

18th Annual New Zealand Molecular Ecology Conference 27 – 29 November 2015

ABSTRACTS

The beautiful Te Tai ō Marokura will be our meeting place this year, where the University of Canterbury is very privileged to run a field research station.

We have a fantastic line-up for the weekend. It is wonderful to see the diversity of work being presented this year.



Emergent patterns of genetic diversity across the Indo-Pacific Ocean

<u>Libby Liggins</u>, Eric D. Crandall, Cynthia Riginos, Michelle R. Gaither, S. Connolly, Eric A. Treml, J. David Aguirre, Chris E. Bird, Rob J. Toonen, Diversity of the Indo-Pacific network (DIPnet)

The Indian and Pacific Oceans contain much of the world's marine biodiversity, but species and genetic lineages are not evenly distributed throughout this region. Patterns of species richness tend to follow a bulls-eye pattern, peaking at the intersection of the Indian and Pacific Oceans. Genetic studies have also identified genetic diversity gradients for many Indo-Pacific marine species that decline toward the western and eastern limits of the Indian and Pacific oceans, respectively. Understanding the generality of such genetic patterns across all marine species is important for describing the evolution of biodiversity in the Indo-Pacific and for spatial conservation planning. However, synthetic analyses of genetic diversity patterns in marine species have previously been impeded by data accessibility and methods of analysis. In my talk I will describe the efforts of colleagues and myself to advance the state of this research, with the support of the National Evolutionary Synthesis Center and on-going funding from the National Science Foundation as a Research Coordination Network. I will also discuss some of the preliminary results we have for genetic diversity patterns of marine species across the Indo-Pacific Ocean.

Food webs and biological control. A review of molecular tools used to reveal trophic interactions in agricultural systems.

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Modern monocultural agro-ecosystems can be perceived as a simplification of natural ecosystems, where a single plant species is usually grown over vast areas. In these systems, it is often assumed that a linear food web (food chain) can describe the relationships between an insect pest and its control agent, despite their complexity in terms of ecological interactions between other species (food webs) and their surrounding habitats. However, identifying, analysing and quantifying these complex multi-trophic interactions is very difficult using solely old methods such as dissection and visual gut content identification. An emerging field of study using molecular tools to analyse prey DNA in predators as well as parasitoid DNA within their hosts can now overcome these impediments and help to better understand multi-trophic dynamics. In this article, we reviewed the scientific literature related to the use of molecular tools to reveal trophic interactions in agroecosystems, published between 2000 and 2014, using the ISI Web of Science search engine. A total of 193 articles were found and a clear increase in the published literature was found during the period studied. Based on the analysis of those publications, we propose future avenues in which advanced molecular tools can contribute to a mechanistic understanding of biological control, suggesting how this approach could help design agricultural systems based on agroecological techniques.

Which pattern do you follow? Population genetics of the New Zealand endemic invertebrates, cat's eye snail and half crab.

Vanessa Arranz Martinez, Rachel Fewster & Shane Lavery

School of Biological Sciences, University of Auckland & Leigh Marine Laboratory

Connectivity levels among populations of marine organisms around the New Zealand coast are a key issue for conservation. Much current research examines which populations are 'closed' and rely predominantly on self-recruitment, which are 'open' and rely mostly on external recruit, and what are the major forces driving the geographic patterns of connectivity. In order to adequately define the number or extent of marine protective areas required, it is crucial to have reliable information about the levels of population connectivity.

Even though there are some common patterns in population structure of coastal species around NZ, many species have revealed subtly different patterns of connectivity. One relatively common pattern found by many studies is a distinction between northern and southern populations that are divided somewhere near Cook Strait.

We are investigating the population genetic and phylogeographic structure of the New Zealand endemic cat's eye snail *Lunella smaragdus* and half crab *Petrolisthes elongatus* in order to determine: (1) if they exhibit geographic restrictions to genetic connectivity and if so, where and (2) if they follow one of the common patterns of connectivity previously displayed by other species. These two species would be expected to differ significantly in both their dispersal abilities and their patterns of connectivity.

We have obtained sequences of the mitochondrial cytochrome c oxidase I region from 580 *L. smaragdus* from 27 locations around North, South and Stewart Island and from 320 *P.elongatus* from 20 locations. I will present the results so far from these two different species and highlight the patterns of connectivity they follow.

Why do inbred males fire blanks?

Helen R. Taylor¹, James V. Briskie², Neil J. Gemmell¹

In the midst of the current extinction crisis, severe population bottlenecks have become increasingly common. These bottlenecks can lead to loss of genetic diversity, and increased inbreeding and inbreeding depression. Although the negative impacts of low genetic diversity and inbreeding are clear, the underlying mechanisms driving loss of fitness are not yet fully understood. One neglected area in which inbreeding depression often manifests is male reproductive fitness. Sperm quality (sperm count, motility and percentage of abnormal sperm) has been shown to be related to fertility in a variety of species and is known to be negatively associated with inbreeding. However sperm health (in particular motility) can be challenging to measure in wild populations. We are conducting the first ever assessment of bird sperm health in New Zealand, a country where the majority of native species have experienced recent population bottlenecks. We have developed a new "mobile lab" facility that can be taken to remote sites and used to assess bird sperm motility in situ, almost immediately on collection of semen samples. Here, we focus on the South Island robin (Petroica australis australis), which has experienced a number of translocation-induced bottlenecks in the past few decades. Inbred robins are known to exhibit higher nest failure than less inbred individuals and our sperm data will help decipher whether these failures are due to infertility as a result of reduced genetic diversity and increased inbreeding.

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Can marine eDNA detection be implemented in a New Zealand monitoring program?

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Continuing anthropogenic impacts have resulted in biodiversity loss and decreased ecosystem health. Ecological assessments are key to implementing a targeted approach to combat this global decline. Informed decisions regarding biodiversity loss and ecosystem health can only be made if data on total biodiversity is available. Traditional monitoring methods typically base decisions on one functional group or selected key species, and novel methods are required to provide more reliable data for whole ecosystems. Recently, environmental DNA (eDNA) detection has emerged as a promising tool for monitoring the complete diversity in an environmental sample. However, the implementation of eDNA detection in the marine environment has been slower than in terrestrial biomes, due to the unknown dynamics of aquatic eDNA over a range of environmental conditions. During my PhD, I will investigate the following key features of marine eDNA detection: (1) I will attempt to establish an optimized extraction and sample handling protocol to assure quality data and the possibility of comparing studies over space and time. (2) I will determine the spatial and temporal resolution of marine eDNA by spiking the environment with alien DNA and sampling greatly differentiated community assemblages in close proximity to each other. (3) I will attempt to validate marine eDNA detection as a monitoring program by comparing it with an existing monitoring program. My aim is to determine whether marine eDNA detection can be implemented in a New Zealand marine biodiversity monitoring program.

Origins of Mice: DNA testing of mice to evaluate feasibility of eradication from Waikawa Island

<u>Ellie Bradley</u> and Mary Morgan-Richards Massey University (Palmerston North)

Waikawa (formally Portland) is a small near-shore island off the east coast of New Zealand that is home to a variety of threatened and at risk seabirds, including the nationally critical New Zealand shore plover (*Thinornis novaeseelandiae*). Waikawa experiences occasional invasion of *Rattus* spp. that have catastrophic effects on island wildlife. The presence of mice on the island causes problems during rat eradication efforts, which have so far been successful. If mice were eradicated from Waikawa it is thought future *Rattus* reinvasions could be more easily managed. However, removing mice would only be effective if mice only rarely arrive on the island. We used a combination of morphological and molecular data to determine whether the mice populations on Waikawa Island are isolated from the mainland mice populations. Mitochondrial DNA sequences revealed that our island sample had two haplotypes, indicating at least two female mice have dispersed to Waikawa. Comparison with mice samples from Hawkes Bay showed that Waikawa island mice are genetically distinct, suggesting gene flow is low. Size and weight comparisons also revealed the island population as distinct, probably due to age structure differences. With low levels of dispersal between the mainland and Waikawa Island detected, we suggest mouse eradication might be sustainable long term.

Phylogeny of three new red algal parasites from New Zealand

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Red algal parasite have a reduced morphology and unique development and are common on other red algae. Red algal parasites are often closely related to their hosts but also switch hosts. Previously parasites were grouped as closely or distantly related to their hosts. There are nine parasites described in New Zealand but preliminary studies indicate a much higher number of red algal parasites. This study details three undescribed parasites growing on *Polysiphonia aterrima*, *Cladhymenia oblongifolia* and *Phycodrys novae-zelandiae*. Microscopy and molecular markers (mitochondrial, nuclear, plastid) were used to study the morphology and genetic variation of these parasites. All three parasites show a close relationship to their hosts: 1) the *Cladhymenia* parasite and the *Polysiphonia* parasite are sister species to their hosts, and 2) the *Phycodrys* parasite is nested within the host species. The close relationships between all parasite and host combinations suggests that each parasite evolved from its host and relatively recently. Parasite were previous group as closely or distantly related to their host but our data shows clearly that there is a continuum between closely to distantly related species.

Sympatric speciation in freshwater green algae: the Cladophorales species flock in ancient Lake Baikal

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Species are the fundamental units in taxonomy, evolution, ecology and conservation. However, understanding the way in which a single species diversifies into two or more lineages remains one of the biggest challenges in biology. Sympatric speciation, where speciation occurs without physical barriers separating populations, is a poorly understood mode of diversification. While it has been widely accepted that sympatric speciation is possible, the debate is now focused on how common it is in nature and how many reliable examples exist. Recently, a new system for studying sympatric speciation has been discovered, the endemic green algae of the order Cladophorales in ancient Lake Baikal, Russia. These filamentous algae are classified in 17 taxa and five genera, but preliminary molecular studies show that they are actually a monophyletic group with low genetic divergence and high morphological diversification. The main goal of this study is to determine if this group does represent a case of sympatric speciation. Microsatellites obtained from NGS data will be used to recover the population structure of the morpho-species, to detect reproductive isolation and hybridization, and to investigate linkage disequilibrium. Polyploidy will also be investigated as a possible cause of speciation. As most studies on sympatric speciation patterns have focused on animals, this project will provide new insights into speciation patterns and processes in algae and plants.

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Genotype-based estimates of local abundance and effective population size for Hector's dolphins in Cloudy Bay

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Conservation considerations are often based on demographic estimates of abundance (N), and occasionally on genetic estimates of effective population size (N_e). We used genotype-based methods to estimate both parameters from the same set of samples, while also providing information on demographic closure and the genetic structure of Hector's dolphins in Cloudy Bay, New Zealand. Using a two-occasion recapture model based on genotype identifications, the abundance of individuals age 1 or older was estimated to be N_{1+} = 269 (CV = 0.12). This was similar to the estimate of N = 230 (CV = 0.30) that resulted from the more traditional analysis using contemporaneously collected photo-identifications, however the genotype-based analysis had much higher precision. The assumption of closure in Cloudy Bay was supported by the lack of genetic differentiation between the two years of the recapture surveys and the absence of any genetically detectable migrants from other populations. N_e of the parental generation was estimated to be 191 (95% CL: 123-362), and the N_e/N_{1+} ratio of 0.71 was in reasonable agreement with species of similar life history characteristics. The N_e estimate was, however, below the recommended threshold ($N_e = 500$, recently increased to ≥ 1000) thought to be necessary to preserve long-term evolutionary potential in perpetuity. Previous studies suggested that a low level of stepwise gene flow connects adjacent local populations of Hector's dolphins. Our findings highlight the importance of preserving corridors for gene flow in order to maintain the genetic diversity and evolutionary potential of each small steppingstone population, and ultimately the subspecies.

Gene expression differences among three alternative sexual phenotypes of the bluehead wrasse.

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In many marine fishes, sexual fate is plastic and influenced by the social environment. Bluehead wrasse are small social reef fish that exhibit three sexual morphs as adults. As diandric protogynous (female first) hermaphrodites, individuals either mature directly as male (primary males) or develop first as females with the capacity to change sex to male (secondary males) later in life. Primary males mimic females in morphology and behaviour and employ a 'sneaker' mating strategy. The large, brightly coloured secondary males exhibit aggressive behaviour and actively court females. Development of alternative sexual phenotypes in this species is sensitive to social cues and, therefore, is not fixed by an underlying genotype and must reflect differential gene regulation. RNA sequencing (RNAseq) is a genomic technology that enables studying all RNA types expressed in a sample, and was used here to profile the molecular basis of alternative sexual phenotypes in the bluehead wrasse. Specifically, we used RNAseq to identify genes differentially expressed in the brain and gonad between primary males, secondary males, and females. Secondary males up-regulate more genes overall compared to females and primary males, suggesting more genes are involved in maintenance of this phenotype. Genes found differentially expressed between phenotypes included those related to cognition, social behaviour and sex hormone regulation. This work is part of a larger

investigation using protogynous wrasses as models for studying the molecular mechanisms underlying reproductive plasticity in teleost fishes.

Incongruent genetic connectivity patterns for demosponges off New Zealand: implications for the management of vulnerable marine ecosystems

<u>Cong Zeng¹</u>, Michelle Kelly², Ashley A. Rowden², Malcolm R. Clark², and Jonathan P. A. Gardner¹

The understanding of genetic connectivity among populations in the deep sea is important because it indirectly reveals dispersal patterns of the fauna, which may provide information for the designation of marine protected areas. Currently, patterns of deep sea genetic connectivity throughout New Zealand's EEZ are not well documented. To elucidate such patterns, the mitochondrial markers cytochrome oxidase I, cytochrome b and 12s rRNA were utilised to detect the genetic differentiation among populations of two species of demosponges. For Poecillastra laminaris (Sollas, 1886), significant genetic differences existed between regions north and south of the Chatham Rise, at ~44 °S in the Pacific Ocean. In contrast, no significant population or regional genetic structure was detected for *Penares palmatoclada* (Lévi, 1993) across the EEZ. For both P. laminaris and P. palmatoclada, greatest haplotypic diversity for cytochrome oxidase I and cytochrome b was observed along the Chatham Rise, suggesting that this a hotspot of genetic diversity for these two species. We interpret the regional genetic diversity of P. laminaris as being influenced by oceanic currents, with gene flow from the north and from the south meeting and mixing in the vicinity of the Chatham Rise. The absence of such a pattern for the P. palmatoclada populations may be attributable to differences in life-history and reproductive strategies between the two species or to differences in sample sizes. The results of this study are discussed in terms of the conservation of vulnerable marine ecosystems, the designation of marine protected areas and the management of impacts from fishing and mining in the deep sea off New Zealand.

Seminal fluid proteins and speedy sperm: sperm competition in the chinook salmon (*Oncorhynchus tshawytscha*). Michael Bartlett

Males of many species make rapid adjustments to ejaculate quality in response to social cues, however the molecular mechanisms allowing such rapid adjustments in ejaculate quality remain unknown. Male salmon form dominance hierarchies, with dominant males gaining direct access to females by chasing away rivals, while subdominant males invade spawning pairs subjecting them to high levels of sperm competition (multiple ejaculates competing to fertilise a single set of eggs). The outcome of competitive fertilisation in salmonids is primarily determined by sperm velocity, as males that have faster swimming sperm sire a greater proportion of eggs. Seminal fluid proteins (SFPs) can have considerable influence on the outcome sperm competition and fertilisation in insects. Less is known about SFPs in fish except that SFPs < 50 KDa influence sperm velocity. Here I present preliminary results for the proteomic analysis of the seminal fluid from 17 males, for which I experimentally manipulated social status. I characterise the seminal fluid proteome and compare sperm velocity parameters, SFPs and the outcome of competitive fertilisation success among these males with the aim to produce a candidate list of proteins that may influence sperm velocity.

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Chatham Island black robin

Natalie Forsdick, Marie Hale, Melanie Massaro. University of Canterbury.

The Chatham Island black robin (*Petroica traversi*) was once regarded as the world's most endangered bird, with the species totalling five individuals. Through intensive management, the robin has recovered to around 290 individuals on two islands, but remains endangered. In my study, I investigate how this intense bottleneck has affected the robin's genetic diversity, and whether thirty years of isolation between the two populations has resulted in any genetic differentiation. A previous study using genetic markers developed for a different species found insufficient variation to answer these questions. I developed microsatellite markers specific to the black robin, and found a lower level of diversity in black robin than in the Chatham Island tomtit, sister-species to the black robin. I have also found moderate levels of genetic differentiation between the isolated island populations. Next, I intend to use these markers to analyse local population structure, relatedness, and whether there is evidence of extra pair paternity in this endangered bird.

Conservation genomic management of a critically endangered New Zealand bird.

Stephanie Galla¹ and Tammy Steeves¹

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Endangered species management can utilise captive breeding for translocation as a technique to prevent extinction and enhance species recovery. Captive breeding for translocation programmes often make pairing decisions based on available pedigree data to minimise inbreeding and maximise genetic diversity in an effort to maintain evolutionary potential. However, captive breeding for translocation programmes face challenges with pedigrees, with many being incomplete, shallow, or error-prone. Genetic markers (i.e., microsatellites) have been used to estimate relatedness for species with poorly-developed pedigrees, but emerging evidence suggests that these estimates are poor indicators of genome-wide diversity and genomic data (i.e., single nucleotide polymorphisms or SNPs) may be better suited for estimating relatedness. Here, we use the critically-endangered kakī (Himantopus novaezelandiae) in a proof-of-concept study to demonstrate the utility of genomic data in captive management. Our aim is to compare relatedness values and resulting pairing recommendations from the kakī pedigree, 8 microsatellite markers, and ~15,000 SNPs derived from a biospectra-bysequencing (i.e., BBS) approach to evaluate which technique is most effective for captive breeding for translocation programmes. While this project is still in its infancy, we believe that collaboration, including those with researchers outside of academia (e.g., Crown Research Institutes like AgResearch and Plant and Food), will be key to bridge the 'conservation genomic gap' to produce, manage, and interpret large genomic datasets to inform the conservation manangement of threatened taonga species like kakī. Additionally, we aruge that building partnerships with relevent iwi-Māori will be essential to develope effective conservation programmes for New Zealand's living treasures.

Genomic basis of complex cognition in New Caledonian crows

Dr. Nic Dussex, Dr. Jochen Wolf, Dr. Christian Rutz, Prof. Russell Gray, Prof. Neil Gemmell

The discovery of complex avian cognitive abilities has revolutionized our understanding of the evolution of intelligence. However, the genetic basis of these abilities is unknown. One possibility is that numerous changes in genes across a wide range of functional domains are required for the evolution of complex intelligence. Alternatively, only a limited number of genetic tweaks might be required. Corvids are well known for their cognitive abilities such as episodic-like memory, problem solving, and tool manufacturing and are therefore an ideal model to study the genetic basis of complex cognition. Using genome-wide tests of selection we are comparing the tool-manufacturing New Caledonian crow (*Corvus moneduloides*) with closely related, non-tool-using crows to identify genomic regions associated with tool-using lifestyle. Once these broad genomic regions are identified, we will refine them using brain transcriptome from the closely-related white-billed crow species (*Corvus woodfordi*) in order to identify regions that are likely linked to cognition. Finally, we will use crow and other avian genomes to test hypotheses on gene family evolution.

POSTER

Assessment of eDNA metabarcoding for the monitoring of benthic communities near offshore oil and gas activities.

Olivier Laroche, PhD candidate at the University of Auckland Supervisors: Dr Louis Tremblay, Dr Xavier Pochon and Dr Gavin Lear

To comply with the marine management regime of New Zealand, oil and gas companies performing offshore activities are required to monitor the environment's health near their operation sites. At present, the resulting impacts are determined by measuring chemical properties of sediment and changes in infaunal diversity. Microscopic examination of infauna is time-consuming, expensive and relies on taxonomic expertise. Moreover, classification of damaged or incomplete specimen is often impossible and complex developmental cycle involving multi morphological stages can render identification difficult to achieve.

Recent breakthroughs in DNA sequencing technology now allow species richness to be rapidly estimated from small amounts of sediment. Referred to as environmental DNA (eDNA) metabarcoding, this new molecular tool has incredible potential for studying global ecosystem biodiversity through its ability to identify a wide range of organisms simultaneously and automatically. Additionally, metabarcoding is less reliant on taxonomic expertise, standardized protocols can be developed, and the results can be audited. All these qualities could make eDNA metabarcoding a cost-effective, reliable and expeditious solution to morphological classification.

My project proposes to assess eDNA metabarcoding for the monitoring of the benthic ecosystem health near offshore oil and gas operations. By screening the genetic material of soil samples, it aims to provide information on microfauna communities and on the presence/absence of various bioindicators. To identify which stressors originating from drilling fluids and produced water are responsible for microbial community changes, specific gene expression pathways will be investigated by quantitative PCR (qPCR). Finally, I aim to provide recommendations to enhance the environmental management of offshore oil and gas activities by reviewing and comparing national and international key environmental legislations and policies.