

11th Annual Molecular Ecology Meeting 28th-30th November, Lake Okataina



Programme and Abstracts

University of Waikato
Department of Biological Sciences

2008

Name:

Stephane



THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

PROGRAMME & ABSTRACTS

Eleventh Annual New Zealand Molecular Ecology Meeting
28-30 November 2008

Lake Okataina Outdoor Centre, Rotorua

Speakers and Abstracts

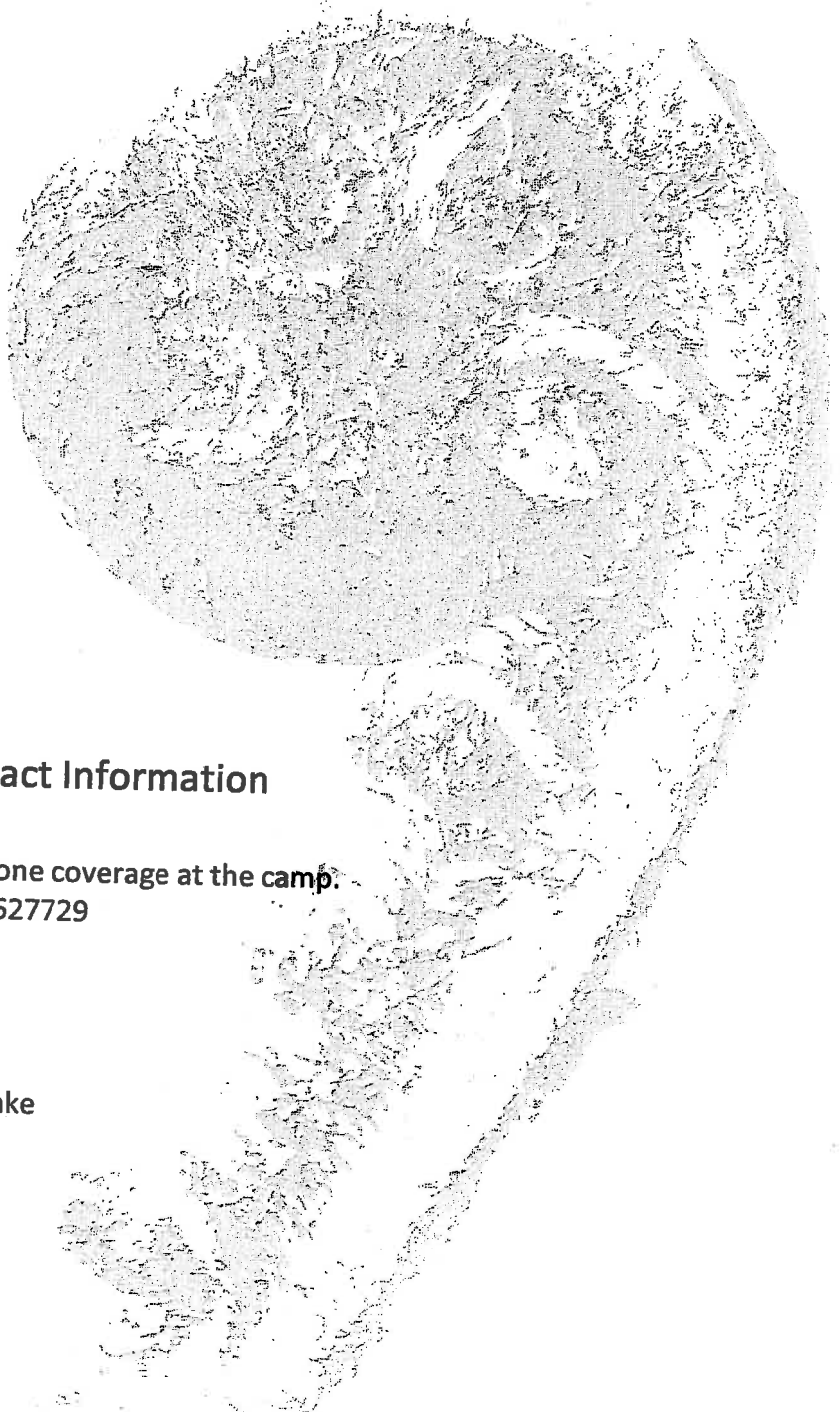
Robin Atherton
Jawad Abdelkrim
Samuel Brown
Lisa Bryant
Susan Carrodus
Ritendra Nath Das
Monica Gruber
Elizabeth Heeg
Simon Hills
Fiona Hodge
Olivia Holland
Gary Houliston
Beatrix Jones
Sarah Kelly
Hayley Lawrence
Jagoba Malumbres-Olarte
Mary Morgan-Richards
Jennifer Nickel
Sebastien Rioux Paquette
Ngaire Phillips
David Pontin
Lara Shepherd
Daniel White

Emergency & Camp Contact Information

There is minimal to no mobile phone coverage at the camp.
The camp phone number is: 07 3627729

Organising Committee

Phil Ross Cindy Cadman-Satake



Programme

Friday 28 November		
1700	Arrivals and registration	
1900	Diner	
Saturday 29 November		
0745	Breakfast	
0900	Welcome by Ian Hogg	
Session 1	Chair: Ian Hogg	
0915	Simon Hills (Massey, Palmerston North)	The Strange Case of <i>Alcithoe wilsoni</i> and <i>Teremelon knoxi</i>
0930	Beatrix Jones (Massey, Auckland)	Blocks of linked SNPs for parentage analysis
0945	Daniel White (Otago)	The Mitochondrial Bottleneck and Inheritance of Mitochondrial Heteroplasmy in Chinook Salmon (<i>Oncorhynchus tshawytscha</i>)
1000	David Pontin (Lincoln)	Identifying New Zealand's <i>Physalia</i> complex using neighbour networks
1015	Elizabeth Heeg (Victoria)	The population genetics and rapid evolution of rainbow trout from Lake Taupo catchment, New Zealand
1030	Jawad Abdelkrim	Fast, cost-effective development of species-specific microsatellite markers by genomic sequencing
1045	Morning Tea	
Session 2	Chair: Shane Lavery	
1115	Jogoba Malumbres-Olarte (Lincoln)	A DNA database for completing "incomplete" ecological studies on spiders
1130	Lisa Bryant (Victoria)	DNA barcoding of pelagic amphipods: A preliminary phylogeny of Antarctic Hyperiidea and a tool for identification
1145	Lara Shepard (Massey, Palmerston North)	Hybridization and phylogeography of New Zealand's <i>Pseudopanax</i> trees.
1200	Ngaire Phillips (NIWA)	Genotype-dependent recovery to heavy metal contamination in the freshwater clam <i>Sphaerium novaezelandiae</i> Deshayes 1853
1215	Susan Carrodus (Waikato)	Identification and the role of hybridisation in <i>Pittosporum</i>
1230	Lunch	
1400	Shuttle departs for rafting	
1430	Rafting/bush walks/etc	
1600	Afternoon Tea	

Session 3	Chair: Rob Cruickshank	
1720	Jennifer Nickel (Waikato)	Investigating the genetic diversity and dispersal range of New Zealand's seahorse, <i>Hippocampus abdominalis</i> .
1735	Monica Gruber (Victoria)	Reduced genetic diversity is associated with increased population densities of invasive yellow crazy ants in Arnhem Land, Australia
1750	Ritendra Nas Dath (Victoria)	Molecular diversity of moko disease causing strains of <i>Ralstonia solanacearum</i>
1805	Mary Morgan-Richards (Massey, Palmerston North)	Rapid morphological evolution or mtDNA introgression?
1830	Soccer/Football	North vs. South
1930	Dinner	
2030	Pub quiz	
Sunday 30 November		
0745	Breakfast	
Session 4	Chair: Jonothan Banks	
0900	Gary Houlston (Landcare)	Conservation genetics of <i>Olearia gardneri</i> ; a critically endangered tree daisy
0915	Olivia Holland (Landcare)	Impacts of genetic variation in the possum major histocompatibility complex on response to immunocontraceptive vaccines
0930	Robin Atherton (Massey, Palmerston North)	Tracing the domestication history and whakapapa (relationships) of cultivated New Zealand plants using DNA markers.
0945	Sam Brown (Lincoln)	Molecular systematics of South Pacific <i>Carpophilus</i> (Coleoptera: Nitidulidae)
1000	Morning Tea	
Session 6	Chair: Mary Morgan-Richards	
1030	Sarah Kelly (Waikato)	A Phylogeographic Investigation of the Origin of Rock Snot in New Zealand.
1045	Sebastian Paquette (Victoria)	Investigating male-biased dispersal in tortoises with microsatellite data
1100	Fiona Hodge (Victoria)	Hybrids and hybrid zones in <i>Carpophyllum</i>
1115	Hayley Lawrence (Landcare)	Conservation Genetics of NZ's Rarest Seabird, Hokopapa o tch Tchäik, Whakapapa o te Täiko
1200	Lunch	
1300	Cleanup	
1400	Departure	

ABSTRACTS

Fast, cost-effective development of species-specific microsatellite markers by genomic sequencing

Jawad Abdelkrim, Bruce Robertson, Jo Ann Stanton, Neil Gemmell

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Microsatellites are the genetic markers of choice for many population genetic studies, but must be isolated *de novo* using recombinant approaches where prior genetic data is lacking. Here we utilized high-throughput genomic sequencing technology to produce millions of base pairs of short fragment reads which were screened with bioinformatics toolsets to identify primers that amplify polymorphic microsatellite loci. Using this approach we isolated 13 polymorphic microsatellites for the blue duck, a species for which limited genetic data were available. Our genomic approach eliminates recombinant genetic steps, reducing significantly the time and cost requirements of marker development compared to traditional approaches

Tracing the domestication history and whakapapa (relationships) of cultivated New Zealand plants using DNA markers.

Robin Atherton, Pete Lockhart, Nick Roskrige, Peter de Lange, Lara Shepherd

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The domestication of plants and animals was the most important development in human history during the past 13 000 years. Most crops are thousands of years old, with aspects of their initial domestication indeterminable. However, the relatively recent settlement of New Zealand by Māori provides a unique opportunity to investigate the early phase of crop domestication. We are using genetic analyses of five endemic New Zealand plants cultivated by Māori to examine recent domestication. We aim to detect the number of times each species was brought into cultivation and the way in which domestication has reduced genetic diversity in the cultivated populations. Genetic data will also be combined with traditional oral histories, where available, in order to reflect and illuminate pre-European Māori settlement routes and mobility.

Molecular systematics of South Pacific *Carpophilus* (Coleoptera: Nitidulidae)

Samuel Brown

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The cosmopolitan sap beetle genus *Carpophilus* Stephens consists of over 200 species, of which around 27 have been recorded from the South Pacific. Preliminary parsimony, maximum likelihood and Bayesian phylogenies of 12 species from the Pacific, Australia and North America were inferred from 600bp of each of the 5' COI mitochondrial and 28S D1-D2 rRNA nuclear gene regions. These initial results indicate the monophyly of the South Pacific endemic species *C. oculatus*, and the sister-species relationship of *C. oculatus* and *C. maculatus*. The overall phylogeny and interesting features within the *C. oculatus* clade will be discussed.

DNA barcoding of pelagic amphipods: A preliminary phylogeny of Antarctic Hyperiidea and a tool for identification

Lisa Bryant¹, Els Maas², Simon Davy¹, Ken Ryan¹

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² NIWA

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Amphipods are well known as ubiquitous taxa in the Antarctic marine system. Despite their obvious importance to Antarctic ecosystems, researchers often have difficulty accurately and confidently identifying all but the most common species, leading to much confusion in ecological studies. As well as being one of the most speciose crustacean orders, problems also occur due to the fact cryptic speciation is not uncommon in the Amphipoda, and yet other species exhibit relatively high levels of polymorphism. These issues have also left many groups within the Amphipoda in desperate need of systematic revision. The 650-base fragment of the 5' end of the mitochondrial cytochrome oxidase I subunit (mtCOI, or *cox1*) gene is a very useful taxonomic and phylogenetic marker for many taxa, including Amphipoda, and is commonly referred to as a 'DNA barcode' for fauna. Here I will present the first preliminary phylogeny of Antarctic members of the pelagic amphipod suborder Hyperiidea collected in the Ross Sea, Antarctica, and discuss the value of the mtCOI gene as an identification tool for amphipods.

Identification and the role of hybridisation in *Pittosporum*

Susan Carodus, Chrissen Gemmill and Bruce Clarkson
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The genus *Pittosporum* has many closely related species and a number of hybrids, and also contains a number of heteroblastic species (with different juvenile and adult forms). Godley (1985) proposed that species which have a distinct juvenile and adult

form may be derived from hybridisation between two species which resemble the 'hybrid' at each stage; and proposed that the rare species *Pittosporum turneri* is a putative hybrid, as it exhibits a divaricating juvenile phase which is totally distinct from the non-divaricating adult form. This study aims to identify whether *Pittosporum turneri* is derived from hybridisation between a divaricating shrub (*Pittosporum divaricatum*) and a non-divaricating tree (*Pittosporum colensoi*).

Molecular and morphological methods as well as a cross-pollination experiment have been employed to identify hybridisation in *P. turneri*. Cross pollination between the putative parents of *P. turneri*; *P. colensoi* and *P. divaricatum* was undertaken in the wild between female flowers of *P. divaricatum* and male flowers of *P. colensoi* to demonstrate whether hybridisation between these species is possible. Analysis of the maternally inherited TrnT-TrnL region of chloroplast DNA was undertaken to compare with data from the biparentally inherited internal transcribed spacer of nuclear ribosomal DNA (ITS) for all New Zealand *Pittosporum* species, to identify incongruencies between two phylogenies based on biparentally inherited and maternally inherited DNA sequences. Additionally, inter simple sequence repeats (ISSR's) and allozymes were used to attempt to identify hybridisation between *P. colensoi* and *P. divaricatum*.

Molecular diversity of moko disease causing strains of *Ralstonia solanacearum*

Ritendra N. Das¹, Mark Fegan² and Lindsay I. Sly³

¹Victoria University of Wellington

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Moko disease, caused by *Ralstonia solanacearum* race 2, is a devastating disease of bananas and plantains. *R. solanacearum* can be divided into four broad genetic groups called phylotypes. *R. solanacearum* race 2 strains belong to phylotype II. A Phylotype-specific multiplex PCR was used to verify the phylotypes of 69 moko and 21 reference strains of *R. solanacearum*. Within phylotype II, *R. solanacearum* race 2 strains belong to four genetic groups termed sequevars. A sequevar-specific multiplex PCR revealed that of the 69 moko strains used in this study, from various geographical locations, 30 belonged to sequevar 3, 21 to sequevar 4, 9 to sequevar 6 and 9 to sequevar 24. The sequevar 3 strains were from Costa Rica, Honduras, Panama and the Philippines. The sequevar 4 strains were from Peru, Costa Rica and Cuba. The sequevar 6 strains originated from Honduras, Hawaii, Venezuela, Guyana and Australia (eradicated). All sequevar 24 strains were from Brazil. The genomic fingerprinting methods rep-PCR and pulsed field gel

electrophoresis (PFGE) were employed to identify the genetic variation within moko disease causing strains of *R. solanacearum*. As expected PFGE was more discriminatory than rep-PCR for strains belonging to Phylotype II, sequevars 3 and 24. Both methods were equally discriminatory for Phylotype II, sequevar 6 strains. However, rep-PCR was found to better discriminate strains belonging to phylotype II, sequevar 4. Overall a large amount of genetic diversity was revealed within *R. solanacearum* race 2 moko disease causing strains.

Reduced genetic diversity is associated with increased population densities of invasive yellow crazy ants in Arnhem Land, Australia

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The reduction of genetic diversity common during biological invasions does not preclude the success of exotic organisms in a new environment. Reduced genetic diversity in invasive ants is hypothesized to be associated with increased population densities and abundance and greater impacts on invaded communities. In north east Arnhem Land the invasive yellow crazy ant forms two colony types which have distinctly differing population densities. We used microsatellite data to investigate the genetic differences between colony types using a combination of analyses including traditional and Bayesian approaches to population structuring and bottleneck tests. Overall microsatellite diversity was low in the study populations. Colonies with lower genetic diversity were associated with higher population densities. Population structuring suggested a transition between colony types associated with changes in genetic diversity. Management that results in incomplete eradication could therefore result in genetic bottlenecks that might enhance invasive success and impacts. However, the introduction of ants from genetically divergent populations could be explored as a method to reduce the ecological impacts of colonies with low genetic diversity, until eradication can be effected. Our study supports the hypothesis that loss of genetic diversity is correlated with increased population densities in highly invasive ants.

The population genetics and rapid evolution of rainbow trout from Lake Taupo catchment, New Zealand

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¹Victoria University of Wellington

²Department of Conservation,

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The rainbow trout (*Oncorhynchus mykiss*) of Lake Taupo provide an exceptional opportunity to explore the rapid evolution of spawning time in salmonids, since they have a long history of management and were introduced from California 100 years ago. Recently their spawning time has shifted to later in the season, over a shorter period, and increasingly smaller fish have been observed in the fishery, possibly due to fishing pressure or imposed size regulations. This talk will focus of the results to date of our study of the genetic subdivision within the Taupo populations, part of a larger study of the distribution of genotypes between early and late spawn times. We've analyzed approximately 50 samples from 6 locations throughout the lake's catchment at 5 microsatellite loci to begin to ascertain population structure. A summary of the preliminary results of our genotyping of the *clock1b* locus will also be presented. A better understanding of the population genetic structure and how fishing activity has affected the spawning time of these fish will assist the Department of Conservation in the management of an important resource, and will further our understanding of the species' evolutionary response to novel environments and harvest pressure.

The Strange Case of *Alcithoe wilsoni* and *Teremelon knoxi*

Simon Hills

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Use of the fossil record to calibrate molecular phylogenies relies on our ability to identify species, both extant and extinct. The deepest fossil calibration point for New Zealand volutes is based on a 10 million year old *Teremelon knoxi* fossil that is morphologically identical to extant *T. knoxi*. In the extant fauna *T. knoxi* has been considered distinct enough to be a different genus from *Alcithoe*, the predominant lineage of volutes in New Zealand waters. However, some forms of the highly morphologically variable species *Alcithoe wilsoni* are similar to some *T. knoxi* specimens. In addition to differences in key features of shell morphology, these species had been thought to be ecologically separate, with *T. knoxi* in deeper water (450m – 600m), and *A. wilsoni* in shallower water (50m – 500m). Molecular analysis indicates that these two entities are one highly morphologically variable species. Haplotype network analysis shows very little genetic structure, with no correlation between haplotype and distribution or depth. Given the lack of genetic structure it is likely that the great variety in shell shape is a result of eco-phenotypic differences, and it is possible that some of these differences can be elucidated by specific shell characters. These analyses indicate that it is appropriate to assign the 10 million year calibration

point to *Alcithoe wilsoni*. In initial molecular clock analysis of the *Alcithoe* this calibration is consistent with the molecular data. This work highlights potential hazards with species identification in the fossil record, and shows how molecular analysis of extant species can inform interpretation of extinct species.

Hybrids and hybrid zones in *Carpophyllum*

Fiona Hodge

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Hybridisation is an important evolutionary process. It is also interesting ecologically as it creates 'instant' variation, both in genotypes and phenotypes, and unusual distributions. Many studies document 'hybrid zones' where hybrids occupy a discrete and narrow habitats between the parent species ranges. Investigating morphology and distributions within a hybrid complex can allow hypothesis to be generated about the relationship between distribution, phenotype fitness and environmental conditions. Molecular work has confirmed hybridisation to be occurring between the common brown algae *Carpophyllum angustifolium* and *Carpophyllum maschalocarpum* using nuclear encoded ITS markers. This study investigates the morphology and distribution of the parental taxa and the hybrids in the high subtidal. It finds the hybrids have distinctive morphologies, and intermediate distributions. Furthermore there appears to be evidence of introgression between the hybrids and *C. angustifolium*. This work helps us understand the ecological and potential evolutionary implications of hybridisation in this system.

Impacts of genetic variation in the possum major histocompatibility complex on response to immunocontraceptive vaccines

Olivia Holland^{1,2}, Phil Cowan¹, Dianne Gleeson¹, Larry Chamley²

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² University of Auckland

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The brushtail possum (*Trichosurus vulpecula*) is a major invasive pest in New Zealand. One option for its control is the use of immunocontraceptive vaccines, a form of fertility control. Initial trials of vaccines have shown individual variation in immune response. The use of vaccines on wild populations could result in selection for possums that remain fertile because of low or no immune response and raises the possibility that a vaccine resistant population could evolve. Understanding the basis of this variation is therefore important. The major histocompatibility complex (MHC) is an important influence on the nature of immune responses. The aim of this research was to investigate the genetic

variation in MHC loci in the possum and to identify variants associated with different individual response to immunocontraceptive vaccines. High levels of polymorphism and evidence of population structuring were found in the possum MHC loci. The analysis of possible associations between MHC alleles and response to immunocontraceptive vaccines has identified potential predisposing haplotypes. Specifically, two MHC haplotypes were found to be significantly associated with differences in response to immunocontraceptive vaccines, Hap 6 was associated with a reduced response and Hap 9 was associated with an increased response to vaccines. The results from this study indicate that the MHC has a role in differing possum responses to immunocontraceptive vaccines and demonstrates that the design of contraceptive vaccines for possum control should take into consideration MHC variability.

Conservation genetics of *Olearia gardneri*; a critically endangered tree daisy.

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Thirteen populations of the critically endangered tree daisy, *Olearia gardneri*, were studied using AFLP markers. This represented almost all of the extant individuals of this plant in the wild. Genetic variation was at similar levels to other long-lived tree species in New Zealand and also like many studies to date had poor correspondence between genetic and geographic distance.

Genetic factors like inbreeding depression and the loss of genetic diversity might lower fitness and have substantial consequences for evolution and survival of rare threatened plants. With less than 160 individual plants, *Olearia gardneri* is the third-rarest tree in New Zealand and a good model to study evolutionary process in fragmented endangered plants.

Due to the obvious decline of this species in recent time it is imperative that conservation measures are undertaken for this species, including revegetation work. While there has been considerable emphasis on "eco-sourcing" in plant recovery programmes there is strong evidence that this may not be the best strategy for *O. gardneri* due to breeding system considerations.

Blocks of linked SNPs for parentage analysis

Beatrix Jones¹ and Anthony Fiumera²

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Single nucleotide polymorphisms (SNPs) are plentiful in most genomes, and amenable to high throughput genotyping. However, because they are biallelic,

their per-marker parentage information is typically low. Thus, in non-model organisms the process of identifying an adequate number of unlinked SNPs can be daunting. This talk explores the possibility of using blocks of tightly linked SNPs as highly polymorphic molecular markers for reconstructing male genotypes in polyandrous organisms with moderate (5 offspring) to large (25 offspring) clutches of offspring. Haplotypes are inferred for each block of linked SNPs using the programs HAPLORE and PHASE 2.1. Each multi-SNP haplotype is then treated as a separate allele, producing a highly polymorphic, "microsatellite-like" marker. A simulation study is performed using haplotype frequencies derived from empirical data sets from *Drosophila melanogaster* and *Mus musculus* populations. We find that the markers produced are competitive with microsatellite loci in terms of single parent exclusion probabilities, particularly when using 6 or more linked SNPs to form a haplotype. These markers contain only modest rates of missing data and genotyping or phasing errors and thus should be seriously considered as molecular markers for parentage analysis.

A Phylogeographic Investigation of the Origin of Rock Snot in New Zealand.

Sarah Kelly, Chrissen E.C Gemmill, S. Craig Cary

The University of Waikato

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Didymosphenia geminata, commonly known as "Didymo" or "Rock Snot", is a freshwater diatom, a photosynthetic alga with a silica shell. It attaches to rocks and plants by its mucilaginous stalks, its large blooms often covering all available substrates. In the last few decades, Didymo has been increasing in abundance and geographical range worldwide, altering river ecosystems and biodiversity.

Didymo, first discovered in New Zealand in October 2004, has spread rapidly through the South Island. As part of the response to the spread of Didymo in New Zealand, the University of Waikato developed Didymo-specific primers to enable a highly sensitive detection method using quantitative polymerase chain reaction (qPCR). We are using these primers to conduct a phylogenetic study of Didymo samples from around the world. We are looking at the internal transcribed spacer (ITS) region, which displays enough variation to distinguish intraspecific differences within *Didymosphenia geminata*.

This study will reveal which samples are most similar to New Zealand's Didymo, determining its most likely source. It may also determine whether there have been multiple introductions of Didymo to New Zealand. Preliminary results indicate that North America is the most likely source of New Zealand's Didymo. Future studies will include more local and

international samples and other molecular markers will increase the confidence of our results. Such information is useful in targeting control to prevent future introductions and spread.

Conservation Genetics of NZ's Rarest Seabird, Hokopapa o tch Tchäik, Whakapapa o te Täiko

Hayley Lawrence¹, Graeme Taylor², Craig Millar³ and David Lambert⁴

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² *Department of Conservation*

³ *University of Auckland*

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The Chatham Island Täiko (Tchäik, *Pterodroma magentae*) is one of the world's most endangered seabirds. Genetic techniques were used to investigate aspects of Täiko biology and relationships in order to aid Täiko conservation. Mitochondrial DNA markers were sequenced in almost the entire known Täiko population. The level of genetic variation revealed in Täiko was unexpectedly high. Sequencing of ancient DNA from subfossil Täiko bones showed a large proportion of the genetic diversity of the extinct Täiko populations was retained in the remnant population. However, genetic variation in Täiko chicks was low, thus genetic diversity in the population could be lost in just a few generations. DNA sexing revealed that the many non-breeding Täiko were almost all male, which signified a potential Allee effect (i.e. that a reduced density of potential mates is decreasing population productivity). Further understanding of the Täiko mating system and behaviour was obtained by parentage, sibship and pairwise relatedness analyses of genotypes at eight microsatellite DNA loci. It is important that Täiko are found so they can be protected from introduced predators. Results indicated that there are likely to be more Täiko breeding in undiscovered areas. Analysis of philopatry using genetic markers can assist conservation by the identification of areas to search for these undiscovered individuals.

A DNA database for completing "incomplete" ecological studies on spiders

Jagoba Malumbres-Olarte, A. Paterson, R.

Cruickshank & C. Vink

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Many ecologists are still reluctant to use molecular technology in the belief that it will not add any necessary or even relevant information to their research. A number of studies, however, could benefit enormously from such technology. Entomological, and in particular arachnological (spiders), studies are often limited by the inability to identify juvenile specimens morphologically. The

consequences of such limitations go beyond simple underestimation of species richness, reducing resources for practical aspects of a study and even drastically changing conclusions relevant for conservation management decisions.

Although available techniques such as high resolution melting (HRM), single-strand conformational polymorphism (SSCP) and restriction fragment length polymorphism (RFLP), have the potential to help identify juvenile individuals of some taxa rapidly and at a low cost, they require basic data on the sequences of the species under study. Unfortunately this data is not available for many key ecological taxa, such as spiders, in New Zealand. This is one of the many reasons why we have started a DNA database of the spider species present in New Zealand.

Rapid morphological evolution or mtDNA introgression?

Mary Morgan-Richards¹, Simon Hills¹, Bruce Marshall², James S. Crampton³

¹ *Massey University*

² *Te Papa Museum*

³ *GNS Science*

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Rates of morphological evolution are not constant but hybridisation can also mislead us about genetic divergence. The lined whelk, *Buccinum colensoi* is a common snail found under rocks from low tide to 100m, but only on the east coast of North Island, New Zealand. This species is morphologically distinct from two sub-species of *Buccinum vittatum* but genetically it falls within the diversity of the southern sub-species *B. v. littorinoides* (sequences of ND3 (mtDNA)). We are using nuclear genetic data to distinguish between introgression and rapid morphological evolution. Has *B. colensoi* captured the mitochondrial genome of *B. v. littorinoides* via hybridisation? Or does the distinct shell of *B. colensoi* represent a rapid morphological change due to selection on a distinct substrate found on the East Coast?

Investigating the genetic diversity and dispersal range of New Zealand's seahorse, *Hippocampus abdominalis*.

Jennifer Nickel, Ray Cursons and Chrissen Gemmill
The University of Waikato.

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New Zealand's waters are home to a single, highly morphologically variable, species of seahorse, *Hippocampus abdominalis*. This species is listed as 'endangered' and 'data deficient' on the 2003 IUCN Red List of Threatened Species and, along with all other seahorse species, is included on CITES Appendix II. To contribute to the knowledge about

this species, we have undertaken a study to investigate the genetic diversity and dispersal range of this species.

We have collected approximately 140 dorsal fin clips from both wild and aquarium specimens from all areas around New Zealand. Both nuclear (four microsatellite loci) and mitochondrial DNA (cox1, cytochrome B, control region) variation has been analysed, together encompassing both the population and phylogenetic level. Preliminary data show that the control region and cox1 gene are more phylogenetically informative than the cytochrome B gene, and the microsatellite loci are highly polymorphic.

Hatched juveniles are thought to go through a month long pelagic phase, but how far the ocean currents carry them along the New Zealand coastline is not known. The data generated by this study will also provide inferences as to the dispersal ranges of this species along the coast and the extent of gene flow between populations.

Current and possible future threats to this species, in a purely New Zealand context, are also being investigated. Collectively this study provides important data which will contribute to future conservation management strategies.

Investigating male-biased dispersal in tortoises with microsatellite data

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Sex-biased dispersal is well documented in birds and mammals, but has seldom been investigated in other taxa, including reptiles in general and, more specifically, non-marine chelonians. In these species, nest-site fidelity observations are frequent but remain to be associated with female natal homing, a behavioral trait associated with male-biased dispersal. Here, we tested the hypothesis that dispersal should be male-biased in the radiated tortoise (*Astrochelys radiata*) from southern Madagascar, a result that would point towards natal homing in this species. Relying on data from 13 microsatellite markers, we investigated the genetic structure of male and female tortoises from two distinct populations. We found clear evidence that geographically close female tortoises are more related than males. All Mantel tests conducted with matrices of genetic distances or relatedness values and matrices of geographic distances indicated significant structuring in females and the absence of structure in males. Furthermore, two analytical approaches designed to assess general trends in sex-specific dispersal strongly supported male-biased dispersal in the two radiated tortoise populations. These exciting new results should form the basis of future research on natal homing in tortoises. They also highlight the importance of identifying nesting

sites for conservation initiatives, and should also be considered in recovery plans of depleted radiated tortoise populations, characterized by male-biased sex ratios due to the selective harvesting of females by poachers.

Genotype-dependent recovery to heavy metal contamination in the freshwater clam *Sphaerium novaezelandiae* Deshayes 1853

Ngairé Phillips and Chris Hickey

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Urban streams are exposed to a complex array of factors which lead to degradation of stream function and ecological values. In addition to diffuse and point sources of contaminants, habitat alterations can alter biotic integrity. In extreme situations this can result in changes to fundamental life-support functions (e.g. dissolved oxygen). In such cases, contamination effects are likely to be secondary. However, the more common situation in urban streams is where stream function is only partially impacted and biotic communities retain some degree of intactness. But how resilient are such communities to long-term low level and highly time-variable contaminant exposures? Such effects may not be seen within a single life time and require measures that integrate over longer time scales. Currently used methods are largely only suited to assessing responses within short time frames. In contrast, the genetic structure of populations retains responses to contamination for at least one generation following elimination of the contaminant source. High stress levels may reduce or eliminate genetic diversity, resulting in a reduction in the ability of populations to adapt to changing environments. The aim of this investigation was to determine the genetic basis for resistance and resilience to a single contaminant (zinc). We found evidence for genotype-dependent responses to contaminants. Additionally, we found that energy production (as measured by activity levels) was correlated with genotype, providing a link between genotype and individual fitness. These findings have implications for the predictability of chronic effects of stormwater contaminants on aquatic ecosystems.

Identifying New Zealand's *Physalia* complex using neighbour networks

David R. Pontin and R.H. Cruickshank

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Physalia physalis (Portuguese Man O War) and *P. utriculus* (bluebottle) have both been described as present in New Zealand waters, however reports are often conflicting and the presence of either species has never been confirmed. Fifty four specimens

collected from 13 locations from around New Zealand and Australia were sequenced for both the mitochondrial gene, Cytochrome c oxidase I (COI), and the internal transcribed spacers (ITS) of the nuclear ribosomal gene cluster. The sequences were analysed using split decomposition neighbour networks to determine conflict between clades. Three clades were identified from both the COI and ITS sequences, none of which correspond to *P. physalis*. The results suggest that molecular phylogenetics of *Physalia* is complex and it appears likely that hybridisation has occurred as clades were not consistent between genes. Furthermore there is a strong potential that there are three or more species present in New Zealand and only one of these is possibly a named species. It may be necessary to undertake a global review of the genus to establish the correct taxonomy and clarify what species are present.

Hybridization and phylogeography of New Zealand's *Pseudopanax* trees.

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AFLPs, microsatellites and chloroplast DNA sequences were used to investigate hybridization between coastal five-finger (*Pseudopanax lessonii*) and lancewood (*P. crassifolius*). These two species hybridize extensively wherever they co-occur, producing a wide array of morphologically intermediate hybrids. Our data suggests that little introgression is occurring between the two species and that the majority of hybrids are later-generation hybrids. Preliminary phylogeographic patterns are presented for coastal five-finger and lancewood as well as for the threatened fierce lancewood (*P. ferox*). The phylogenetic relationships between all 12 *Pseudopanax* species, as inferred by AFLP and chloroplast sequences, will also be discussed.

The Mitochondrial Bottleneck and Inheritance of Mitochondrial Heteroplasmy in Chinook Salmon (*Oncorhynchus tshawytscha*)

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Vertebrate, mitochondrial heteroplasmy is less abundant than one would expect, in the absence of external factors. One such factor is a mitochondrial bottleneck at early stages of development, believed to be an effective means of ridding mtDNA populations of harmful mutations. Although its

existence is widely acknowledged, it is contentious whether it is at embryogenesis or oogenesis that the effects of the bottleneck are most strongly felt. To address this, we have measured mitochondrial heteroplasmy at various stages of development across one generation of Chinook salmon (*Oncorhynchus tshawytscha*), including somatic and gametic tissue of a mother fish, and the somatic tissue of fry. A combination of likelihood and Bayesian analysis of the variance in heteroplasmy measures between developmental stages revealed the effective number of mitochondrial molecules (N_e) transmitted to mother's oocytes to be 109.3 (95% CI 62.4 to 189.6), and between mother and fry to be 105.4 (95% CI 70.3 to 153.1). Thus, the mitochondrial bottleneck is most strongly felt at oogenesis in Chinook salmon, with a reduction of total to effective mtDNA population size in the order of 10^9 to 10^2 . Using $2N_e$, a predicted time to fixation is approximately 210 generations. These results are considered in light of other vertebrate systems, and the impact of heteroplasmy persistence on interpretations of evolutionary and ecological analysis.

