

LENScience Senior Biology Seminar Series
Ancient Secrets in the Seaweed
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Google CLIMATE CHANGE and you will see an array of headlines, blogs, web sites all with differing information, issues and opinions.

Cycles of Climate Fluctuation

Scientists understand that **Cycles of Climate Fluctuation** are a natural and on-going part of Earth's Climate Systems. However, since the early 1970's scientists have been concerned at the effect of increased greenhouse gases in the atmosphere resulting from Human activities. These increased greenhouse gases are raising temperatures and destabilising the Earth's climate systems. They are changing the natural climate cycles.

Natural Climate Cycles are an important part of the Earth's systems that cause changes in the physical (abiotic) environment, and through these changes, impact on the biotic environment within ecosystems.



These environmental changes may alter selection pressures that could result in changes to the distribution patterns of species.



Fig 1. Ceridwen with a Kelp **Holdfast**, Falkland Islands

Allan Wilson Centre Post Doctoral Fellow **Ceridwen Fraser** has recently completed her PhD, which saw her spend three years unravelling a story of the impact of climate cycles that is hidden in the DNA of **Southern Bull Kelp** (*Durvillaea antarctica*). Using modern biotechnologies to analyse DNA from Bull Kelp samples collected from the coastline of New Zealand, Southern Chile and Sub-Antarctic Islands, Ceridwen has discovered evidence that in the last ice age the sea ice was more extensive than previously thought, and that this has affected the distribution of populations of Kelp that we see today.

Southern Bull Kelp is one of the world's largest seaweeds, commonly found in the inter-tidal zone around the southern coastlines of New Zealand, the sub-Antarctic islands, Chile and the Falkland Islands. Anchored to the rocks by a strong **holdfast**, strands of this macro-algae can stretch out more than 10m in length, being tossed around by the strong waves and currents but held by this incredibly strong anchor. The holdfast creates a rich habitat for a large numbers of different **invertebrates** such as limpets, snails and crustaceans. Māori as well as people in Chile have traditionally made use of Bull Kelp both as a food and a material for making food bags and waka. Importantly, *Durvillaea antarctica* is **buoyant**. If pulled away from the rocks it will float, creating enormous **rafts** that carry their own invertebrate community with them.

Not all species of Kelp are buoyant.



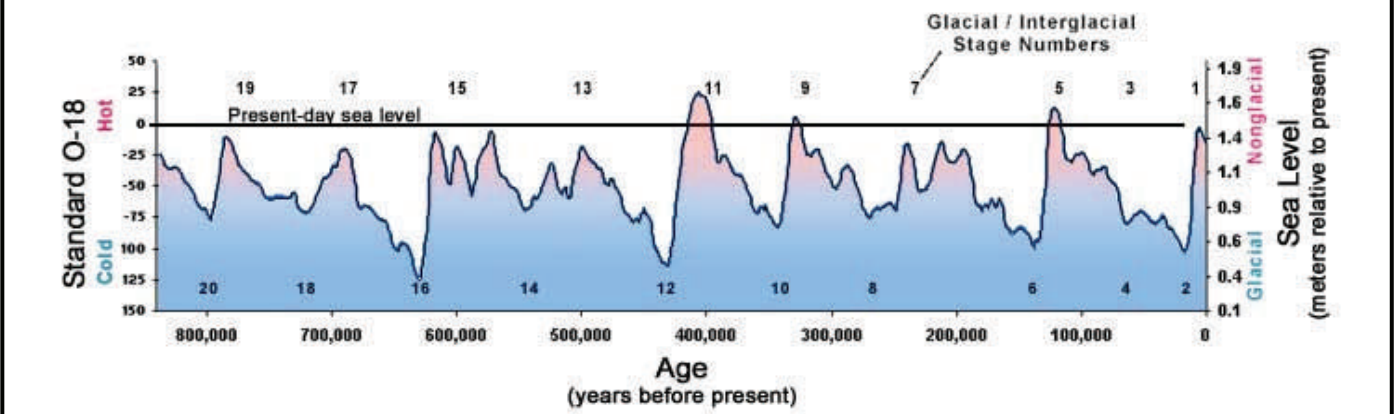
Fig. 2 Beach-cast *Durvillaea antarctica* (left) with **holdfast** showing **invertebrates** living within it (right)

Understanding Climate Cycles

Climate cycles over geological time are thought to be caused by variations in the Earth's orbital patterns (e.g. changes to the Earth's axial tilt). These changes in climate can be traced by scientists through geological studies. Evidence shows that the Earth moves between **glacial** (frozen) and **interglacial** (warm) periods at relatively regular intervals lasting tens of thousands of years. Currently we are at the peak of an interglacial (warm) period. But the use of fossil fuels by humans is dramatically increasing the rate of global warming within this interglacial period.

Fig 3: Oxygen isotope records are used to reveal the ice age cycles, serving as a proxy for sea level changes. Two isotopes of oxygen, O-18 and O-16 are studied. O-16 evaporates easily, entering the water cycle. During a cold period, O-16 is locked frozen in glaciers, leaving less in sea water and therefore less in shells of marine organisms. Higher proportions of O-18 in the shells of marine organisms indicate colder climatic conditions.

Image used with permission from Scripps Institution of Oceanography <http://coastalchange.ucsd.edu>



Glacial Periods and Extinction Events

As the Earth enters a **glacial period**, the decrease in temperature causes an increase in the area covered by ice. As well as ice expanding over previously habitable areas, sea levels fall as water is frozen into glaciers covering the land. Sea ice also expands across oceans, changing marine habitats; in the last glacial period the sea level was more than 120 metres lower than it is today. Figure 4 shows what New Zealand is thought to have looked like in the last glacial period, approximately 20,000 years ago. During a glacial period habitats can be altered, **creating new selection pressures** which can cause **extinction events**, particularly when a species is living near the edge of its habitat range.

Interglacial Periods: colonization and possible speciation

Interglacial periods are the warm parts of the climate cycles. Ice melts and glaciers retreat, creating the potential for species to move back into habitats that were occupied by their ancestors thousands of years before. Species that have survived the glacial periods are often able to increase their range, colonizing new habitats, which can, over time, lead to speciation events. In addition, the melting glacial ice raises **sea level**, often creating islands out of previously connected land. These islands can provide isolated habitats (such as we know in NZ), which are known to encourage speciation events.

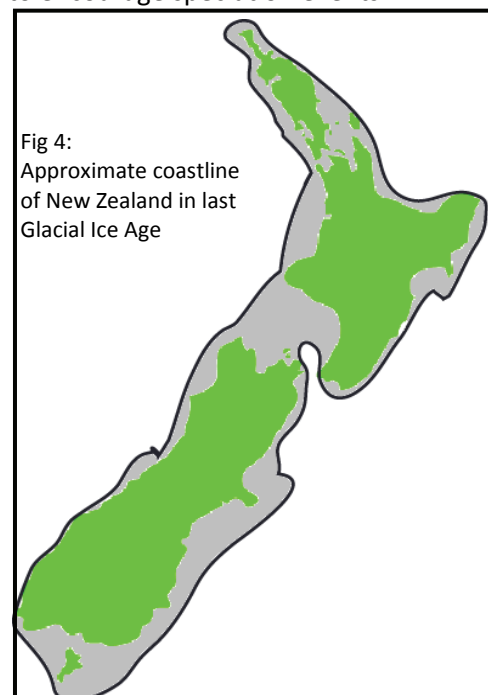
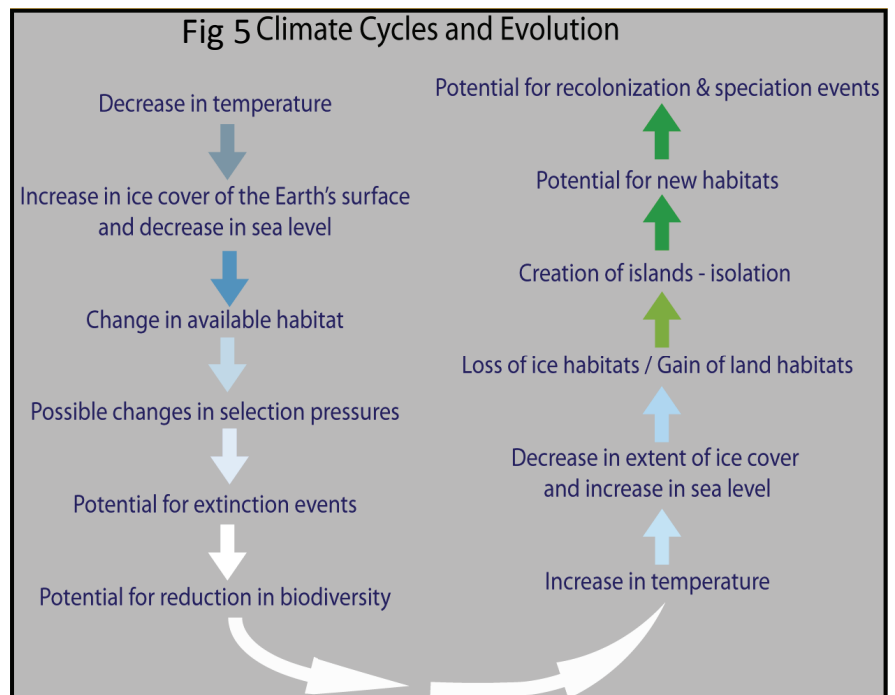


Fig 4:
Approximate coastline
of New Zealand in last
Glacial Ice Age



A Scientific Investigation

Ceridwen is part of a team of scientists who have been using genetics to investigate Southern Hemisphere Bull Kelp populations, looking at how closely the populations are related to each other. The team consisted of Ceridwen, Jon Waters, Hamish Spencer and Raisa Nikula of the Department of Zoology at the University of Otago. **They set out to understand how these populations may be genetically connected as a result of the kelp's ability to float long distances.** Excitingly, they also discovered new evidence about climate change processes.

Aim / Research Question

The specific questions that Ceridwen wanted to answer through her part of the project were:

- Does Southern Bull Kelp (*Durvillaea antarctica*) disperse via rafting?
- Was kelp wiped out by ice in the subantarctic during last ice age?

By finding the answers to these questions, Ceridwen has contributed to what we understand about the effects of past climate change periods. This information can help predict the effect of future climate change.

Methods

The process of finding out starts with **extensive planning and background research**. Once an appropriate research plan had been formulated, Ceridwen began **collecting her samples** from sites around New Zealand, the subantarctic islands, Chile and the Falkland Islands. Ceridwen travelled to these places over a period of two years, with the help of other scientists that had been contacted during the planning process. This work enabled her to make observations and collect samples of kelp, as well as invertebrates found living in the Kelp, to bring back to the lab.

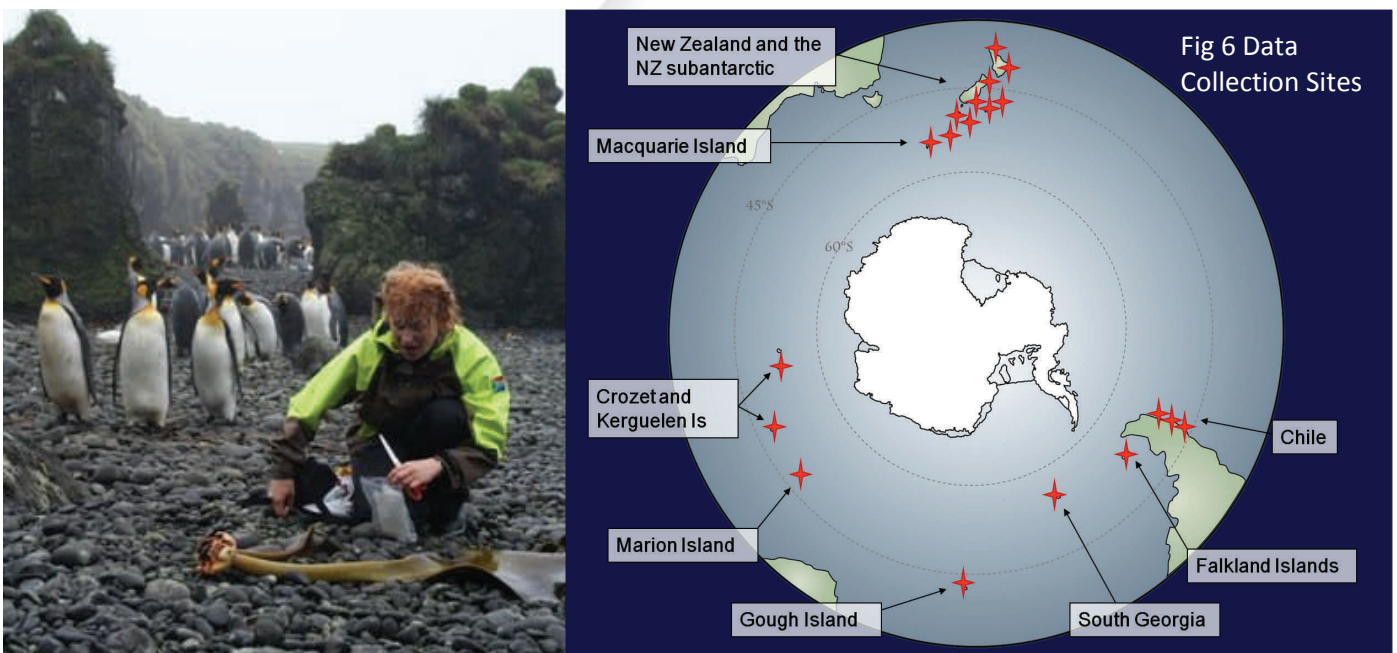
Of course, most people can't bring samples of seaweeds into New Zealand because of biosecurity regulations, so Ceridwen needed special permits from the Ministry of Agriculture and Forestry. Ever since, these samples have been carefully stored in isolation within a specially regulated laboratory.

Back in the lab Ceridwen and the team used modern molecular biotechnologies to find out how closely related the different species were. These analyses allowed Ceridwen to **infer** what had happened to this species during the last glacial period.

The techniques used were

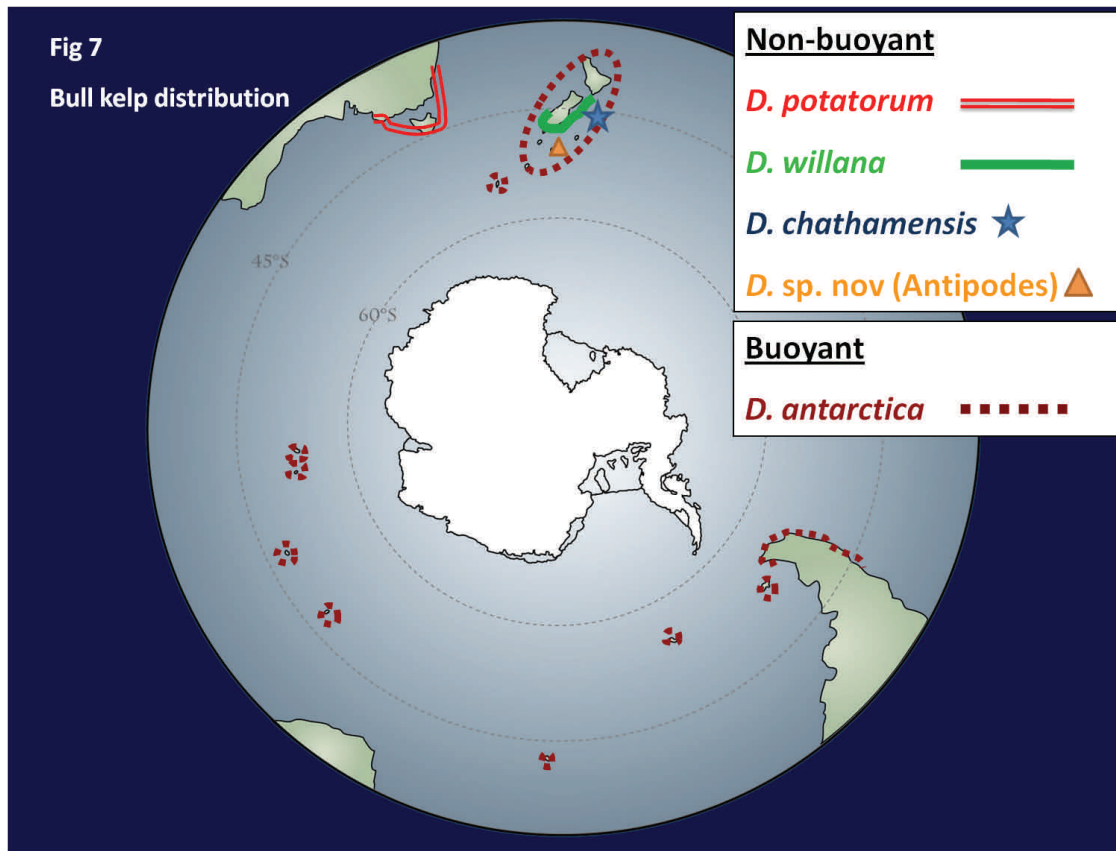
- Extraction of DNA
- Polymerase Chain Reaction to amplify specific DNA targets
- DNA sequencing
- Analysis of variation in genes
- Phylogenetic analyses to reveal relatedness

By using these techniques Ceridwen and the team were able to build up a picture of the genetic relatedness of the different populations of kelp.



Distribution of Buoyant and Non-buoyant Bull Kelp Species

The genus *Durvillaea* contains a number of species in the Southern Ocean area in addition to *D. antarctica*. Bull Kelp species can be split into two groups - one of **buoyant** species that can float, and one of **non-buoyant** species. Fig 7 shows that the non-buoyant species are restricted to the coasts of New Zealand and southern Australia. In contrast, the **buoyant** species *D. antarctica* (**Southern Bull Kelp**) is found throughout the region that was studied. This pattern suggests that the ability of *D.antarctica* to float allows it to occupy a larger geographical range.



Computing and Mathematical Power

With screeds of raw data, Ceridwen and the team harnessed the power of computers and mathematics to make sense of it all.

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Laminaria.AJ344328 tcttggacacagcgtgctgctgcttattcggttgcaattggcctagctcctggaaatcaatcttttaggggggaaatcaatcagctgtaaacgttatgtaactgcccacgcttt
Fucus.AV494879 tattaggaacagcctatgctgctgcttattagattgacagctgcaaacctcgggaaatgcttttagggggcaatatacagctttacaagcttatgtaaacgctcaatgctttt
chat.c.1 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCAGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
chat.c.2 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCAGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
chat.c.3 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCAGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
chat.c.4 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCAGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
chat.c.5 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCAGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
chat.c.6 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCAGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
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chat.c.10 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCAGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
fen.A.1 ?????GGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
fen.A.2 ?????GGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
fen.A.3 ?????GGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
fen.A.4 ?????GGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
fen.A.5 ?????GGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
fen.A.8 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
fen.A.9 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
fen.A.10 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
fen.A.11 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
fen.A.12 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
pot.b.1 ?????GGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
pot.b.2 ?????GGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
pot.b.3 ?????GGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
pot.b.5 ?????GGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
pot.b.101 TATTGGGTACAGTTATGCTGTTCTTATTAGATTACAGCTTGCAAGTCCGGAAATATGTTTTAGGGTGGTAAATATCAACTATATARTGTTATTGTAACTGCTCATGCTTTT
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This raw data shows the DNA sequences that were identified from the target genes. Ceridwen needed to be able to **identify patterns of similarity and difference** in these sequences so that she could find out how closely related the different species of Bull Kelp were. To do this without the power of a computer would take years! Thanks to computing technology that has been developed by scientists around the world to cope with this type of data, Ceridwen was able to analyse the data over a period of many months to create the evolutionary tree that you can see on the next page.

Interpreting an Evolutionary Tree

The evolutionary or **phylogenetic** tree that Ceridwen has drawn shows the relationship between the different genetic variants (called **haplotypes**) of Bull Kelp, *Durvillaea antarctica*, that she sampled.

1. COI (~700pb)

Sequences of mitochondrial DNA of around **700 base pairs** were used to look for patterns.

6. There are two ways of deciding whether each group is different. The lower number is called a bootstrap value. The closer this number is to 100, the more certain we are of the branch.

Durvillaea antarctica

COI (~700bp)

Bold values: Bayesian Post. Prob.

Grey values: ML bootstraps (1000)

Outgroups trimmed.

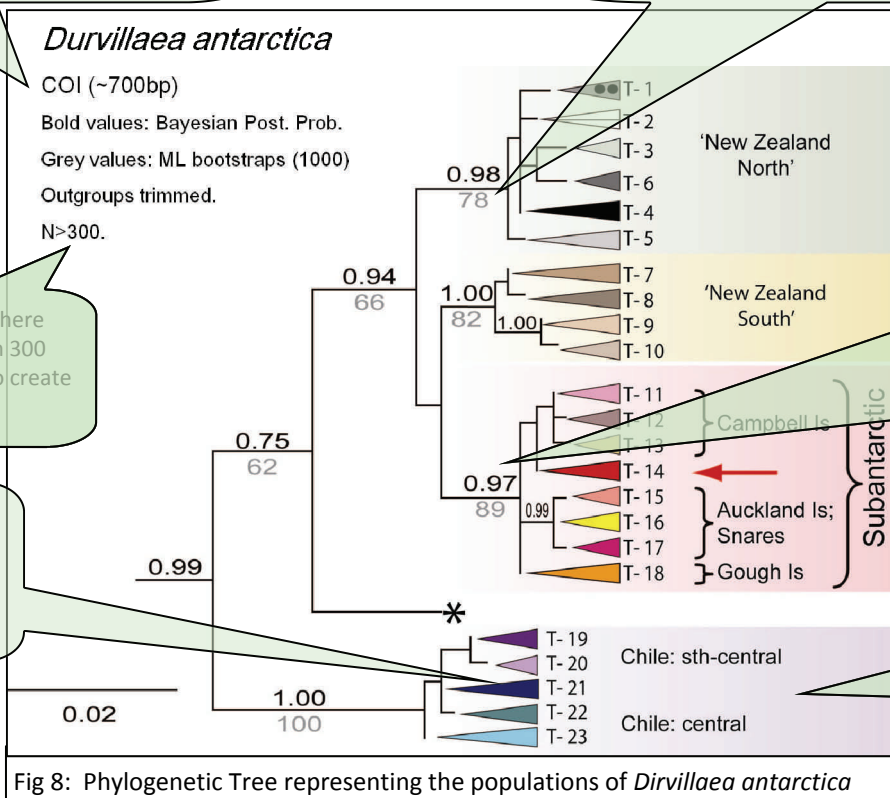
N>300.

2. N> 300 means there were more than 300 samples used to create this tree.

3. Each triangle represents a different **haplotypes** of Bull Kelp

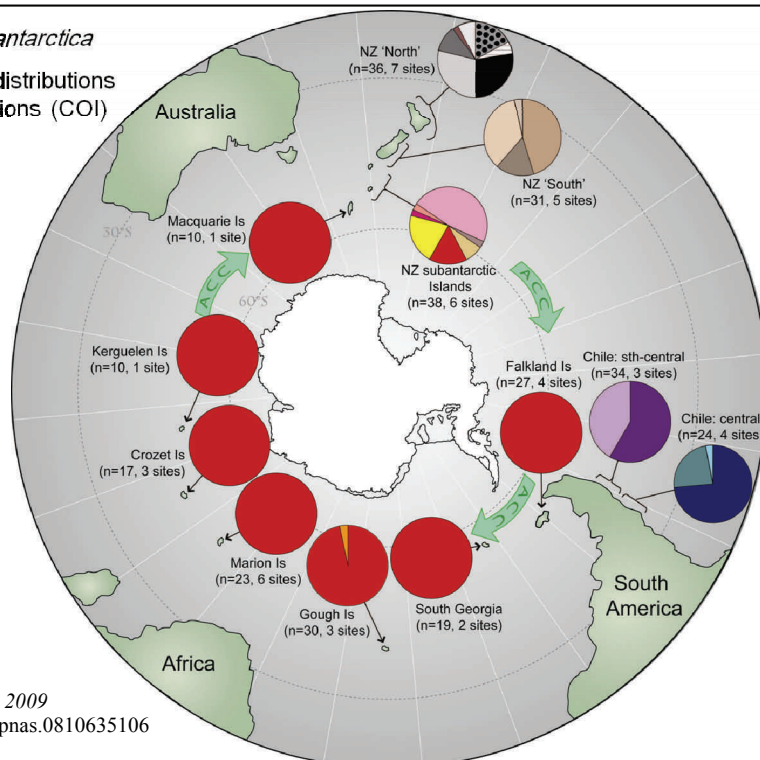
5. The computer programme can estimate the probability that each branch of the tree is true. An estimate of 1.0 is perfect. The closer the number is to 1.0, the more certain we are of the difference between the parts of the tree at this branch.

4. Each block represents **haplotypes** that are found in each geographical region.



The colours in the diagram below, representing the different **haplotypes**, match those in the **phylogenetic tree** above. Each pie shows the proportions of different haplotypes at each location on the map. This diagram shows that one haplotype (the red one) dominates the subantarctic. This almost-complete uniformity indicates that all these populations must have arisen from a common source population and that this event must have occurred relatively recently, suggesting that these islands were previously unoccupied by Bull Kelp.

Fig 9 *Durvillaea antarctica*
Haplotype distributions and proportions (COI)



From Fraser et al PNAS March 2009
www.pnas.org/cgi/doi/10.1073/pnas.0810635106

Why would these habitats have been available?

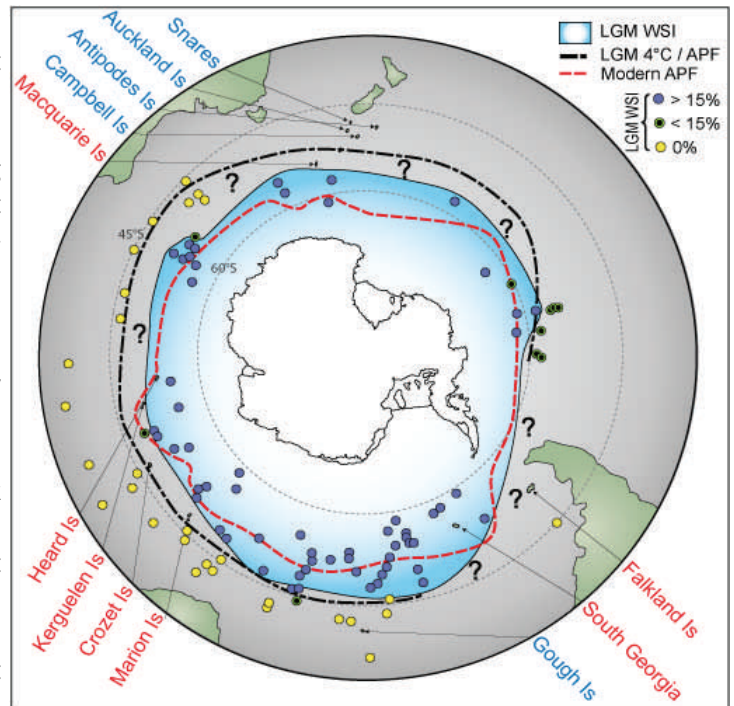
This diagram at the right shows the estimated extent of winter sea ice (WSI) in the Southern Ocean at the peak of the last ice age, the so-called **Last Glacial Maximum (LGM)**. These estimates were made using geological data compiled from sea-floor sediment cores containing fossil diatoms. Diatoms are single-celled algae, which occur in many habitats, and some species are most commonly found in sea ice. Geologists *infer* whether a core comes from an area that was under sea ice at the Last Glacial Maximum by looking at which diatom species are in the core. The blue dots on the diagram show the location of cores that indicate sea ice was present; the green-and-black dots show where the edge of the sea ice is thought to have been, and yellow dots represent cores that showed no evidence of sea ice.

Studies previously published by other scientists (researched by Ceridwen in her planning) show that Bull Kelp cannot survive where sea ice occurs. Could sea ice explain the genetic patterns found in Bull Kelp? Subantarctic populations could have been destroyed by sea ice during the last ice age. When the climate warmed and the ice receded, the kelp could have recolonised the newly-freed islands by floating across the oceans from somewhere beyond the reach of the sea ice. This scenario could certainly explain the **near-complete genetic uniformity** of the kelp populations throughout much of the subantarctic, versus the **higher genetic diversity** in places further north (such as in New Zealand and Chile) that remained free of ice.

The problem with this hypothesis was that sea ice at the Last Glacial Maximum was not thought to extend as far north as many of the supposedly recolonised islands (see Figure 10, above). The islands labelled in red are those that seem to have been recently recolonised by Bull Kelp, but some – such as Marion and Macquarie Islands – lie beyond the estimated range of Last Glacial Maximum sea ice. These islands, however, are in areas that have little geological data supporting the estimated limit of the sea-ice. You can see from the diagram that, although geologists have taken numerous cores, data is still lacking in many places. **Sea ice might, therefore, have been more extensive at the Last Glacial Maximum than previous studies have suggested.**

This exciting research not only shows how organisms can be affected by climate change, but also how biological studies can give us great insights into past environmental conditions. Understanding ancient climate change and its evolutionary impacts is a crucial part of making good predictions about the effects of future climate change – whether natural or human-induced.

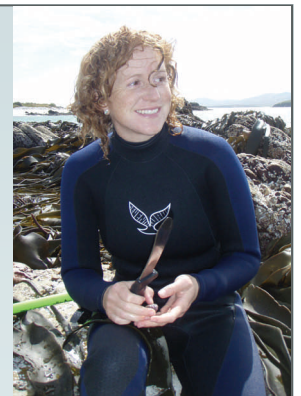
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From Fraser et al PNAS March 2009 www.pnas.org/cgi/doi/10.1073/pnas.0810631106

Profile of a Scientist

Ceridwen grew up in Canberra, Australia, and early in high school decided she wanted to become a marine biologist. Many tried to discourage her, saying there were few jobs in marine science, so Ceridwen did a university degree in art conservation—but she eventually had to admit that biology was still her dream, jobs or no jobs. She started a degree in Marine Science at James Cook University in tropical northern Australia, finished it off at Macquarie University in Sydney, worked for a year in the marine invertebrate research group at the Australian Museum, and then began a PhD in the Department of Zoology at the University of Otago, New Zealand, which she completed in 2009. She is now a postdoctoral researcher with the Allan Wilson Centre for Molecular Biology and Evolution. Since starting her degree in marine biology, she has never looked back, and instead looks forward to an exciting career filled with discovery and adventurous fieldwork.



ALLAN WILSON CENTRE FOR MOLECULAR BIOLOGY & EVOLUTION

Further Reading:

Fraser, C. (2009) An Icier Age. *Australian Science* <http://www.control.com.au/bi2009/305Ice.pdf>

Fraser et al (2009) Kelp genes reveal effects of subantarctic sea ice during the Last Glacial Maximum *PNAS* March 3, 2009 vol. 106 no. 9 3249–3253 (A copy of this paper is available for students on the seminar webpage at <http://lens.auckland.ac.nz>)

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