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ALLAN WILSON CENTRE

INSIDE THIS ISSUE

What your relatives say about you: Moa may have flown to New Zealand

The latest research findings from studies on moa, seabirds, and song birds challenge historical theories on the evolution of New Zealand birds. This research was undertaken by AWC Alumnus Dr Matthew Phillips, in conjunction with Professor David Penny and his PhD student Gillian Gibb.

Allan Wilson Centre researchers have discovered the closest relatives of the extinct flightless moa are a flighted group of South American birds called tinamous. This finding implies moa must have flown to New Zealand, and changes our whole understanding of ratite biogeography.

What are ratites?

Ratites are a group of large flightless birds distributed throughout the Southern Hemisphere. Ostriches live in Africa, rheas in South America, the extinct elephant bird lived in Madagascar, emus and cassowaries live in Australia, and kiwi and the extinct moa are found here in New Zealand.

The closest relatives of the ratites were thought to be the tinamous, a group of small chicken-like birds that can fly, although not very well. Ratites and tinamous together form the palaeognaths, or 'old jaws', and are the sister group of all other living birds (known as neognaths, or 'new jaws')

Because ratites are large and flightless, it was thought their current distribution resulted from the breakup of Gondwana. As the continent split up and drifted apart, each group of ratites evolved in isolation on its own landmass from the flightless common ancestor into the different species we see today. This concept is known as vicariance biogeography.

Allan Wilson Centre researchers used long mitochondrial DNA sequences and new computational methods to take a closer look at how all palaeognaths are related to each other. They were very surprised to discover that tinamous are not the 'cousins' of the ratites, but in fact fall within the ratite group, and are the closest relatives of the moa.

The finding of flying tinamous within the flightless ratite group means that each ratite lineage must have independently lost flight. If the ancestors of modern ratites flew, they could have flown to their current location before becoming large and flightless.

When did ratites become flightless?

It is quite hard to estimate when ratites became flightless, and it may have happened at different times in each lineage. Our analyses suggest the common ancestor of moa and tinamous lived 60 million years ago. Zealandia (the land that would become New Zealand) had already rifted from Antarctica and Australia by this time, so the ancestor of moa must have flown to New Zealand less than 60 million years ago, then become flightless. How much less than 60 million years ago? That is still an open question.

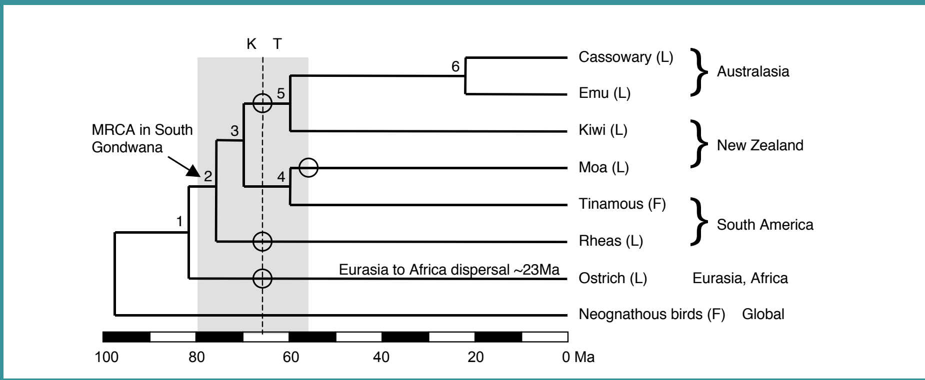
By the same token, our analyses also suggest ancestors of kiwi flew to New Zealand, escaping from its Australian relatives, the emu and cassowary.

Why did ratites become flightless?

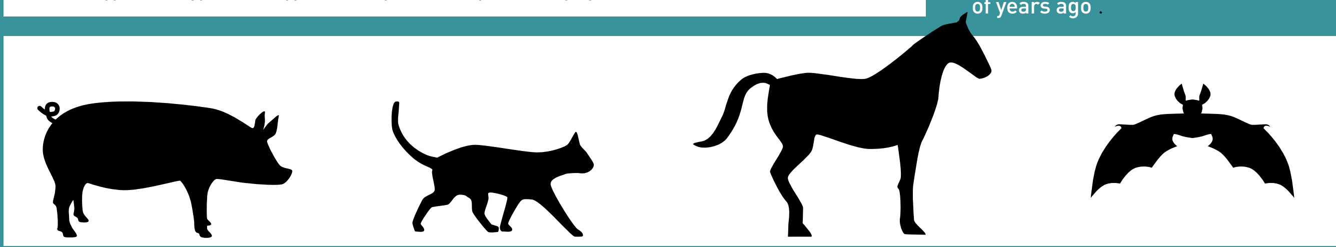
Flightlessness is not an unusual occurrence among birds. There are more than 100 cases of rail species (the rail family includes the weka, pukeko and takahe) becoming flightless as they colonized predator-free islands in the Pacific. Flight is costly, even for birds (think of the muscles needed). So maybe in the absence of predators birds could avoid this cost. While the actual cause of flightlessness and increase in size in ratites is not known, we can speculate that the extinction of mid-large terrestrial dinosaurs at the Cretaceous/Tertiary boundary (65 million years ago) left vacant ecological niches that ratites filled. This hypothesis is still open to testing.

For further information please contact Gillian Gibb on g.c.gibb@massey.ac.nz.

Moa illustration from Extinct Monsters by Rev. H. N. Hutchinson, illustrations by Joseph Smit (1836-1929) & others. 4th ed., 1896. Plate XXIII between pages 232 & 233. (www. .wikipedia.org). Tinamus major, engraving from Birds (The Cambridge Natural History) by Arthur Humble Evans (1855-1943, author), George Edward Lodge (1860-1954, artist), New York, London, MacMillan 1899



The family tree of palaeognaths, showing moa and tinamous as each other's closest relative. F and L specify flighted and flightless birds respectively and circles indicate when flight may have been lost in each linage. The asteroid impact at the Cretaceous-Tertiary boundary (K/T, dotted line) and the South Gondwana break-up period (shaded) are shown. MRCA indicates 'most recent common ancestor', and Ma stands for 'millions of years ago'.



RESEARCH

Phylogenetics:

How are species related to each other?

Much of the research in the Allan Wilson Centre is underpinned by phylogenetics. What is phylogenetics and how is this method employed?

Phylogenetics is the study of evolutionary relatedness among groups of biological organisms – working out how species are related to each other to make a family tree.

Phylogenetics can be done using different kinds of data such as morphological characteristics (for example shapes of bones, or how many legs) and more recently DNA sequences. The more similar the characteristics are between groups of individuals, the more closely related they are to each other.

Sometimes relationships can be confused by convergent evolution where distantly related organisms can evolve very similar body types, perhaps to fill similar niches. For example, dolphins and whales swim like fish, but are mammals, and related to hippopotamuses. Bats fly like birds, but are part of a very diverse group of mammals (called Laurasiatheria) that also includes cats, horses and pigs, to name a few.

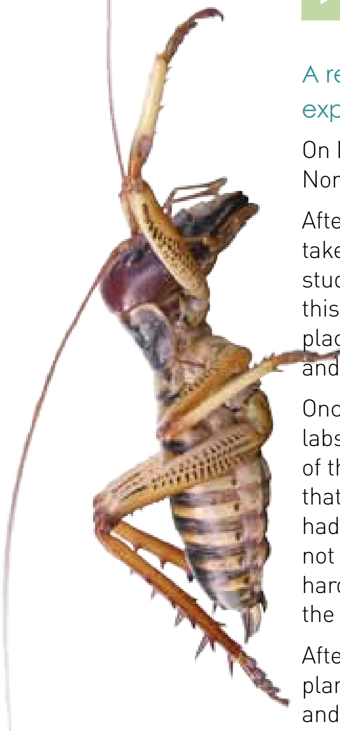
DNA sequences are less prone to problems of convergent evolution than morphology. By aligning DNA sequences from the same gene for multiple species, we can look for nucleotide differences that show patterns of relatedness. After creating a family tree from the DNA sequences (a phylogeny), morphological characteristics (such as loss of flight) can be mapped onto it, and an evolutionary picture uncovered.

In the case of ratites, the morphological characteristics associated with being flightless appeared to group ratites together to the exclusion of the flying tinamous. We now understand many of those characteristics evolved independently in each ratite lineage. Only the tinamous, which still fly, retain some of the ancestral characteristics of the group.

The first hint that the biogeographic distribution of flightless ratites might not strictly represent their evolutionary relationships came in the early 1990s, when it was discovered that the two New Zealand families (moa and kiwi) were not sister-groups. The study used the emerging science of ancient DNA sequencing to determine the DNA code of a very short piece of DNA from a moa. Although the DNA sequence was not long enough to confidently say who moa were most closely related to, it definitely was not a close relative of kiwi.

Now ancient DNA sequencing techniques have improved and we have much longer DNA sequences from the extinct moa, as well as all other living ratites. We also have DNA sequences from the tinamous of South America, and many more distantly related birds. This allowed us to confidently determine the relationships between ratites and tinamous. Importantly, we also have agreement between two independent DNA datasets (nuclear and mitochondrial DNA) that corroborate each other.

For more information contact Gillian Gibb g.c.gibb@massey.ac.nz



Biology- Hands on

A report from Aperahama Hurihanganui & Tim Easthope, who visited the Allan Wilson Centre to experience science at the coal face.

On Monday 15 February the Level 3 Biology class from Rotorua Boy's High School travelled to Palmerston North to visit the Allan Wilson Centre at Massey University.

After a long five hour bus trip it was a relief to reach the Centre and be introduced to the scientists who had taken time out of their normal routine to talk to us about scientific research. We learned about research studies carried out at the Allan Wilson Centre and got some good ideas for investigations that we could use this year. For example, we learned about New Zealand's tree ferns and how the silver fern is only found in dry places, generally on a slope. The initial session was followed by a chance to have a walk around the campus and visit the student cafeteria for lunch.

Once everyone was fed we went back to the Centre for a session on weta research. We were shown around the labs and introduced to three graduate students who were actually studying the weta. They taught us the basics of the weta lifestyle, explained how they carried out their research and gave us some suggestions for projects that we could carry out for our own internal standard investigating the ecological niche of an organism. We had the chance to handle weta – some of the boys jumped at the chance for weta wrangling while others were not keen to get that close! Truth be told, the male weta was pretty aggressive and bit the research scientist hard enough to draw blood, and we learned first hand that they have a most unattractive habit of vomiting on the unsuspecting weta wrangler when really aggravated!

After we finished playing with weta we moved upstairs where a German graduate student taught us about his plant studies on the New Zealand buttercups. He was looking at why they are found in different environments and their genetic differences.

Our time at the Allan Wilson Centre ended with a wonderful afternoon tea including cake, club sandwiches and juice. They really knew how to host a group of young men! While we were eating, a post-doctoral fellow talked all about his overseas experience and where a career in Biology can take you.

After a great day of learning about plants and weta, and gaining ideas and enthusiasm for carrying out our own research, it was time to return home.

All the boys enjoyed themselves and were happy to have seen Massey University first hand.

Thank you to the Allan Wilson Centre – it was a wonderful experience.

Ben McGrath up close with a male Wellington weta

Aperahama and Mark learning how to carry out a scientific investigation on weta

RESEARCH

Ancient DNA

Lisa Matisoo-Smith is a Principal Investigator in the Allan Wilson Centre. She recently moved from the University of Auckland to the University of Otago. On December 10, 2009, the new, state-of-the-art University of Otago Ancient DNA Laboratory was officially opened.

What is ancient DNA? When people hear the words ancient DNA (or aDNA as it is often referred to) they tend to immediately think about obtaining DNA from extinct species, but technically ancient DNA techniques are applied to any tissue sample that is not fresh or specifically preserved for later DNA extraction – this includes museum specimens that may only be tens to hundreds of years old. Such tissues are typically defined as being "ancient" by having highly degraded DNA.

AWC postdoctoral fellow Michael Knapp processing a sample in the University of Otago Ancient DNA lab.

When an organism dies, the DNA molecules in the tissues begin to break down immediately and thus time since death can be a major factor in the preservation and quality of DNA that can be obtained from a sample. Various environmental factors can speed up or slow down these degradation processes, so determining how well preserved a sample is can be difficult.

As a general rule of thumb, cold and dry conditions are good for DNA preservation and hot and wet conditions are bad, but a constant temperature or environment is also important for preservation. Given good conditions, DNA can be preserved in bone, teeth, tissue, hair, feathers or seeds for thousands of years. Recently an almost complete genome of a woolly mammoth has been sequenced by researchers, dated to approximately 60,000 years old and a Neanderthal dated to around 38,000 years old.

Unfortunately, those working in the Pacific are not blessed with preservation conditions as good as permafrost or the cool, dry caves of northern Europe. The hot, wet and variable conditions found in much of the Pacific are about the worst possible for DNA preservation. Despite these difficulties it has been possible to obtain DNA from Pacific samples as old as 3000-4000 years, but the quality of DNA is very poor. In New Zealand, the conditions are slightly better and Allan Wilson Centre researchers have obtained DNA from moa bones over 6000 years old.

One of the biggest problems with aDNA has to do with contamination. Contamination of a sample with foreign DNA can occur at several points. As soon as an organism dies and begins to break down, it is invaded by foreign organisms involved in that process. Ancient DNA extracted from bones, for example, includes not only the preserved DNA of the organism in question but DNA of bacteria and fungi.

As soon as a sample is exposed to the environment by archaeologists, it is also exposed to modern DNA that is being shed by all of those archaeologists and others on the site or anyone in the museum who might be touching the bones or even breathing over them.

When the sample is taken into the laboratory it is exposed to and potentially contaminated by DNA that has previously been studied in that lab. While contamination is a problem for all aDNA studies, it is the most difficult for aDNA studies involving human remains because the contaminating DNA can easily be confused with the original DNA from the sample being studied.

In order to minimise all of this potential contamination there are several protocols followed in aDNA research:

- In the field, samples are collected as soon as they are exposed and before they are touched by the excavators.
- Ancient DNA laboratories should not be located in buildings where DNA is being amplified or other molecular work is being undertaken.
- Inside the aDNA lab, researchers take numerous precautions to make sure that they are not introducing any modern DNA to the lab – this includes changing out of regular street clothes before entering the lab and then wearing a full protective suit and face mask.
- Other protocols ensure that reagents and lab gear are not introducing contamination.
- Replication of results in independent labs provides further controls.

Several projects involving aDNA have been conducted by Allan Wilson Centre researchers and the establishment of a new aDNA lab at the University of Otago will increase the number and quality of aDNA studies that can be undertaken.

In addition to continuing analyses of the aDNA of commensal plants and animals, this new lab will focus on the analysis of ancient human DNA. Researchers in the Allan Wilson Centre are now working with several iwi and Pacific Island communities to analyse the DNA of their tupuna (ancestors) to address issues such as population origins, health and demography. Representatives of some of these collaborating iwi were present for the opening and blessing of the new lab.

For further information on this research or on ancient DNA please contact Lisa on lisa.matisoo-smith@anatomy.otago.ac.nz.

Drilling pig tooth to obtain ancient DNA

RESEARCH

Origins of NZ skinks revealed

The author of this story, Hilary Miller, is a postdoctoral fellow in the Allan Wilson Centre based at Victoria University of Wellington.

Most New Zealanders can name at least a dozen or so species of native bird, but how many can do the same for our native reptiles? If you start counting and only got as far as one-tuatara you're probably not alone. Although we are missing some of the major groups of reptiles (like snakes and alligators), we do have a diverse array of lizards. In fact New Zealand has around 80 different lizard species in two major groups – geckos and skinks.

Around half of our lizard species are skinks. These are the most commonly encountered native reptiles, being the species most likely to be spotted disappearing under rocks or into long grass on a hot day. Research by David Chapple is improving our understanding of the origins and evolution of our skink fauna.

Chapple investigated the relationships among 32 of our skink species and their closest relatives from Lord Howe Island, New Caledonia and mainland Australia. New Zealand's skinks were previously grouped into two separate genera, *Cyclodina* and *Oligosoma*, which were mostly distinguished by their differing body shapes. However, Chapple's genetic work shows that there are in fact eight different lineages of skinks that likely evolved from a single ancestral species after it colonized New Zealand from New Caledonia.

There is no clear division between *Cyclodina* and *Oligosoma*, suggesting that the differences in morphology that separate these two genera have evolved on multiple occasions. Chapple's research thus spells the end for *Cyclodina* as a recognised genus – all New Zealand skinks (plus their closest relative *C. lichenigera* from Lord Howe Island) are now included in the genus *Oligosoma*.

Chapple was also able to estimate when skinks first colonized New Zealand by using a "molecular clock", which allows researchers to relate the number of changes in a DNA sequence between two species to evolutionary time. His results suggest skinks arrived in New Zealand around 16-19 million years ago, timing which is consistent with the recent finding of an *Oligosoma*-like fossil from the St Bathans area of central Otago dating to this period.

Both the genetic work and the St Bathans fossils point to skinks colonizing New Zealand not long after the "oligocene drowning", a period 25-35 million years ago when much of the present-day New Zealand landmass was underwater. Chapple suggests that after diverging from their New Caledonian cousins, ancestral New Zealand skink species may have survived on now-submerged volcanic islands along the Lord Howe rise and Norfolk Ridge before reaching New Zealand. Of course, this scenario requires skinks to have dispersed across large distances of open water, which you may think would be a problem for a non-flying, terrestrial vertebrate. But this is not as unlikely as is sounds - many of our skink species live in coastal areas amongst material that is often swept out to sea during storms. They have also been observed swimming in rock pools and can stay underwater for up to 20 minutes. So it's not unreasonable to think they could survive a trip across the ocean on a raft of kelp or driftwood.

Chapple's research has not only given us a better understanding of the origin and evolutionary relationships among skink species, but has also resulted in the naming of a number of new species, highlighting just how diverse our skink fauna is.

For more information on this research contact David Chapple at David.Chapple@sci.monash.edu.au.

Skink images: Kim Miller

RESEARCH

Cultural and evolutionary aspects of the cultivation of karaka/kōpi by Māori and Moriori

Robin Atherton is a PhD candidate in the Allan Wilson Centre at Palmerston North. Her research uses new sequencing technology to develop molecular markers for use in tracing the translocation history of *Corynocarpus laevigatus* (karaka in Aotearoa or kōpi on Rekohu/Chatham Islands).

The research also involves collecting historic information of karaka/kōpi in the form of kōrero (oral histories) and rongo/waiata (songs). The regional ethnobotany of the tree in Aotearoa will also be studied.

Karaka/kōpi was an esteemed and staple item of food in the Māori and Moriori diet. The kernel was eaten after careful preparation through steaming and running in water to remove the poison, karakin. Prior to human arrival karaka/kōpi is thought to have been restricted to the northern North Island of Aotearoa. The present occurrence of this plant elsewhere is strongly associated with Māori and Moriori archaeological sites and thought to have resulted from translocations during cultivation.

In mid-February Robin was invited to Rekohu by the Hokotehi Moriori Trust to take part in a series of cultural workshops. The setting was Kopinga Marae (which means a 'grove of kōpi trees'). The marae, with its wharenui Hokomenatai (meaning 'to be gathered together in peace and tranquility'), was opened in January 2005 to re-establish a central base on Rekohu in which Moriori could meet, celebrate, debate and just be together and as a welcoming, peaceful place for visitors to experience Moriori hospitality. Moriori are tchakat henu (tangata whenua/indigenous inhabitants) of Rekohu who live by a long-established code of peace.

Upon arrival in the wharenui Robin was blessed at the central pou, the focal point for this marae. The pou was beautifully carved by Todd O'Hagan and James Dowle using laser scanning. In 1862, Moriori elders compiled a list of all inhabitants they could remember living on Rekohu in 1835; these names are carved into the pou, along with images of rakau momori(dendroglyphs). The marae was the perfect setting for the week's activities. The meeting was made up of a series of workshops designed for Moriori members and covered a range of topics related to cultural identity.

During the week of the visit a team from the Department of Conservation and Otago University were carrying out a survey of rakau momori on kōpi trees and recording them using laser scanners. Rakau momori literally translates to 'memories carved in wood' and it is understood they were carved as a way of creating a memorial to the ancestors. Other non-human images were probably carved or bruised into the trees because they were very relevant to the Moriori way of life.

Shirley King, Chairperson of the Hokotehi Moriori Trust, describes the rakau momori perfectly in a TVNZ6 *Meet the Locals* episode in 2009, "I love their spirit, the power of them, their dignity and strength, the solitude, the peace." Hapupu Reserve, one of only two national historic reserves in New Zealand, is truly a special place, a place that both gladdens and saddens the heart. The rakau momori are a sight you can only appreciate in person, the feeling of direct contact with Moriori ancestors is tangible and moving. Unfortunately disease, strong storms and grazing have destroyed many of the trees and there is only a fraction of the original number remaining. However, there still exist some 180 internationally significant rakau momori at about five sites on Rekohu.

Young Moriori members interviewed elders and recorded the kōpi tree scanning. There were also workshops on taonga conservation and storage and evening lectures on ecology and management planning for reserves. Atherton presented her research to members and discussed kōpi ecology and ways of conserving trees, especially those with rakau momori. She was involved in a week-long bilateral consultation on her research carried out during and between workshops, whilst sample collecting, cooking, eating dinner, photographing taonga, and even whilst cleaning the showers! The Trust wholeheartedly gave her work the thumbs up and she was permitted to collect samples from some of the oldest trees at Hapupu. She also took samples of dead trees to investigate the cause of death of a large number of trees at Hapupu and has been asked to return in six months to report on her findings.

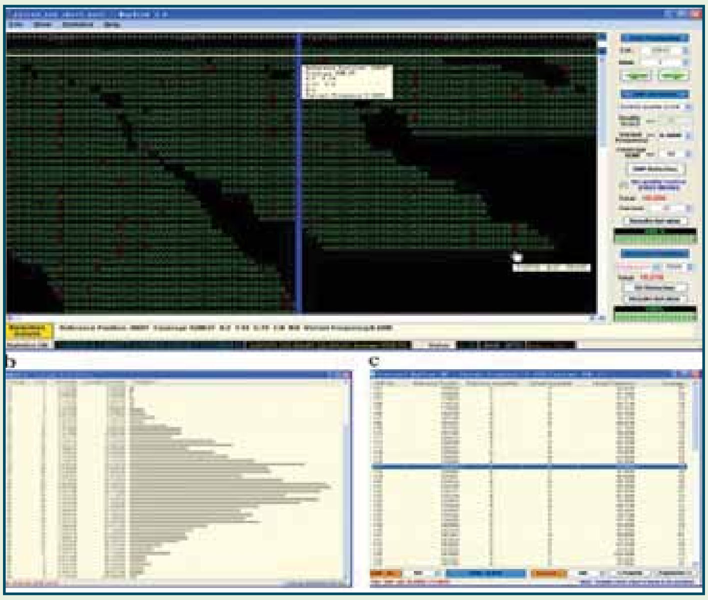
Discussions are underway to devise a collaborative research project between the Hokotehi Moriori Trust and the Allan Wilson Centre to enable a Moriori student interested in kōpi ecology to go Massey University, Palmerston North, to study over summer 2011. The Hokotehi Moriori Trust wish to encourage collaborative research initiatives with researchers from Aotearoa and beyond, and have a research validation and ethical process they would like to see adopted by all who wish to work on the islands. Please contact Susan Forbes, salala@paradise.net, for further details.

For more information on this project please contact Robin Atherton on R.A.Atherton@massey.ac.nz.

NEWS

Update: High-Throughput Sequencing

Dr Lesley Collins is a research consultant for the Massey Genome Service. She has been instrumental in getting the relatively recently acquired Illumina Genome Anal yser up and running.



Training Course: Solexa sequence analysis for Molecular Biologists

The Massey Genome Service is running a training course in *Solexa sequence analysis for Molecular Biologists* on the Palmerston North campus of Massey University on 1 & 2 July 2010.

For further details and to register please go to www.allanwilsoncentre.ac.nz/ISAW.htm. Enquiries regarding the programme should be addressed to Lesley Collins on l.j.collins@massey.ac.nz. Enquiries regarding the organisation of the workshop can be sent to Susan Adams on susan_adams@xtra.co.nz

It has been a few years now since high-throughput sequencing arrived at the Allan Wilson Centre in the form of the Illumina Genome Analyser, able to perform Solexa sequencing on entire DNA genomes or the RNA from them. Today, projects sequencing DNA or RNA from disease-causing bacteria and fungi, plants, animals and other single celled organisms have come through the Massey Genome Service and demand is still high for even more from these groups.

However, there is still a large problem after the sequencing has been done: many of our customers do not know how to begin analyzing the very large datasets that they are sent. To give an idea, most sequencing files (containing ~15 million sequences) are too large to open on a standard PC or Mac system, cannot fit into Excel, and are sent to customers on a 1Tb portable drive. To top it all off, most readily available software is Linux-based, a computer operating system that is largely unfamiliar to molecular biologists. This is where analytical methods of genomes has changed as molecular biologists have enlisted the aid of bioinformaticians to help them get a handle on their data.

Data processing is usually done on computer servers instead of desktops and databases are used to tie sequence to gene information. With this analysis the levels of gene expression (genes turning on or off) can be measured from the same organism in different conditions, genomes compared to find potential disease-related genes, and RNAs discovered that could be used for markers for cancer and other conditions.

As the years go by, we do expect the training of molecular biologists to include more computing skills which will enable them to analyse their data themselves, but those who know computing, mathematics and statistics as well as biology are in very high demand. Genome sequencing is very much an integrated science and it is still early days in this exciting field.

To find out more about the Allan Wilson Centre Genome Service and the Illumina Genome Analyser, contact Lesley Collins on l.j.collins@massey.ac.nz or see www.allanwilsoncentre.ac.nz/AWCGSintro.htm.

Next Generation Sequencing

2010 Next Generation Sequencing Workshop and Scientific Meeting

The Allan Wilson Centre, in conjunction with the University of Otago Genome Service, was pleased to host the first Next Generation Sequencing Workshop and Scientific Meeting at Te Manawa, Palmerston North in August 2009.

The second annual Next Generation Sequencing Workshop and Scientific Meeting meeting will be held at the Otago Museum on 23 & 24 August 2010.

Further information on the event can be found at www.allanwilsoncentre.ac.nz/NGSWorkshop.html. Registrations will open mid-April 2010.

ALLAN WILSON CENTRE

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Visit the Allan Wilson Centre at
www.allanwilsoncentre.ac.nz

Teachers' Workshop

The Allan Wilson Centre is pleased to announce it will be hosting Teachers' Workshops on the Palmerston North campus of Massey University and at the University of Auckland.

This popular event will be held on 21 May 2010 between 4 and 9pm. The Workshops will focus on achievement standards *90716 Describe animal behaviour and plant responses in relation to environmental factors* and *90717 Describe processes and patterns of evolution.*

For further information and to register please see www.allanwilsoncentre.ac.nz and follow the link from the front page of the site to the Workshop registration page.

We look forward to hosting you for what is always an informative and informal evening.

For further information contact Susan Adams on susan_adams @xtra.co.nz

the ROYAL SOCIETY of NEW ZEALAND
TE APĀRANGI

Royal Society of New Zealand Teacher Fellowships

Over the past six years the Allan Wilson Centre has hosted many teachers who have been awarded Royal Society of New Zealand Teacher Fellowships.

This Scheme, funded by the Government and administered by the Royal Society of New Zealand, offers primary, intermediate and secondary teachers the opportunity to improve their teaching through experience in technological, scientific or social sciences practice.

The scheme is open to fully qualified practicing primary, intermediate and secondary teachers whose work can be related to science, mathematics, social sciences and technology. Only New Zealand citizens or permanent New Zealand residents are eligible to receive a Fellowship.

If a year in a research environment appeals to you, and you would like to be hosted by the Allan Wilson Centre at any one of our host or partner institutions, please contact us and we will arrange to meet with you to discuss a possible project. Following discussions we will assist you to complete and submit the application form.

For more on the criteria and application process:
http://www.royalsociety.org.nz/Site/teachersstudents/Funding_for_teachers/

To initiate discussions regarding a project please contact Joy Wood on j.r.wood@massey.ac.nz

TEACHING RESOURCES

Over the years the Allan Wilson Centre has produced a number of resources that can be used in the classroom.

Allan Wilson Evolutionary: biochemist, biologist, giant of molecular evolution

This documentary film was aired on the Sky Documentary Channel during 2009. It profiles the remarkable career of Allan Wilson and explores the enduring impact of his ideas on anthropology, molecular biology and all the natural sciences. Duration: 40 minutes.

pheno

Back issues of *pheno* are available either in hard copy or online at
www.allanwilsoncentre.ac.nz

Biotechniques

This DVD demonstrates through explanations and animations the biotechnological techniques of DNA extraction, gel electrophoresis, PCR, restriction enzyme digestion, DNA sequencing, ligation and cloning, DNA chips and tissue culture.

There are also innumerable recorded lectures downloadable from
www.allanwilsoncentre.ac.nz

To receive your own copy of one of our resources please contact Joy Wood on j.r.wood@massey.ac.nz