistant than control lines, suggesting negative genetic control for resistance. Correlation analysis shows a positive association between the strains' performance in both stresses, a finding that concurs with previous studies on selected lines. Changes in the stress responses of the lines at different temperatures suggest substantial genotype-environment interactions. Several strains exhibited strong resistance to the stresses tested, and the chromosomal aberrations particular to these putative resistant strains will be discussed with regard to putative location of resistance genes.



Mitochondrial DNA as an informative marker for the conservation of Murray-Darling Basin (Australia) fish.

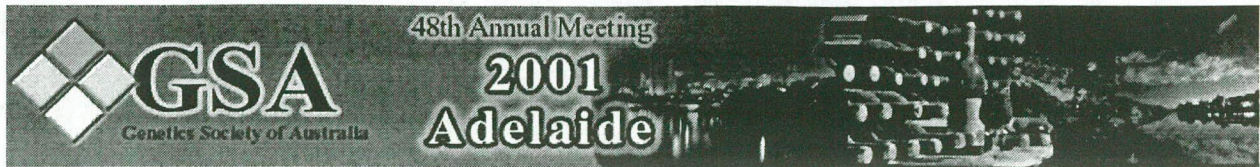
Tikel, D.¹ & Apte, S.¹.

¹Queensland Department of Primary Industries, Bribie Island Aquaculture Research Centre.

This work is part of the "Conservation Genetics for Murray Darling Fish Project", funded by the Natural Heritage Trust (NHT), Murray-Darling Fish Rehabilitation 2001 Program, administered by the Department of Agriculture Fisheries & Forestry of Australia.

In order to assess the genetic variability of captive and wild populations of three depleted fish species of the Murray-Darling Basin, approximately 450 bases the control region of mitochondrial DNA was sequenced in the silver perch (*Bidyanus bidyanus*), Murray cod (*Muccullochella peelii*) and trout cod (*Maccullochella macquariensis*). Outgroups including other members of the Australian Percichthyidae were sequenced to obtain an indication of among species variability for this gene, including: Mary River Cod, Golden Perch, Australian Bass, Black Fish, Cat Fish, Nile Perch, Barramundi, Welch's Grunter, and Macquarie Perch. The objective of the study will be the provision of baseline information to aid conservation and hatchery managers to make informed decisions on improving hatchery and restocking practices, and conservation management of freshwater fish in the Murray-Darling Basin.

We found that the control region is too variable for a convincing alignment between species, hence it is not useful for interspecific systematics. However, this makes control region sequences an effective species marker, and likely to be appropriate for determining population structure. As the sample sizes for each of the species of interest was too low from the pilot investigation to make any significant inferences regarding phylogeography, it does appear as if the control region will be appropriate as a gene marker to examine population structure. Preliminary impressions of intraspecific genetic diversity and population structure for these fish species, will be presented. The development of microsatellite markers are intentioned for this study. It is expected that the microsatellite markers will provide a higher resolution for the population structure and dynamics of the fish, and the future sampling regime for the microsatellite markers will be based on the phylogeography suggested via the mitochondrial DNA sequences.



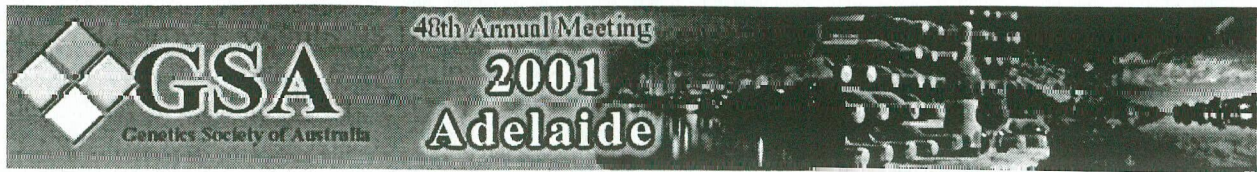
Transposition of chloroplast DNA to the nucleus

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²*CSIRO Plant Industry, GPO Box 1600, Canberra, ACT, Australia 2601*

Nuclear DNA with strong sequence similarity to chloroplast DNA is abundant in the genomes of many plant species [Ayliffe et al., *Mol. Evol. Biol.* 15, 738-745 (1998)] and this so called promiscuous DNA has been well characterised in a small number of cases. It is supposed that they originate by transposition of DNA from the chloroplast and integration into the nucleus. In tobacco these nuclear integrants appear to be particularly long and there is evidence that some transposition events are very recent [Ayliffe and Timmis, *Theor. Appl. Genet.* 85, 229-238 (1992)]. However, the frequency of transposition is unknown. It is now possible to insert genes into the chloroplast genome by biolistic transformation with vectors designed to integrate by homologous recombination [Zoubenco et al., *Nucl. Acids. Res.* 22, 3819-3824 (1994)]. Using the vector pPRV111A which encodes spectinomycin resistance for selection in the chloroplast, a nucleus specific reporter gene has been targeted to the inverted repeat region of the plastid genome. Within this construct is nested a second reporter gene that encodes neomycin phosphotransferase (NPTII) under the control of the 35S nuclear constitutive promoter. An intron sequence derived from the potato ST-LS1 gene [Eckes et al., *Mol. Gen. Genet.* 199, 216-224 (1986)] was also included within the NPTII gene such that it can only be expressed following correct intron splicing that can only occur if the gene is transcribed and processed in a nuclear environment. Several independent transplastomic lines have been produced and brought to homoplasmy by several cycles of leaf regeneration by spectinomycin selection. Experiments have confirmed that the NPTII gene does not confer kanamycin resistance to transplastomic tobacco shoots. The homoplastic plants have yielded seeds which are currently being tested for kanamycin resistance.

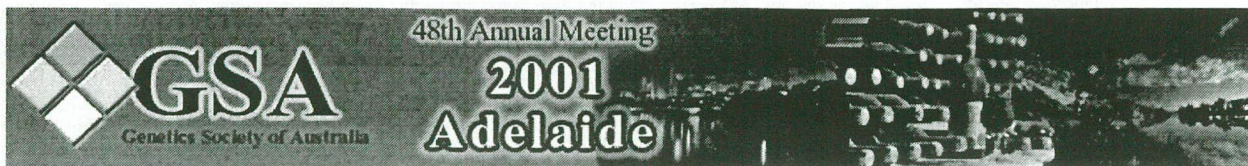


The *Penicillium marneffei* TUP1 homologue represses both asexual development and yeast morphogenesis to allow vegetative filamentous growth.

Richard B. Todd, Michael J. Hynes, and Alex Andrianopoulos.

Department of Genetics, The University of Melbourne, Parkville 3052.

The opportunistic human fungal pathogen *Penicillium marneffei* is dimorphic, showing growth as two different morphological forms. At 25°C the free-living saprophyte grows vegetatively as multinucleate filamentous hyphae and may undergo asexual development (conidiation) by the production of conidia (spore)-bearing conidiophores. At 37°C growth occurs as uninucleate yeast cells that are pathogenic. Little is known about the molecular events involved in the establishment and maintenance of the developmental states in *P. marneffei*. We have cloned *tupA*, the homologue of the *Saccharomyces cerevisiae* pleiotropic repressor *TUP1*, from *P. marneffei*. The TupA protein is highly conserved with homologues in other filamentous and yeast-like fungi. Deletion of the *tupA* gene results in pleiotropic effects. The *tupA* deletion mutant shows partial derepression of a subset of carbon catabolite (glucose) repressible metabolic activities, at 37°C formation of irregularly shaped yeast-like cells, and at 25°C inappropriate developmental switching. At 25°C prolific asexual development and production of uninucleate yeast-like cells occurs, indicating that *tupA* plays a role in vegetative filamentous growth by repression of both asexual and yeast developmental programs. These data contrast with both the lack of asexual development and the constitutive filamentation observed in the equivalent mutants in the mono-morphic fungus *Neurospora crassa* and in the dimorphic fungal pathogen *Candida albicans*, respectively. Although TUP1 homologues in fungi are structurally conserved and regulate analogous developmental and metabolic processes they appear to differ in their specific roles.



Sexual dimorphism in the varied magpie (*Gymnorhina tibicen dorsalis*): the role of natural selection.

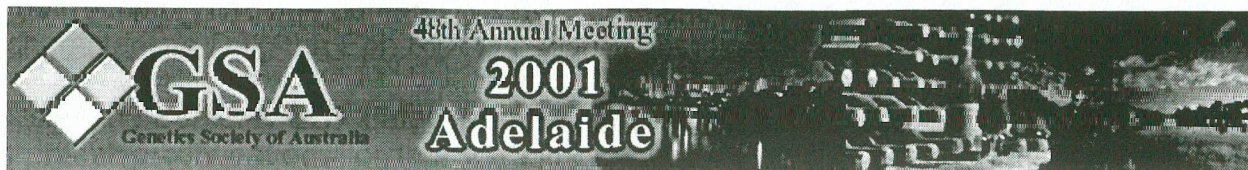
Alicia Toon¹, Jane Hughes¹, Andrew Baker² and Peter Mather²

¹Australian School of Environmental Studies, Griffith University, Nathan, Q. 4111

²School of Life Sciences, QUT, Gardens Point, Q.

Geographic variation in plumage pattern is evident throughout the range of the Australian magpie (*Gymnorhina tibicen*). Based on plumage colour and beak length, between four and six distinct races of the Australian magpie are recognised across mainland Australia. Recent analysis of a contact zone between two races in eastern Australia (black-back magpies and white-back magpies) revealed no genetic subdivision suggesting that the different races arose *in situ* via differing selection pressures. The aim of this study was to assess the extent of geneflow across the contact zone between the varied magpie (*G. t. dorsalis*) and the western black back magpie (*G. t. longirostris*) to uncover the origin and maintenance of plumage colour in Western Australian magpies.

The extent of geneflow between *G. t. dorsalis* and neighbouring morphs was assessed using mitochondrial sequence data. No haplotypes were shared between the varied magpie and eastern magpies, suggesting geographic isolation between eastern and western forms. However, extensive geneflow was observed north to south between varied magpies and the western black-back magpies with no evidence of historical or contemporary structure between these morphs. The results are consistent with a primary origin hypothesis for the north to south plumage pattern within the western region. Environmental constraints, predator-prey associations and sexual selection may contribute to maintenance of morph distributions in the Australian magpie.



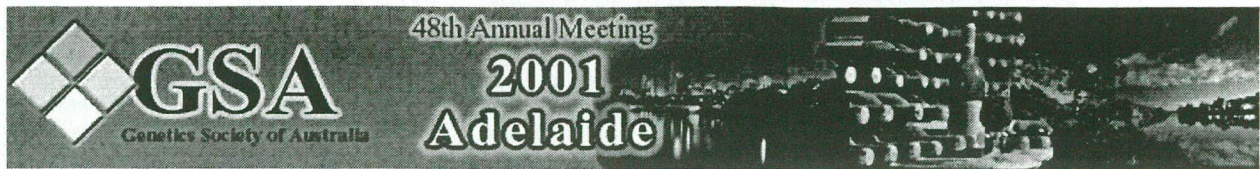
D-Ephrin, An Ephrin Homologue In *Drosophila Melanogaster*

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In vertebrate systems the Eph/ephrin family has been implicated in a number of key events in nervous system development. These include axon guidance, neural cell migration and are found to colocalize with PDZ proteins at synapses (Torres *et al.*, 1998). Furthermore, Eph/ephrins has been shown to be involved in other non-neural developmental processes such as angiogenesis. The Eph subfamily is the largest of the receptor tyrosine kinases (RTK). Recently Scully *et al.*, (1999) isolated and characterised *Dek*, the Eph RTK *Drosophila* homologue.

Using the Berkeley EST database we have isolated a potential ligand to Dek, according to naming conventions for the Eph family this molecule is designated *d-ephrin*. The *d-ephrin* transcripts are located on chromosome 4 according to genomic information in the database. *D-ephrin* contains a number of features consistent with ephrin ligands found in other species; including four conserved cysteine residues, and an ephrin signature. *In situ* mRNA analysis demonstrated expression in the ventral nerve cord and brain of the developing embryo, Similar to that of *Dek*. Expression in the developing nervous system of *Drosophila* raises the hypothesis that *d-ephrin* may be involved in important axon guidance events in the visual and commissural pathways also found in vertebrates.

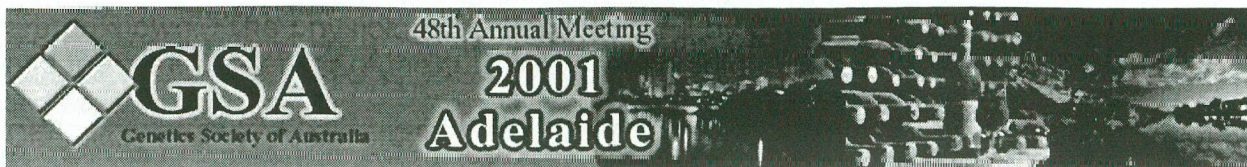


Clinal variation in the *period* gene of *Drosophila melanogaster* may facilitate behavioural adaptation to photoperiod in different climatic regions

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¹ Centre for Environmental Stress and Adaptation Research and School of Biological Sciences, Monash University, Melbourne, 3800 Australia, ² National Aging Research Institute, Parkville, 3052 Australia

The *Drosophila period* gene underlying the circadian clock produces a large protein that cycles in neural tissues from near absent at dawn to high levels in the late evening. A sequence in exon five of *period* is polymorphic in worldwide samples. It encodes for a variable number of tandem repeats of the amino acid pair threonine-glycine (TG), and has been the subject of numerous investigations attempting to elucidate the role of this region of the protein. Strong associations of the frequency of the *period* TG17 allele with latitude have been reported for populations throughout Europe. We found this allele to be associated with latitude along a near-linear Australian eastern coast transect, spanning 3,000 km over 26° of latitude. Unlike latitude, location differences in frequency of the *period* TG17 allele were not robustly associated with location differences in temperature and rainfall statistics. Since at any point in time photoperiod is related directly to latitude, and since a major phenotype influenced by *period* is the circadian rhythm of daily locomotor activity, we have looked for relationships between TG-repeat variation and both photoperiod and the locomotor activity rhythm. Three different photoperiod regimes, 6 hours light, 12 hours light and 18 hours light have been established for replicate laboratory populations, and these are being maintained at each of two constant temperatures, 18°C and 25°C. We will report on our sampling of these populations for *period* TG-repeat variation over 10 to 15 generations of such laboratory culture, and on variation in measures of their daily locomotor activity. Daily locomotor activity in this species has two peaks, a minor peak in the morning around sunrise and a major peak around dusk, and these are completely absent in *period* null mutants. We examined locomotor activity in a number of isofemale lines that were fixed for one of the three common TG-repeat alleles. These lines were a subset of those used for our latitudinal transect, or were derived from them without inbreeding. Clear differences occurred in the extent and daily pattern of locomotor activity among the allelic groups suggesting the presence of genetically based latitudinal differences in locomotor behaviour.



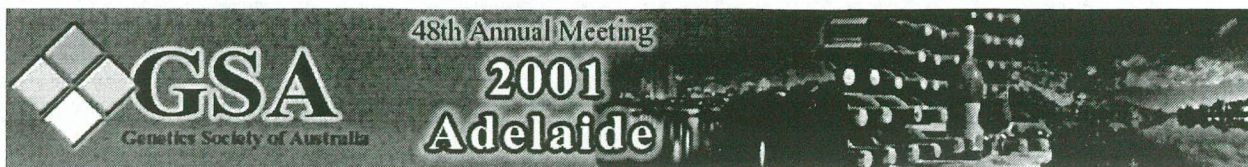
Positional cloning of a cyromazine resistance gene in *Drosophila melanogaster*

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*Centre for Environmental Stress and Adaptation Research, Genetics Department,
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The insecticide cyromazine is classified as an insect growth regulator. It has been used to control pest species such as the housefly (*Musca domestica*), the Australian sheep blowfly (*Lucilia cuprina*) and the pea leafminer (*Liriomyza huidobrensis*). The molecular mode of action of cyromazine has not been determined and mechanisms of resistance are unknown. The physiological effects caused by cyromazine include necrotic lesions and irregular hardening of the cuticle, elongation of the larval insect body and death.

Chemical mutagenesis studies have been identified at least four genes conferring cyromazine resistance in *Drosophila melanogaster*. One of these genes, *Rst(2b)Cyr*, had previously been mapped to chromosome 2 within an interval of 1,263 kilobases. DNA in the cytological region 48A to 49D. Using polymorphic molecular markers (RFLPs and microsatellites) and recombination mapping, we have refined the location of *Rst(2b)Cyr* to region of 109 kilobases which contains fifteen putative open reading frames (ORFs). Eleven of these ORFs are of unknown function. The remaining four encode enzymes (protein kinase, peptidase, ubiquinol-cytochrome c reductase, and diacylglycerol kinase). Further mapping within this region is in progress. It is hoped that the identification of the *Rst(2b)Cyr* gene will provide an understanding of the mode of action of cyromazine and of potential resistance that may arise in natural populations.



Evolutionary Analysis of Uroplakin 1B (UPK1B), a Member of the Tetraspanin Group of Proteins

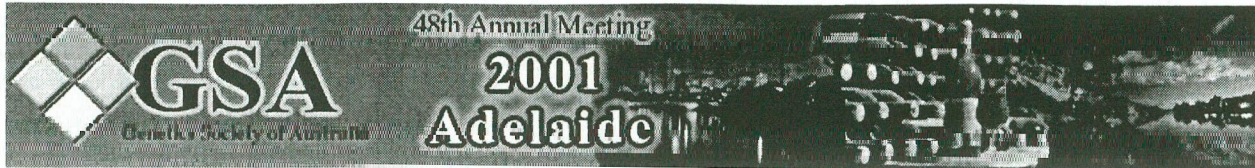
A.E. Varga¹, H.D. Campbell², P.A. Cowled¹

Department of Surgery¹, Adelaide University, The Queen Elizabeth Hospital, Woodville SA 5011, Australia, and Molecular Genetics & Evolution Group², Research School of Biological Science, Australian National University, Canberra ACT 2601, Australia

A range of biological and prognostic markers, including a family of proteins termed uroplakins, have been proposed for bladder cancers (reviewed by Stein *et al.*, 1998). There are four uroplakins, UPK1A, 1B, 2 and 3, which form hexameric complexes within the luminal plasma membrane of bladder epithelium (urothelium) in mammals. Of the four uroplakins, uroplakin 1B (UPK1B) is our focus. UPK1B is a member of the tetraspanin (TM4/TM4SF/transmembrane 4 superfamily) group of proteins. Other family members include CD9, CD63 and CD81. Alterations in expression of tetraspanins have been linked to metastasis and poor prognosis in a variety of cancers, including breast and prostate cancers, and melanoma. The aims of our study were (i) to identify homologues of UPK1B in other species and to analyse their relationships in both parsimony and neighbor-joining analysis, and (ii) to use hydrophilicity plots to investigate conservation in the second extracellular domain. Diplomo analysis (Weiller and Gibbs, 1995) and ClustalW sequence comparisons showed that a partial *Xenopus* sequence has homology with mammalian UPK1B proteins, with 69% homology to human UPK1B. This indicates that UPK1B function as a structural element of the mammalian urinary bladder may be conserved in the amphibian bladder. Diplomo analysis revealed that a phylogenetic tree of the UPK1Bs, including the *Xenopus* sequence, could be derived. UPK1B parsimony and evolution-based neighbor-joining trees were constructed with the programs 'eprotpars' and 'eneighbor' respectively. Both trees showed that while the partial *Xenopus* sequence is somewhat evolutionarily divergent to the mammalian UPK1Bs, it is closely related to this subgroup of the tetraspanins. Hydrophilicity plot analysis, which showed conservation in protein structure for both *Xenopus* and mammalian UPK1B proteins, supported the Diplomo findings. In conclusion, *Xenopus* contains a UPK1B homologue. Although it is related to the other members of the tetraspanin family of proteins, Diplomo analysis, hydrophilicity plots and sequence comparisons have indicated that it belongs within the UPK1B group. The findings suggest that the function of this protein may be conserved over a significant evolutionary distance.

Stein J.P., Grossfeld G.D., Ginsberg D.A., Esrig D., Freeman J.A., Figueroa A.J., Skinner D.G., Cote R.J. (1998). Prognostic markers in bladder cancer: a contemporary review of the literature. *J Urol* **160**: 645-659

Weiller G.F., Gibbs A. (1995). DIPLOMO: the tool for a new type of evolutionary analysis. *Comput Appl Biosci* **11**: 535-540



Is the t(1;29) Centric Fusion a Relic from the Pre-domestication Population of *Bos taurus*?

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The t(1;29) centric fusion in cattle appears to be an ancient translocation and may pre-date domestication. The evidence is as follows:

- a) The t(1;29) centric fusion, or Robertsonian translocation, is found in all breeds of cattle.
- b) The two examples of the 1;29 fusion chromosome so far tested do not show any trace of the Satellite I centromeric repeat DNA which is found in abundance on the procentric regions of all autosomes of *Bos taurus*. Satellites 2-4 are found on the 1;29 centric fusion chromosome.
- c) In the cattle t(14;20) centric fusion, characteristic of the Simmental breed, the Satellite 1 centromeric repeat DNA is retained on both chromosome arms. This is also the case for the t(9;10) centric fusion in sheep, *Ovis aries*.
- d) In the domestic sheep, *Ovis aries*, the largest centric fusion chromosome (OAR1) shows no Satellite I and the other two centric fusion chromosomes (OAR2 and OAR3) show reduced amounts of Satellite I compared with the other autosomes. In the Barbary sheep, *Ammotrogus lervia*, the single large centric fusion chromosome also shows no Satellite I.
- e) Some wild populations of artiodactyls and other mammals are polymorphic for centric fusions, which have been fixed by domestication.

It is problematic to account for the entire loss of Satellite I from the 1;29 fusion chromosome by any of the usual mechanisms of chromosomal rearrangement. A slow ablation over a long period of time seems to be required, as has apparently happened, to varying degrees in the centric fusion chromosomes of the sheep. As to why the 1;29 centric fusion has remained polymorphic in cattle is more difficult to argue. While the rate of non-disjunction during spermatogenesis in 1;29 fusion carriers is normal, there is evidence of early embryo loss causing a 10-20% reduction of fecundity in carriers.



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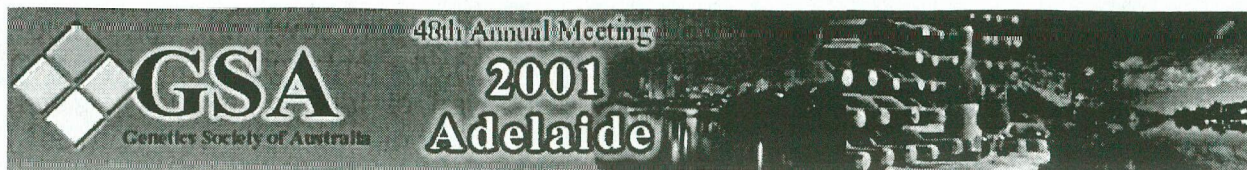


A classical approach to association mapping

B.S. Weir and D.M. Nilesen

Program in Statistical Genetics, NC State University

We have adopted the classical linear model for quantitative traits to study association mapping methods for human diseases. Disease susceptibility depends on the individual's genotype at a trait locus, but it can also be modeled as depending on the marker genotype. The relationship between direct effects at the trait locus and associated effects at the marker locus depends on linkage disequilibria between the loci. Our general framework helps to clarify the nature of case-control and TDT tests, and reveals the relationship between measures of association and coefficients of linkage disequilibrium. In particular we can show the consequences of additive and non-additive effects at the trait locus on the behavior of these tests. We gain additional insights into these tests by relaxing the assumption that both trait and marker loci have only two alleles.



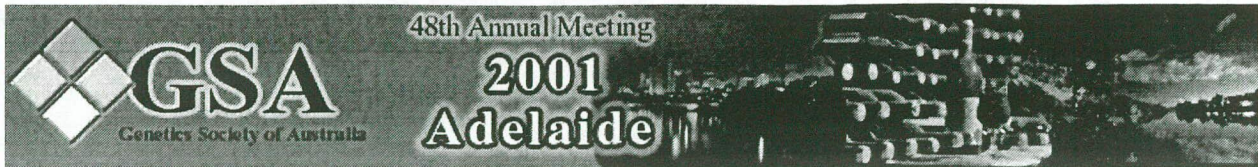
**Molecular relationships of the Banded Hare-Wallaby
(*Lagostrophus fasciatus*) and the Desert Rat-Kangaroo
(*Caloprymnus campestris*).**

M. Westerman¹, A. Burk², M. S. Springer²,

¹Genetics Dept., Latrobe University ^{2,3} Biology Dept., U.C. Riverside, California.

The Australasian marsupial superfamily Macropodoidea comprises three extant families - Macropodidae, Potoroidae and Hypsiprymnodontidae, the latter represented by a single living species. Morphological data suggests that Macropodidae contains two subfamilies, of which the Sthenurinae has only one extant species, the banded hare-wallaby *Lagostrophus fasciatus*. Loving potoroids belong to the one subfamily Potoroinae with two tribes, Potorini and Bettongini. The desert rat Kangaroo, *Caloprymnus campestris*, which is thought to be extinct, having not been seen since before 1950, is thought to have been the sister taxon to the rufous rat-kangaroo (*Aepyprymnus rufescens*) in the bettongini.

We have obtained mitochondrial 12S rRNA gene sequences from both *Lagostrophus* and an alcohol preserved museum specimen of *Caloprymnus* which were used to test phylogenetic relationships of both taxa.



The conservation of synteny in insect genomes: A comparison between *Lucila cuprina* and *Drosophila melanogaster*.

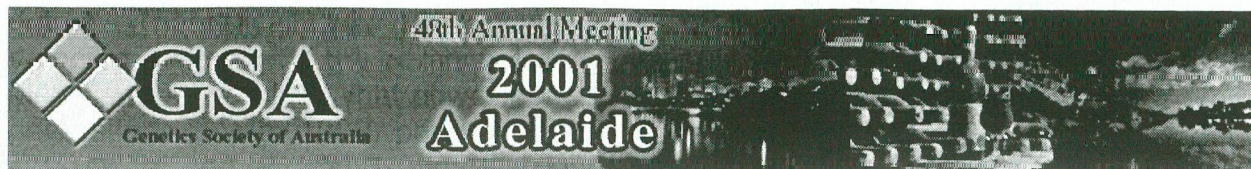
Lee Willoughby, Jason Fair, Charles Robin and Philip Batterham

Centre for Environmental Stress and Adaptation Research, Genetics Department,
University of Melbourne, Parkville, Victoria 3052

While chromosome number is variable within the genus *Drosophila*, six linkage elements have remained conserved. Each linkage element is present in *Drosophila* species as either an entire chromosome or a chromosome arm. Weller and Foster (1993) found the six linkage elements to be conserved among other member of higher Dipteran genera - *Musca domestica*, *Ceratitis capitata* and *Lucilia cuprina*. This conclusion was based on observations with a limited number of phenotypic markers. We are aiming to investigate the degree of linkage conservation between *L. cuprina* and *Drosophila melanogaster* using molecular markers. Potential markers are being identified from *L. cuprina* genomic DNA sequences or from Expressed Sequence Tags (ESTs) from a *L. cuprina* embryonic cDNA library. We are generating molecular maps of *L. cuprina* chromosome 3, (corresponding to the *D. melanogaster* X chromosome) and *L. cuprina* chromosome 4 (corresponding to the *D. melanogaster* chromosome 3R).

Reference

Weller, G.L and G.G. Foster, 1993, Genetic maps of the sheep blowfly *Lucilia cuprina*: linkage-group correlations with other dipteran genera, Genome 36: 495-506

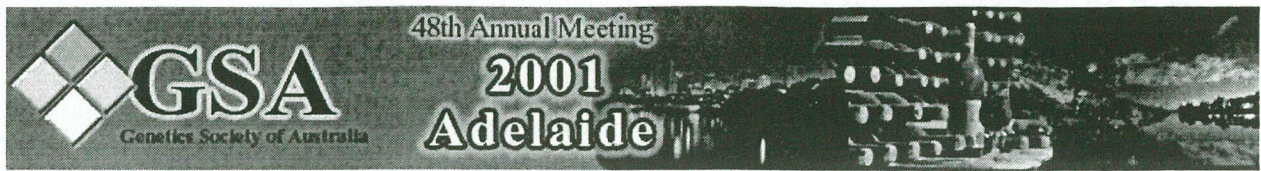


Dingoes: what levels of introgression can be detected?

Alan Wilton

School of Biochemistry and molecular Genetics, University of New South Wales

Dingoes are not only under threat from the Queensland government but also by replacement by domestic dogs through hybridisation. A large proportion of dingoes, particularly those in populated east coast, are hybrids. We have developed a set of 20 microsatellite markers and the mitochondrial D-loop that can be used to differentiate between genetic material of dingo origin and that of domestic dog origin. But what is the power of these tests. What proportion of hybrid animals will these tests be able to detect? Theoretically with 100% power 98.8% of animals with 20% dog ancestry will be detected but only 87.8% of animals that are 10% dog and 64.2% of 5% dog will be identified as hybrids. What power do tests have to detect backcross animals of several generations? With 100% power to resolve ancestry for each test, 93.1%, 72.5% and 47% in 3rd, 4th and 5th backcross generations respectively would be detected. With 50% power in each test the proportions detected would be 72.5%, 47% and 27% respectively. Does the power to detect hybrids increase with the number of loci tested? It increases strongly with the number of test. For example, likelihood of detecting dog ancestry in an animal with 5% dog genes is 0.642, 0.785, 0.871, 0.923 and 0.994 for 20, 30, 40, 50 and 100 loci respectively. Lod scores comparing probability of an individual's genotype coming from a pure dingo to probability it comes from a three-quarter dingo can be used as a practical indicator of likelihood of dog ancestry. If a cut off of 1 lod is used 3.2% of pure animals would be falsely assigned as hybrids and 3.2% of hybrids falsely predicted to be pure in current tests. This is based on the gene frequencies in the captive dingo populations which themselves may not be pure. A source of more reliably pure dingo material would reduce the amount of assignment errors.



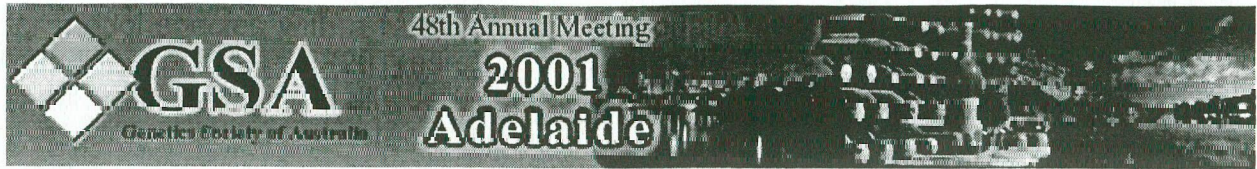
Recombination initiated at *cog* can cross a region of substantial sequence heterology without reducing conversion frequency at *his-3*

P. Jane Yeadon, J. P. Rasmussen, F. J. Bowring and David E. A. Catcheside.

School of Biological Sciences, Flinders University, Adelaide, Australia

Recombination between *his-3* alleles is strongly dependent on the activity of the recombination hotspot *cog*⁺ located about three kilobases 3' of this locus. Events initiated by *cog*⁺ must pass the intervening sequences in some way in order to reach *his-3* and generate recombinant progeny. We have constructed stocks each of which includes a different DNA sequence inserted between *his-3* and *cog*. The three insertions have different lengths and no homology to each other or to the native sequence they replace.

Analysis of crosses heteroallelic *his-3* K26/K480, homozygous *cog*⁺ *rec-2* and either homozygous or heterozygous for the inserted constructs has shown that lack of sequence homology over several kilobases has no significant effect on the frequency of *his*⁺ recombinant progeny. Heterozygosity for length in this region yields a recombination frequency like that of a cross homozygous for the shorter interval, clearly showing that recombination initiated by *cog*⁺ tolerates high levels of sequence variation.



The Effects of Genetic Self-incompatibility on the Population Viability of Plants with Varied Life Histories

Andrew Young¹, Peter Thrall¹, Susan Hoebee^{1,2}

¹ CSIRO Plant Industry, ² Department of Forestry, The Australian National University.

Several recent studies have identified low genetic diversity at self-incompatibility (SI) loci as a cause of reduced seed set in small populations of endangered plants. Here we use a biologically realistic, spatially explicit, simulation model to explore the genetic and demographic consequences of three kinds of self-incompatibility: gametophytic; sporophytic with codominant S alleles; sporophytic with linear dominance of S alleles, for model species with a range of birth and death rates simulating annuals through to long-lived perennials (20 years). Results show that SI systems can constrain the viability of small genetically depauperate plant populations through their effects on mate availability and seed set and that this affect varies with life history strategy. As approximately one half of the angiosperms are thought to have genetically controlled SI systems the implications for conservation of endangered plants species are significant.

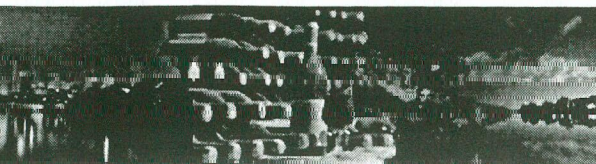


Polymorphisms of Human Microsatellite Markers in Non-Human Primate Populations

Ziino M¹, Ewen-White K¹, Temple-Smith P², Barlow J¹.

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Pedigree records of captive, non-human primate populations are used to make vital animal management decisions and often these records are based on behavioural observations rather than genetic relationships. This study investigated the use of a commercial set of primers for human microsatellites to determine the accuracy of familial relationships and the genetic variation within and between three populations of Hamadryas Baboon (*Papio hamadryas*). Two captive populations were studied (15 animals, Royal Melbourne Zoological Gardens [RMZG]; 92 animals, Australian National Baboon Colony) and one large commensal population (23 animals, Saudi Arabia). Of 400 human microsatellite markers tested, 231 primer pairs successfully amplified Hamadryas baboon DNA and 142 of these were polymorphic. These polymorphic microsatellite primers were grouped into 13 panel sets, which were used to study the baboon populations. Pedigree analysis using the results from this investigation confirmed or excluded recorded relationships within the populations, along with the level of allele sharing amongst individuals. This genetic study found numerous inaccuracies in pedigree records, including 80% relationship exclusion within one population (RMZG). This study highlights the importance of genetic studies for the accurate determination of familial relationships for reliable management of captive animal populations.



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