Acknowledgements

Organising Committee

Graham Wallis (funding, venue and co-ordination)
David Winter (program)
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Jon Waters (venue)
Rebecca Cumming (transport)
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Ken Miller
Department of Anatomy and Structural Biology

Venue

Carol Geissler & Wendy Dewe (catering)
Alan Dewe (grounds and buildings)

Prizes

Genetics Otago

Financial Support

Department of Zoology

Timetable

Friday

5pm	Check in and mixer
6pm	Barbeque Dinner

Saturday

8:50	Welcome

Session 1

9:00 - 9:12	Tatiana Escovar
9:12 - 9:24	Shane Wright
9:24 - 9:36	Shelly Myers
9:36 - 9:48	Mary Morgan-Richards
9:48 - 10:00	David Winter

Break

Session 2

10:30 - 10:42	Hilary Miller
10:42 - 10:54	Jolene Sutton
10:54 - 11:06	Catherine Grueber
11:06 - 11:18	Caroline Antolik
11:18 - 11:30	Monika Zavodna
11:30 - 11:42	Brendan Bycroft
11:42 - 11: 54	Rupert Collins

Lunch

Session 3

1:00 - 1:12	Ceridwen Fraser
1:12 - 1:24	Raisa Nikula
1:24 - 1:36	Rebecca Cumming
1:36 - 1:48	Peter Martin
1:48 - 2:00	Nick Demetras

Break

Session 4

2:30 - 2:42	Leah Tooman
2:42 - 2:54	Elizabeth Hegg
2:54 - 3:06	Mariana Mahood
3:06 - 3:18	Stephane Boyer
3:18 - 3:30	Tim Page

Break

Timetable

Saturday

Session 5

4:00 - 4:12	Peggy Macqueen
4:12 - 4:24	Rod Hitchmough
4:24 - 4:36	Jo Fitness
4:36 - 4:48	Naema Shibani
4:48 - 5:00	Nic Dussex

Dinner

7pm	North Island v South Island soccer championships

Sunday

Session 6

9:00 - 9:12	Kristina Ramstad
9:12 - 9:24	Sebastian Rioux Paquette
9:24 - 9:36	Matt Taylor
9:36 - 9:48	Amy Marshall
9:48 - 10:00	Rosalynn Anderson-Lederer
10:00 - 10:12	Tom McCowan

Break

Session 7

10:42 - 10:54	Davon Callander
10:54 - 11:06	Ngaire Phillips
11:06 - 11:18	Ben Myles
11:18 - 11:30	Jawad Abdelkrim
11:30 - 11:42	Raazesh Sainudiin
11:42 - 11:54	Reema Jain

Lunch

Pack up, clean out and go home!

Differences in the toxicity and phylogenetic relationships of “Poison Dart” frogs of the genera *Phyllobates*

Tatiana Escovar & Amézquita, A
Universidad De Los Andes, Bogota, Colombia

The poison frogs of the family Dendrobatidae have developed toxicity as a mechanism of defense against predators. Approximately one-third (11 genera) of dendrobatids secrete powerful toxins from dermal granular glands. The genus *Phyllobates* was historically used by the Emberá Indians of Chocó, western Colombia to poison the darts of their blowguns and are recognized for their high level of toxicity. Taxonomically, five species are grouped within the genus *Phyllobates* based on their shared production of the powerful Batrachotoxin. However, there is a high degree of difference in the level of toxicity between these species. Theoretical background and empirical evidence indicates that the species to species difference in toxicity may be correlated to evolutionary origin. In this study we compared the species to species differences in toxicity and phylogenetic signal. To establish a species’ level understanding of *Phyllobates* toxicity we conducted clinical toxicity trials by injecting mice with extracts from three species of *Phyllobates* . We also indentified and quantified the myriad of alkaloids in their extracts through gas chromatography and mass spectrometry. Genetic distances were calculated from two mtDNA genes (COI and 16S) and were compared to inter-species and inter-population differences in toxicity and alkaloid composition. Our results indicate evidence of differential evolution in toxicity levels between different populations of *Phyllobates* sp. Our conclusions, that toxicity is poorly explained by phylogenetic processes, suggests that it may be attributed to strong local selective pressures. However, this hypothesis remains to be tested.

Energy and the tempo of evolutoin in amphibians

Shane Wright
School of Biological Sciences, University of Auckland

Following research demonstrating more rapid molecular evolution for plants [Wright et al. (2006) PNAS 103, 77187722] and foraminifera [Allen et al.(2006) PNAS 103, 91309135] living in warmer climates we have now tested forthe generality of this effect. We have done this by making an investigation using a large global dataset - in a separate kingdom - for a class of ectothermic vertebrates. This research compared rates of molecular evolution between amphibian species that are spatially separated in either latitudinal or elevational dimensions. Thus, we report here on our findings for a group of animals whose phylogenetic and trophic contexts are remote from those ofthe plants and forams. The study utilised paired contrasts for 188 species across 18 families including anurans and caudates - with the widely applied mitochondrial RNA genes 12S and 16S. Under the constraints of the biogeographic and phylogenetic selection criteria that we applied, thesecontrasts represent all possible comparisons using those genes from data available in the public domain. All paired contrasts were between phylogenetically proximate sister species that shared a distributional overlap of no more than 25%. As with plants and forams, we found that amphibians living in warmer climates have a more rapid microevolutionary tempo. The consistency of these results with the two previous studies - and in this instance using different genes within an evolutionarily remote kingdom of vertebrates - suggests that this is a ubiquitous pattern in nature

Investigating hybrid origins of *Acanthoxyla,* New Zealand's parthenogenetic genus, using combined genetic techniques

Shelly Myers & Mary Morgan-Richards
Ecology, INR, Massey University, Palmerston North

Although both hybridization resulting in speciation and parthenogenetic reproduction in eukaryotes are rare, both of these phenomena are strangely common in stick insects. Recently, the New Zealand stick insect *Acanthoxyla* has been under investigation and is of particular interest as the entire genus of eight species is parthenogenetic. *Acanthoxyla* have low COI-II diversity and karyotypes consist of 2n=36 or 38. The same chromosome number occurs in sexually reproducing insects implying they are diploid. A hybrid origin of *Acanthoxyla* was suggested involving the sexual species Clitarchus hookeri. More recently Buckley et al. (2008) have suggested things are more complicated and that some *Acanthoxyla* are triploid. I will be presenting my honours research on the genetic structure of *Acanthoxyla,* its implications on evolution of hybrid species, and how combining genetic techniques enables a better understanding of the currently debated theories about this unusual parthenogenetic genus.

Tree weta phylogeography and the trouble of paralogs

Mary Morgan-Richards, Steve A Trewick, Sofie Welvaert
Ecology, INR, Massey University, Palmerston North.

Tree weta distribution is thought to have ebbed and flowed with glacial cycles; the Auckland tree weta expanding southwards in warmer inter-glacials, and the Wellington tree weta expanding north in cooler glacial phases. We think competitive exclusion operates to limit sympatry. Initial DNA sequence data from the Wellington tree weta was used to infer the southern-most chromosome race with 2n=19 might be derived from the chromosome race 2n=15. This hypothesis of chromosome evolution is rather unusual as fusions are significantly more likely than fissions. New data suggests that we were mislead by nuclear copies of genes originating in the mitochondrial genome. Although chromosome evolution is unexceptional in this species, we have new evidence of range expansion from the south, followed by range contraction, leaving isolated populations in cold northern sites.

Speciation Without Separation?

David J Winter[1], Fred J. Brook[2], Martyn Kennedy[1], Graham P. Wallis[1] & Hamish G. Spencer[1]

[1] *Department of Zoology, University of Otago, Dunedin*
[2] *PO Box 3123, Onerahi, Whangarei, New Zealand*

Sympatric speciation - the idea that species can form without a period of geographical isolatoin is one of the most contentious ideas in evolutionary biology. We work on a remarkable landsnail radiation from the small, isolated island of Rarotonga in the Cook Islands. Here we present evidence that 7 morphologically distinct forms of Rarontongan *Lamprocystis* represent distinct species. Molecular phylogenetic and molecular clock analyses show these species most likely arose within Rarotonga. Finally a intraspecific phylogeographic study in the widespread species *L. venosa* is used to assess whether the mountainous terrain of Rarotonga is capable of driving reproductive isolation in *Lamprocystis* species

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Genetic diversity and differentiation at MHC genes in island populations of tuatara (*Sphenodon* spp.)

Hilary C. Miller & Charles H. Daugherty

Allan Wilson Centre for Molecular Ecology and Evolution, School of Biological Sciences, Victoria University of Wellington, Wellington,

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Neutral genetic markers are commonly used to understand the effects of fragmentation and population bottlenecks on genetic variation in threatened species. Although these markers are useful for inferring population history, measuring levels of diversity at functional genes may be required to determine the significance of any observed geographic differences in variation. The genes of the Major Histocompatibility Complex (MHC) are well-known examples of genes of adaptive significance, and are particularly relevant to conservation because of their role in pathogen resistance. In this study we survey diversity at MHC class I loci across a range of tuatara populations. We compare levels of MHC variation with that observed at neutral microsatellite markers in order to determine the relative roles of balancing selection, diversifying selection and genetic drift in shaping patterns of MHC variation in isolated populations. Tuatara populations are highly differentiated at MHC genes, but in general patterns of MHC variation both within and between populations correlate with microsatellite DNA variation. These results indicate that population bottlenecks and isolation have a larger influence on patterns of MHC variation in tuatara populations than balancing selection

Do neutral and functional genes tell the same story?

Jolene Sutton, Ian Jamieson, Bruce Robertson

Department of Zoology, University of Otago, Dunedin, New Zealand

Microsatellites are often the marker of choice for conservation genetics. But do they always tell us what we really want to find out? How does selection act on functional genes, and do neutral markers act as surrogates for diversity across the genome? To answer these questions, we are investigating the effects of population bottlenecks on neutral (microsatellite) and functional (major histocompatibility complex; MHC) loci in two groups of New Zealand birds with different histories of decline. Severe bottlenecks have lead to loss of both neutral and functional diversity in some other vertebrate species, but patterns are not always similar between the two marker types. Given that MHC is associated with immune response, selection might maintain genetic diversity at these loci even when it is lost at microsatellites. New Zealand saddlebacks underwent severe bottlenecks and experienced greater loss of diversity at microsatellite loci than robins, which experienced more moderate bottlenecks. It is likely that for saddlebacks, functional diversity will also be greatly reduced from historic levels, but in robins different patterns may exist between microsatellites and MHC loci. Although microsatellites are now “relatively easy” to work with, there are still several issues involved with genotyping MHC loci. If we can successfully navigate around the MHC hurdles, our research could aid with understanding the genetic effects of population bottlenecks on both neutral and functional genetic diversity in species with different histories of decline. It could also help to further address the utility of different molecular marker types in conservation.

If I don't have a pedigree, will DNA do?

Catherine Grueber, Ian Jamieson, Jon Waters
Department of Zoology, University of Otago, Dunedin, New Zealand

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Inbreeding depression is an important consideration in threatened species management as it can impact population viability. Pedigrees are the most effective method for evaluating inbreeding depression, but when they are not available molecular methods is the only option. However, the relationship between molecular heterozygosity and inbreeding coefficient is complex, so are molecular methods adequate for evaluating inbreeding depression in an endangered species? Previous analyses tackling this question have met with difficulties, usually the result of poor statistical power. We have been working to answer this question in the endangered takahe, a rare example of a free-ranging population with both pedigree (21 years) and molecular (24 microsatellites) data. Detailed analysis using generalised linear mixed modelling revealed strong inbreeding depression of both survival and reproductive traits in the population, as predicted by pedigree-based inbreeding coefficients. In addition, the molecular markers showed the expected negative relationship between microsatellite heterozygosity and fitness. However, the relationship between individual pedigree-based inbreeding coefficients and molecular heterozygosities is significant, but weak. It is unclear why this is so, given the concordance between both of these metrics in their relationships with fitness. We suggest reasons for this interesting result, and invite comment from the audience as to additional explanations.

Frequency and heritability of recombinant mitochondrial DNA

Caroline Antolik

Department of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand

Mitochondrial DNA (mtDNA) is commonly used in population and evolutionary studies due to its rapid rate of mutation, maternal inheritance, and lack of recombination. However, recent work has shown evidence of paternal inheritance, heteroplasmy, and recombination in the mtDNA of various animal species including humans. mtDNA is used in a wide range of studies spanning taxonomy, phylogeography, population structure through to the relationships among individuals, and recombination in the genome could raise uncertainty regarding population histories, particularly in near time. Therefore, further investigation is needed to better understand the frequency of mtDNA recombination and determine potential ways to account for its occurrence in evolutionary studies. Here, 454 technologies will be used to test for mtDNA recombination in chinook salmon at various stages of life history, which will allow for determination of recombination frequency and level of inheritance of recombinant molecules. This information will provide insight into how strong an influence mtDNA recombination may have on interpretation of the evolutionary history of animal populations based on mtDNA-based studies.

What is the role of genetic recombination in microsatellite evolution?

Monika Zavodna[1], Andrew T.M. Bagshaw[2] , Neil J. Gemmell[1]

[1]*Department of Anatomy and Structural Biology, University of Otago, Dunedin*

[2]*Department of Pathology, Christchurch School of Medicine, University of Otago, Christchurch*

Microsatellites are short DNA sequences in which motifs of 1-6 bases are tandemlyrepeated. Their polymorphism derives mainly from variability in repeat number.Generally, they are considered to be neutral markers and inherited in a Mendelianmanner. As a codominant and highly polymorphic system, microsatellites havebecome instrumental as genetic markers in areas such as population genetics,parentage analyses, genetic mapping, or forensics. However, conclusions drawn fromsuch studies depend on assumptions about how microsatellites evolve. Current modelsof microsatellite evolution are rather simplistic. In particular, genetic recombination,whilst known to be the major generator of genomic variability, is widely regarded as aminor contributor to microsatellite evolution. However, recent studies have shown astrong association between microsatellite abundance and sites of high recombinationactivity in yeast and human genomes. Moreover, microsatellites located in the vicinityof meiotic hot spots appear to be more polymorphic than microsatellites that aredistant from a hot spot, all suggesting that genetic recombination might play a role inmicrosatellite evolution. To examine this further, we have located and designedprimers for more than 250 microsatellite loci (in both, recombination hot spots andcold spots) in yeast (*Saccharomyces cerevisiae*) genome. The frequency and nature ofmutations at these loci will be compared over 700 generations in near identical *S.cerevisiae* strains that do or do not engage sex. This study is in

Markov Chains on Trees for Sampling Distributions of Population Genetic Statistic

Brendan Bycroft

Department of Maths and Stats, University of Canterbury, Christchurch

We present a C++ class library for a fully customisable simulation of repetitive and non-repetitive DNA on structured ancestral recombination graphs. This library allows species-specific and locus-specific mutation models to produce the sampling distributions of most population-genetic statistics. The talk will focus on the capabilities of the library and a user-friendly tour.

DNA barcodes: can they really be black and white?

Rupert A. Collins

Bio-Protection Research Centre, Lincoln University

Effective bio-protection in New Zealand is dependant upon fast, accurate and reliable identification of unwanted organisms entering the country. Significant environmental and economic impacts could result from insufficient risk-assessment and monitoring of invasive or disease-carrying species. Due to the variety of species currently present in the international aquarium trade, accurate identification of imported fishes is becoming increasingly important and molecular technologies using standardised protocols offer an effective solution to identify imported fishes. The DNA barcoding procedure using the mitochondrial COI gene sequence has been demonstrated to differentiate taxa, including fishes, at the species level. Here, I will test the efficacy of COI barcodes as a species identification tool, by assembling a reference database of high-risk ornamental fish species. A critical issue of DNA barcode analysis in"real world" applications, is the effect of incomplete sampling, and the subsequent confidence in species determination of divergent query specimens. I will assess distance, character-based, and coalescent methods to ascertain the whether DNA barcodes can really be black and white. Another major challenge to the mitochondrial COI approach, however, is the inability to identify hybrid aquarium fishes with mixed genealogies. I will review appropriate species level nuclear markers, and develop a sequence-based test as a means of detecting hybrid specimens, applicable across a wide range of taxa. Finally, recent advances in molecular technologies have allowed the amplification of species-specific DNA fragments from environmental samples; this opens the prospect of a non-invasive screening procedure, using aquarium water to identify exotic species in mixed consignments.

Seaweed: barometer of historic climate change?

Ceridwen Fraser, Hamish Spencer, Jonathan Waters

Allan Wilson Centre for Molecular Ecology and Evolution, Department of Zoology, University of Otago, 340 Great King St, Dunedin

Seaweed has long been used to predict weather changes: dangle a dry piece outside your hut, and if it goes limp it's time to rush the washing in before the storm arrives. So seaweed can tell us about imminent weather change – but can it tell us anything about large-scale, long-term climate change? Our recent phylogeographic analyses of southern bull-kelp (*Durvillaea antarctica*) around the Southern Hemisphere revealed intriguing contrasts in genetic diversity in high- versus low-latitude regions. Near-negligible diversity in the south indicates the species has recolonised these latitudes relatively recently. What could have wiped the kelp off subantarctic islands? Was it sea ice at the Last Glacial Maximum? If so, were other species similarly driven from these subantarctic shores? We here present the conclusions of this exciting study and outline some of the future directions our research will take.

Does kelp rafting convey regular gene flow among populations of kelp epifauna?

Raisa Nikula , Hamish Spencer, Jon Waters

Allan Wilson Centre for Molecular Ecology and Evolution, Department of Zoology, University of Otago, Dunedin

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Cavities in the holdfasts of the southern bull kelp (*Durvillaea antarctica*) are inhabited by numerous intertidal invertebrate species that either lack active long-distance dispersal means altogether or only have a short pelagic larval stage. The presence of obligate kelp epifauna in remote subantarctic islands and their large-scale phylogeographic patterns across the Southern Ocean suggest that macroalgal rafting is an efficient long-distance colonization mechanism for sedentary kelp epifauna. But do detached kelp plants make a vehicle for regular gene flow among once established populations of kelp epifauna? To test for this, we are comparing genetic structuring among populations of two kelp-dwelling mollusk species (*Sypharochiton sinclairi, Diloma durvillaea*) to that of their rock-dwelling sister species (*S. pelliserpentis, D. arida*). We have developed a set of polymorphic microsatellite markers for each species pair and sampled and genotyped populations along the eastern coast of the South Island from Banks Peninsula to Stewart Island. Preliminary results will be presented.

Phylogeography of a subantarctic brooding gastropod – evidence for long-distance rafting

Rebecca Cumming & Jon Waters
Department of Zoology, University of Otago, Dunedin

During the Last Glacial Maximum, ice scour is thought to have eliminated many intertidal species from the remote subantarctic islands. It is unclear, however, whether extant subantarctic species are recent colonizers, or species that survived in glacial refugia. The subantarctic gastropod *Onchidella campbelli* often utilizes holdfasts of southern bull-kelp for shelter and egg-laying, an association which suggests potential for long-distance dispersal via macroalgal rafting. While genetic data suggest bull kelp itself was totally eradicated from the subantarctic during the LGM, it is not clear if this was also the case for the hold-fast-dwelling *O. campbelli*, which also inhabits the intertidal zone on rock platforms. Phylogeographic analyses based on mtDNA COI indicate close genetic relationships between distinct subantarctic island populations of *Onchidella*, with particularly small genetic distances between Falkland Islands and NZ subantarctic samples. This recent common ancestry for populations separated by thousands of kilometres of open ocean supports the hypothesis that trans-oceanic kelp-rafting has promoted colonization of the subantarctic. Although in contrast to findings for other kelp-dwelling invertebrates it appears that the presence of *Onchidella* in the subantarctic could in fact pre-date the end of the last glacial – so when did these events take place and by what mechanism?

The phylogeography of *Lessonia* (Laminariales)

Peter Martin

School of Biological Sciences, Victoria University of Wellington, New Zealand

The genus *Lessonia* is solely distributed in the southern hemisphere, with several species found in South America and Australasia. As five species are described in each region we have to consider two possible centres of origin. The goal was: to determine the taxonomic and phylogeographic relationships between *Lessonia* species from the southern hemisphere and to develop possible scenarios of species distribution. To resolve the southern Hemisphere relationships we combined mitochondrial, chloroplast and nuclear markers in a comprehensive dataset. The generic relationships reveal that the South American *Lessonia* species are a sister group to the monophyletic Australasian *Lessonia* suggesting a single dispersal event (west to east) across the Pacific Ocean. However other interpretations are possible and potentially even more likely. The results will tackle the importance of the Antarctic Circumpolar Current on long distance dispersal in the Subantarctic.

Fine scale distribution of mtDNA haplotypes for the springtail *Gomphiocephalus hodgsoni* and the mite *Stereotydeus mollis* in the southern most Dry Valleys, Victoria Land.

Nick J. Demetras, Hogg I.D., Ross P.M., Banks, J.C
University of Waikato, Hamilton

The spatial distribution and genetic structure of *Gomphiocephalus hodgsoni* (Collembola) populations was examined within the Miers, Marshall and Garwood Valleys, a series of small Dry Valleys in southern Victoria Land. Physical, chemical and biological measurements (eg, slope, aspect, conductivity, flora and fauna) were taken at approximately 500 sites throughout the study area from which *G. hodgsoni* were found at 90 sites. A 600 bp, unambiguous region of the mtDNA COI gene was sequenced from 1-5 individuals collected at each site. Multivariate statistics were used to relate the presence of *G. hodgsoni* to physical, chemical and biological characteristics. *G. hodgsoni* were found predominantly on south and west-facing slopes and in proximity to drainage channels or snow melt where soil moisture content exceeded 4%. Six unique mtDNA haplotypes were identified with greatest haplotype diversity found at higher altitude sites within Garwood Valley. The occurrence of areas with high haplotype diversity and unique physical and biological characteristics (eg, elevation, slope, high species diversity, etc) may indicate local ice free habitats that have served as refugia during past glaciation events.

Global mitochondrial population genetics of the invasive pest moth species *Epiphyas postvittana*

Leah Tooman[1], Norman Barr[2], Richard D. Newcomb[1]

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Understanding the mechanisms and routes by which species invade new regions remains a major question for population ecology and a problem for authorities and industries if these species represent pests or pathogens of crops, animals and humans or if they have the potential to modify native ecosystems. Here we investigate the population structure and potential invasion pathways of the lightbrown apple moth, *Epiphyas postvittana*, a horticultural pest in Australia, New Zealand, Hawaii and Europe, with particular reference to its recent invasion of California. A 2216 base pair region of the mitochondrial genome containing the cytochrome oxidase I and II genes was sequenced from 681 individuals sampled from across the moth's global range, including California. Initial phylogenetic analyses revealed a major split between a predominantly Western Australian clade and all other samples, suggestive of a new cryptic species, which we removed from subsequent analyses. Measures of both nucleotide and haplotype diversity were highest in Australian and New Zealand populations with evidence for structuring in both countries. From the 93 haplotypes that were recovered seven were from California of which four are private. The existence of private alleles in California within our dataset, together with haplotype bootstrap resampling analyses, suggest that we have not recovered a majority of the existing haplotypes from any of the regions outside California. This observation highlights the challenge in addressing questions of origin and population genetics in a species were population sizes are potentially very large with the ability to harbour substantial amounts of genetic diversity.

Investigating the source of Lake Taupo rainbow trout (*Oncorhynchus mykiss*)

Elizabeth Rose Heeg[1], Micheal Dedual[2], Fred Allendorf[1], Peter Ritchie[1]

[1]*School of Biological Sciences, Victoria University of Wellington*
[2]*Department of Conservation, Taupo Fishery Area, Turangi*

Determining the source and number of introductions of non-native species is often a difficult task, but is crucial in order to understand the genetic potential of established populations. We investigated the origins of Lake Taupo rainbow trout (*Oncorhynchus mykiss*) translocated from the USA 100 years ago, using data from a panel of 14 microsatellite loci, and the spawn timing candidate locus *clock1b*. We determined the levels of variation within and among Lake Taupo and six sites throughout California, including the two putative populations of origin, McCloud River and Lake Almanor. The Lake Taupo population show significant genetic divergence from all of the Californian populations. Lake Taupo *O. mykiss* have similar mean heterozygosity but significantly fewer alleles per locus than California populations, which suggests that the Lake Taupo populations have been through a bottleneck. Lake Taupo rainbow trout are similar to the McCloud River population in California, and both shared the otherwise rare 345-bp allele at the *clock1b locus*. Therefore, McCloud River is apparently the source population for Lake Taupo.

Genotyping from non-invasively recovered possum (*Trichosurus vulpecula*) DNA using saliva from bitten bait interference devices (WaxTags®)

Mariana. L. Mahood, Rob H. Cruickshank, James G. Ross, Andrew J. Holyoake, Shaun C. Ogilvie, Adrian M. Paterson.

Ecology Department, Agriculture and Life Sciences Faculty, Lincoln University

The brushtail possum is a major agricultural and ecological pest in New Zealand. The WaxTag® (WT), a novel non-invasive DNA sampling tool for detecting its presence was tested. DNA was recovered from saliva left on WT, and two lengths (407 and 653 bp) of the Cytochrome c Oxidase I (COI) barcoding region were amplified by Polymerase Chain Reaction (PCR). Different factors that might affect PCR success rates (PCR (+)) were investigated with samples from captive and wild (non-invasively sampled) possums. The shorter COI amplicon had significantly higher PCR(+) from both captive (99%) and wild (63%) WT than the longer fragment (48 and 23% respectively). PCR (+) were not significantly affected by an indoor overnight delay at RT prior to DNA extraction, the effect of the individual or magnitude of the bite. To determine the reliability of genotyping from WT samples, Tv 5.64 (Nicola Aitken, pers. comm., 2006) was amplified from captive (n=31) and wild (n=16) possum WT samples that were PCR(+) for the 407 bp COI fragment and their corresponding tissue DNA samples. A total of seven Tv 5.64 alleles were detected, with 16 different genotypes. PCR (+) rates of Tv 5.64 were 81% for captive and 67% for wild WT with indoor delay, yielding genotypes that matched the true genotypes by 96 and 100% . We conclude that DNA from saliva traces can be recovered from WT and produce reliable genotyping results. WT are a new tool for non-invasive genetic monitoring of possums, and potentially other wildlife.

Molecular diet analysis of the carnivorous landsnail *Powelliphanta augusta*.

Stephane Boyer[1], Steve D. Wratten[1], Gregor W. Yeates[2], Andrew Holyoake[1], Robert H. Cruickshank[3], Jawad Abdelkrim[4]

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[2] *Landcare Research, Palmerston North (Present address: PO Box 1758, Palmerston North)*
[3] *Department of Ecology, Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln*
[4] *Centre for Reproduction and Genomics, Department of Anatomy & Structural Biology, University of Otago,*

Endemic carnivorous landsnails (*Powelliphanta augusta*) living in the mine footprint were removed ahead of mining for and translocation to adjacent undisturbed areas. The success of these translocation campaigns depends on appropriate food availability in the release area. However, feeding is difficult to observe for these small nocturnal animals. Therefore, with the aim of describing snails' diet, faecal strings from *P. augusta* were collected in the wild for microscopic examination of prey remains and molecular analysis. The main prey remains found in the snails' faeces were earthworm chaeta, which were present in 47 of the 49 analysed faecal strings. Because earthworms are soft bodied only chaeta were retrieved from the faeces but their morphology was not diagnostic for species identification. Molecular techniques appear to be promising alternatives as they are potentially very precise in terms of species identification and applicable to soft-bodied prey. The 16S mitochondrial rDNA was chosen to establish an endemic earthworm DNA library to which faeces-extracted DNA was compared. Group-specific primers were designed to extract DNA from earthworms only and a nested PCR approach was conducted to amplify this DNA. Although sequencing revealed the presence of earthworm DNA in the snails' faeces, the majority of the analysed faeces contained a DNA mixture from different predated earthworm species. The 454-pyrosequencing technique will be used to sequence each earthworm species present in these DNA mixtures.

Using comparative phylogeography of freshwater fauna to inform water resource policy

Timothy J. Page and Jane M. Hughes

Australian Rivers Institute, Griffith University, Nathan, Queensland, 4111, Australia

Molecular methods have proven very useful in informing taxonomy, biogeography and threatened species conservation, but can they be also used in formulating water resource policy? We are using comparative phylogeography to assess levels of evolutionary distinctiveness of populations of freshwater fauna in different catchments in eastern Queensland. We are focusing on approximately 30 species of freshwater crustaceans and fishes. This type of work is important because planned inter-basin water transfers, due to the current drought, greatly increase the risk of transferring locally non-native species and divergent populations between catchments. This can result in a species/population driving others to extinction, with unpredictable ecological effects for the entire system. Catchments which prove to host very distinct evolutionary communities will pose a much higher risk of extinctions, and should either not be included in inter-basin transfers or will require a risk amelioration program. What methods should we use analyse all this data? Hopefully you all can help us with that one.

Phylogeography of the rainforest-dwelling pademelons (*Thylogale*) in New Guinea

P. Macqueen[1], J. Austin[2], J. M. Seddon[3], A. W. Goldizen[1]

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[2]*Australian Centre for Ancient DNA, School of Earth & Environmental Sciences, The University of Adelaide, Adelaide 5005, Australia*

[3] *School of Veterinary Science, The University of Queensland, St Lucia 4072, Australia*

Complex geological beginnings including progressive terrane accretion and recent orogeny are thought to have greatly influenced biodiversity in New Guinea. Glacial cycles of the Plio-Pleistocene period may also have influenced the current distribution of inter- and intra-specific genetic diversity. This is due to changes in habitat distribution and intermittent land-bridge connections with surrounding continental islands. Pademelons (*Thylogale*) are rainforest-dwelling macropods found in both the lowland and high montane forests of New Guinea. Therefore, current genetic structuring within and among pademelon species might be expected to reflect the biogeographic history of New Guinea. We used an extensive collection of museum specimens to sample representatives of the three *Thylogale* species from across New Guinea. Data from the mitochondrial control region and cytochrome b gene show two major clades corresponding to western and eastern New Guinea. Genetic structuring within the clades is congruent with geographic region, but not with current morphological species designations. Low levels of genetic divergence among species may indicate either recent dispersal into new habitats, or, recent hybridisation due to changes in habitat distribution during the Pleistocene glacial cycles. In addition, it is hypothesised that two isolated populations of *T. calabyi* have evolved independently from lowland *T. brunii* to exploit the montane subalpine grasslands. The extensive use of preserved museum specimens for genetic analysis in this study demonstrates the increasing importance of museum collections to genetic studies, particularly for regions where comprehensive sampling is difficult.

Coastal Wellington Geckos meet Wairarapa Geckos; morphological cline within *Hoplodactylus maculatus*

Rod Hitchmough[1], Jo Fitness[2], Mary Morgan-Richards[2]

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The common gecko, *Hoplodactylus maculatus*, is widepread in the North Island and morphologically variable. In previous research the species complex was classified into 11-12 putative species based on analysis of allozymes, morphology and nuclear and mitochondrial sequences. Coastal populations are often at high density, and individual geckos small, compared to geckos collected from inland locations. This effect occurs independently in at least three of the species in the complex. *Hoplodactylus maculatus* (in the strict sense) populations found at Turakirae Heads, near Wellington, are significantly smaller than geckos living just 20 km away at Ocean Beach Wairarapa. Initial morphological data suggest that there is a narrow hybrid zone between two distinct populations along the coast from Turakirae Heads and Ocean beach. A coastal transect highlights the within population variation and identifies a narrow cline in body size.

Wellington Geckos meet Wairarapa Geckos; Genetic cline within *Hoplodactylus maculatus*

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Hoplodactylus maculatus populations were sampled for genetic study from six locations between Turakirae Heads, near Wellington and Lake Pounui, Wairarapa, where a sharp transition from small to large geckos is seen. From each site approximately 20 geckos were genotyped for four independent loci. *Hoplodactylus maculatus* populations contain a great deal of genetic variation probably due to their large size, but dispersal distances appear to be relatively small. The two populations at the extreme ends of our coastal transect are very different from one other, but unique alleles are seen in each population within the 25km transect. There is no loss of heterozygosity in adults compared to juveniles and no evidence of linkage disequilibrium, from which we infer that small Wellington geckos do successfully hybridise with large Wairarapa geckos. Concordance of clines suggests that this is a secondary contact zone although it may be maintained by selection for different phenotypes in different rock habitats

An investigation of patterns of connectivity among populations of the Australian mosquito (*Aedes vigilax*) using mitochondrial sequences and microsatellie loci

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The fluctuating changes in climate during the Pleistocene period played an important role in population genetic structure of many species in northern and southern Australia. I investigated the population structure and history of a widespread Ross River Virus vector, the Australian saltmarsh mosquito (*Aedes vigilax*) across the continent.The genetic structure of 379 individuals of this vector (*Ae. vigilax*) was examined using mitochondrial COI sequence variation and nuclear variation at three microsatellite loci. Bayesian analysis revealed two distinctly divergent clades. Clade-I was only found in eastern Australia (Queensland and New South Wales), whereas Clade-II was found throughout the sampling area (Northern Territory, Western and eastern Australia). A molecular clock calibration was used to estimate the divergence time between the two clades to be 0.924 million years. A recent demographic expansion in the late Pleistocene (6 000 – 13 000 years ago) was inferred from haplotype mismatch distribution analysis. Nested Clade Analysis and Fu's Fs tests also indicated evidence of significant population expansion at most sites. An analysis of molecular variance (AMOVA) showed contemporary structure at different geographic scales. This study demonstrated the importance of contemporary and historical processes in determining the population genetic structure of Australian *Ae. vigilax*, suggests that the arid barriers may also have had an impact on geographic variation of widespread populations of this species to form east-west fragmentation during the Pleistocene period. The data reveal patterns of recent historical population expansion of *Ae. vigilax* populations that current levels of limited gene flow subsequent by secondary contact between the east and west fragments in the eastern region. Based on evidence from microsatellite data, there is no indication that the two clades represent species.

Conservation genetics of the kea (*Nestor notabilis*)

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Many endemic avian species of New Zealand have seen their populations plummet with the arrival of Maori and Europeans due to introduced predators and habitat destruction. However, some species, such as the kea (*Nestor notabilis*), are suspected to have undergone population growth and expansion following the introduction of new food sources (game and sheep) by Europeans. By the late 1800s, the species had become so abundant that at least 150,000 individuals were killed under a governmental bounty scheme. Currently, despite its protected status, the species is still rare and possibly in decline. In this study, we will use mtDNA sequence data and microsatellite data for kea and coalescent approaches to estimate its population size prior to human arrival. Knowledge of the historic population size will allow us to determine if kea had increased during the 1800s from a natural low abundance or were naturally common in the Southern Alps. We also plan to investigate the genetic structure and phylogeography of the kea to document patterns of gene flow and identify management units, which will guide kea conservation.

Serial founder effects and population persistence of little spotted kiwi (*Apteryx owenii)*

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Little spotted kiwi (LSK; *Apteryx owenii*) were once found throughout New Zealand but now number approximately 1600 individuals comprising 8 isolated populations. Kapiti Island is home to the largest population (~1200 birds), and is thought to have been founded by five birds in 1912. All other populations of LSK have been subsequently founded with the transfer of 3 to 40 birds from Kapiti Island and 2 birds from D'Urville Island. Microsatellite and mtDNA data suggest that (1) extant populations of LSK have extremely low genetic variation, (2) recently founded populations are genetically divergent from and have lower genetic diversity than their source populations, and (3) the Long Island population does not harbor novel genetic variation, despite its founders including birds from D'Urville Island. LSK are viewed as being the 'safest' kiwi species because they are the only one where all populations are increasing in size and living in predator free sanctuaries. In the short term, serial founder effects do not appear to have reduced population growth rates among LSK. It is unclear, however, how reduced genetic diversity and the inbreeding effect of small populations may influence long term population persistence and evolutionary potential of the species. The current management aim for LSK is to increase their numbers to 2400 birds by 2018 through additional transfer of birds. This effort should include a strategy for minimizing further loss of genetic variation due to founder effects.

Testing the hoki fishery two-stock hypothesis: Neutral markers suggest the need for higher resolution

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Hoki (*Macruronus novaezelandiae*) is New Zealand's most important commercial fish species, and represents the country's third most lucrative seafood product. Yet, relatively few studies have looked at stock discrimination and population structure in this demersal species. Management of this species assumes a two-stock hypothesis, mostly based on differences in morphometric characters and growth rates between the Hokitika Canyon (HC) and Cook Strait (CS) spawning populations. The two main non-spawning aggregations from the Campbell Plateau (CP) and the Chatham Rise (CR) are believed to comprise adult individuals from HC and CS respectively. Previous genetic studies have failed to detect differentiation, but small sample sizes and low-resolution markers may explain these results. Here, we examined the population structure of hoki with a panel of nine microsatellite loci, analysing samples from HC, CS, CP, and CR. Results suggest extensive gene flow between spawning populations. Furthermore, Bayesian clustering algorithms failed to detect substructure in the dataset. On the other hand, treating data as a single sample lead to deviations from Hardy-Weinberg equilibrium in several markers, and an overall heterozygote deficiency ($F_{IS} = 0.045$; $p = 0.01$), suggesting that New Zealand hoki may not constitute a single panmictic population. It is not uncommon to obtain constrasting patterns of differentiation between neutral and putatively selected genetic markers in marine fishes, and we intend to perform shotgun-sequencing of the whole genome of individuals from HC and CS in order to investigate adaptive divergence between these two stocks.

Genetic population structure of the New Zealand sea lion: implications for species management

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The New Zealand sea lion (NZSL), *Phocarctos hookeri*, is one of the world's rarest sea lion species with a population estimate between 10,000- 14,000 individuals. NZSLs have a very restricted breeding range endemic to New Zealand, with only three significant breeding colonies at the sub-Antarctic Auckland (2) and Campbell Islands (1). Over 99% of pup production occurs within these three colonies. After being driven to extinction on the mainland, NZSLs have begun to recolonise the Otago Peninsula, starting in 1981. To date approximately 40 NZSL pups have been born around the Otago coastline. Despite this local increase, the overall population of NZSL is still in decline (e.g. a 31% decrease in pup production was recorded for the 2008/09 breeding season). A major contributor to this decline is by-catch (mainly females) in the arrow squid fisheries (SQU6T) around the Auckland Island shelf. Here we use microsatellite markers to examine the population structure of the NZSL to identify management units and estimate gene flow between breeding locations. This information is important for NZSL management, as it will indicate if NZSL by-catch is impacting on the entire population or just the colonies of the Auckland Islands and will give us an understanding of the genetic processes involved in colony formation.

Assessing genomic variability in species of high conservation priority

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When species are reduced to small, isolated populations, inbreeding effects (a reduction in genetic diversity and an increased extinction risk) may occur. This compromises the ability of populations to evolve in response to environmental change thus diminishing their chances of long-term persistence. Neutral genetic variability at a panel of variable loci may reflect overall genetic diversity, and consequently is a good indicator of fitness. Initially we are determining if neutral genetic variability detected using microsatellite markers is correlated with disease resistance or susceptibility. The focal species for my project is the New Zealand sea lion, *Phocarctos hookeri.* It is the rarest sea lion in the world and has been a protected species since 1896. It has been the subject of three episodes of mass mortality in recent years due to bacterial infection. We are using a panel of 21 microsatellite markers to determine the role of genetic variability in susceptibility or resistance to these disease epizootics. Heterozygosity of microsatellite loci, at both a global and locus specific scale, will be examined for correlations with individual fitness. However, neutral markers may not always accurately reflect the diversity present in genes related to fitness traits. We have identified several genes, that are good candidates to begin to look for functionally important variability associated with disease resistance and reproductive fitness in NZ sea lions. Our data may help better enable the prediction of the level of genetic imperilment, or future evolutionary potential, in other species of conservation significance, aiding future species conservation.

Redressing black rhinoceros translocation bias for longer-term meta-population management at Hluhluwe-Imfolozi game reserve, South Africa

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As the black rhino species recovery progresses, meta-population management priorities will need to change from an emphasis on population size and growth to that of population quality. Sustained recoveries require the maintenance of genetic diversity within reintroduced populations that are representative of source populations. Nevertheless, after 25 years of black rhinoceros translocation, we still do not know the degree to which capture is genetically biased or how many rhino need to be captured in order to translocate a representative genetic sample. Here, I will present results to date from analyses of the mitochondrial control region (n=71) and the first four of ten neutral microsatellite markers I intend to study of black rhinos (*Diceros bicornis minor*) from Hluhluwe-iMfolozi Game Reserve (source population) and founder populations in South Africa (2002-2008). These results will eventually be used to conduct genetic sustainability analyses for founder populations of various sizes subject to supplementary releases of varying frequency and size using VORTEX software to recommend translocation strategies for genetic rescue

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Genetic assessment of stock enhancement of the New Zealand blackfoot abalone (*Haliotis iris)* in Tory Channel, Marlborough Sounds, New Zealand

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Fisheries stock enhancement involves the release of hatchery raised juveniles into the wild to increase harvestable stocks. This has been successfully undertaken for many species including *Haliotis iris*, the New Zealand blackfoot abalone or paua. To determine the success of stock enhancement, hatchery and wild individuals must be distinguishable. For paua, dietary markers are commonly employed, however these become unrecognisable after approximately 2 years in the wild. Genetic markers such as microsatellite DNA, are an effective means of identifying individuals if potential parents are known, and their utility in assessing the viability of stock enhancement programs is becoming recognised. Our primary aim is to assess the effectiveness of microsatellite DNA markers as a means of tracking the survival and dispersal of reseeded juvenile paua in Tory Channel, Marlborough Sounds. This will involve a reseeding experiment where recaptured individuals will be genotyped using a panel of ten microsatellite markers. Subsequent population and/or parentage assignments should be able to be made to a high degree of probability, ultimately allowing survival rates to be determined. A preliminary assessment of the genetic population structure existent within Tory Channel will also be undertaken. This will provide baseline data to monitor the genetic consequences of stock enhancement on the wild population and determine best breeding practices to achieve maintenance of genetic variation and local adaptations during stock enhancement. Insights from this study may provide steps towards the adoption of stock enhancement as a means of managing and enhancing the productivity of paua stocks in New Zealand.

Mussels in distress: gene expression and environmental stress

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Mussel communities dominate many exposed rocky shores in New Zealand. The ability to understand how increases in temperature affect mussels, therefore, is critical in predictions of how rocky intertidal communities might respond to warmer seas. Many intertidal organisms live at or near their thermal tolerance limits, and so changes in the thermal climate are likely to alter their biogeographic distributions. Mussels are useful in such studies on thermal stress because they may be key habitat-responders and their loss or reduction would greatly reduce intertidal diversity because of the wide range of taxa that rely on them for shelter from hydrodynamic forces and predators. Through a comparative experimental approach, translocating organisms between two different shore heights, this study examines gene expresssion in two species of mussel, *Mytilus galloprovincialis* and *Perna canaliculus*. Collections have been made from around the South Island of New Zealand at various times throughout the year. We are studying the expression levels of stress response genes such as heat shock proteins (HSPs). Their expression levels can be used to assess the physiological condition of these animals. Using quantitative PCR, the relative expression levels of *hsc71* in the spring and summer is being evaluated. This study will highlight physiological responses to thermal stress of ecologically critical marine invertebrates across New Zealand.

Intergenerational responses of aquatic biota to low level contamination in urban streams

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There are many examples of rapid genetic changes or small-scale evolutionary processes (micro-evolution) resulting from exposure to contaminants. These changes are ultimately reflected in an increase in contaminant-tolerant genetic variants, with loss of more sensitive forms. Reduced genetic diversity has also been associated with greater susceptibility to additional/different stressors. Given the importance of genetic diversity as one of the components of biodiversity, a method for detecting the effects of chronic, low-level contamination on multiple generations of aquatic biota would be a powerful tool for elucidating evolutionary scale responses. Utilising a combination of field and laboratory experiments, we examined the effects of stormwater on a previously unexposed adult population of the freshwater clam *Sphaerium novaezelandiae*. We assessed both physiological and reproductive measures of fitness and examined genotype-specific selection within a single generation. We found that exposure to contaminated field-collected stormwater resulted in reduced juvenile production. We also found that pre-exposure to field-derived stormwater conferred reduced adult fitness (survival, reporductive success) when further exposed to zinc in solution. Finally, we observed a shift in genotype frequency between adult and juvenile populations associated with exposure to stormwater contamination, suggesting a selection effect within a single generation. The value of these results for urban managers includes the ability to better predict long-term responses of stream communities to stormwater and also to assess the resilience of these communities to additional/different types of stressors.

Telling the evolutionary time: lichen edition

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The lichenized ascomycetes are a diverse group of ca. 13 500 species, found in a wide range of habitats from every continent on the planet. Like many other groups of organisms, molecular phylogenetic studies on this group have now become commonplace. However, unlike many other groups, lichen systematists have nearly completely avoided the use of molecular divergence dating techniques. This is no doubt largely due to two factors. The first being the highly controversial nature of the molecular clock concept, which some biologists have equated with "reading the entrails of chickens." The second being the extreme poverty of the lichen fossil record which makes reliably calibrating lichen phylogenies a difficult procedure. Here we use the largest family of lichens, the Parmeliaceae, and the most robust method of divergence dating, Bayesian estimation, as a model for studying the latter of these two concerns. The result is a time-calibrated phylogeny that is not exceedingly precise, but that still produces relative dates with high levels of confidence. We argue that modern molecular clock techniques offer much to lichen systematists, and that their ongoing neglect be remedied.

Fine-scale genetic structure of mainland *Rattus rattus* populations: implications for restoration of Puketi Forest Conservation Reserve, New Zealand

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The ship rat or black rat (*Rattus rattus*) is one of the most widespread invasive rodent species on earth and is a known cause of extinction of several endemic species in invaded ecosystems. While some information is available for insular populations, very little is known about the genetic population structure of these rats on mainland areas. In this study, we focused on fine-scale population structure of rats located in the Puketi Forest Conservation Reserve, Northland, New Zealand, to help conservation managers optimise a control program. We used eight microsatellite markers and classical population genetics tools (Fst, clustering methods) as well as individual-based descriptive methods using GPS coordinates for each sample (Genetic Landscape Shape, Bandwidth Mapping) in order to understand whether there was any undetected genetic structure over the 5-km2 area. Very little genetic structure was detected. Nevertheless, a weak but significant isolation-by-distance pattern was inferred. No isolation with external sites (encompassing an area up to 20 km2) was found, suggesting the presence of a contiguous population at even larger scale, exchanging genes mainly between neighbours. We discuss the implications of these findings in terms of management of ship rats to protect native biodiversity.

How to use all the information in the site frequency spectrum about the unknown genealogy?

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This talk will focus on a recent methodology to obtain samples from the posterior distributionover Kingman's unlabeled n-coalescent genealogies on the basis of the site frequency spectrum. The emphasis is on nonparametric approaches to inferring the coalescent epoch times or times to commonancestors. The idea involves the use of controlled Markov chains. The talk will focus on applicationsof this methodology to population genetic inference in the spirit of approximate Bayesian computation.
