

Conservation genomics in practice

Securing biodiversity takes many measures, including the involvement of Indigenous peoples.

Vivien Marx

She's Māori and proud that New Zealand is “a crazy hotspot for biodiversity,” says Amanda Black, a biochemist and soil biologist at Lincoln University in Lincoln, New Zealand, who co-directs [Bioprotection Aotearoa](#). Many New Zealanders care deeply about the country's, and the planet's, environment and biodiversity. For Māori, “it's really close to our culture and how we define ourselves,” she says. Different Māori tribes are *kaitiaki*, or guardians, of *taonga*, or treasured species. Conservation-biology- and biodiversity-oriented projects in New Zealand involve Māori. Respect for Indigenous peoples is integrated into scientific practices in fieldwork and sample handling and when using data to benchmark computational tools. Those tasks are part of conservation genomics, in which methods such as sequencing and sequence analysis are used to study the genomes of people, plants, animals and microbes.

Researcher Lara Urban finds interaction with Indigenous peoples essential. She trained in Europe and just finished a two-and-a-half year Humboldt Research Fellowship at the University of Otago in Dunedin, New Zealand. Scientists must do things “the right way,” she says. “We are not going to be scientific colonialists anymore.”

As Black describes, New Zealand was once part of the supercontinent of Gondwana, which gave rise around 100 million years ago to New Zealand and, for example, Antarctica, Australia, Papua New Guinea and Madagascar. These areas became home to unique flora and fauna and thus desirable destinations for scientists. Many colonizers came to New Zealand with a sense of entitlement. Scientists took organisms and samples for their museums, herbaria and zoos, and performed analyses and published results about organisms or samples, without communicating with those who “have looked after them for centuries,” says Black. That sense of entitlement has to stop, she says.

These days, Indigenous peoples in New Zealand and elsewhere, including the First Nations of North America, South America, Africa, the Pacific Islands and Australia, have a seat at the table when biodiversity projects are planned. Their participation is sometimes window-dressing, she says, but this is slowly changing. Especially in New Zealand. What



Conservation-biology- and biodiversity-oriented projects in New Zealand involve Māori, for whom care for the country's and the planet's biodiversity and the environment is, says Amanda Black, “close to our culture and how we define ourselves.” Credit: LazingBee/Getty Images

fortifies Māori is the Treaty of Waitangi, Te Tiriti o Waitangi. This policy-shaping document governed matters such as trade and missions and was signed in 1840 by a representative of the British Crown and more than 500 Māori tribal chiefs. It transitioned to the document that guides policy between the Crown of New Zealand, the New Zealand government and Māori. Scientists seeking to perform studies of the country's flora or fauna that might involve collecting soil or water, or sequencing samples, need Māori consent and involvement. “In my opinion, the teams in New Zealand are much further ahead in this type of Indigenous people's engagement than many other places,” says Carolyn Hogg, a researcher at the University of Sydney. Such engagement happens in other projects, too, but “there is still a long way to go.”

Active protection

“Biodiversity represents all life on Earth,” says Hogg. It's about understanding the diversity of ecosystems, species and genes. Ecologists have long valued studying ecosystems, and there's a link between agricultural production,

human health and diversity in ecosystems and species. Tools were once lacking to apply genetic diversity in management decisions, but that has now changed. Current technology enables researchers to investigate and understand diversity in genes^{1–3}. She's happy the field has these new tools that “help conservation managers make more informed decisions when it comes to protecting biodiversity.” Hogg has long worked in conservation biology. One species she has studied, the Tasmanian devil, is an at-risk species, threatened in many ways: fragmented habitat, cars, lack of genetic diversity, an infectious cancer. The conservation toolbox, she says, offers many methods, such as captive breeding, translocations and artificial insemination, that need to be applied in different ways for different species.

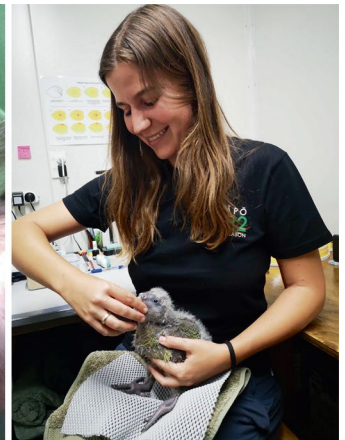
Māori have a holistic way of looking at nature and see organisms as “part of their extended family,” says Black. As a browse of the ‘[Red List](#)’—the International Union for Conservation of Nature's Red List of Threatened Species—reveals, some of this ‘extended family’ is not doing so well. This

collection shows global data about each threatened species: animal, fungus and plant species. The troubled categories span from 'near threatened' to 'vulnerable' to 'critically endangered' to 'extinct in the wild'.

The Auckland snipe (*Coenocorypha aucklandica*) is 'near threatened', and its population is increasing, but the New Zealand lesser short-tailed bat (*Mystacina tuberculata*) is 'vulnerable'. The North Island takahē (*Porphyrio mantelli*), a flightless bird unique to New Zealand, is extinct; the South Island takahē (*Porphyrio hochstetteri*) is endangered. According to New Zealand's Department of Conservation (DOC), there are around 440 individual South Island takahē. Among the conservation projects underway are captive breeding and release of individual birds into the wild. These individuals are radio-tracked, there is food for them, they get checkups with veterinarians.

The kākāpō (*Strigops habroptilus*), a nocturnal flightless parrot, has fared less well than the takahē: it's critically endangered. Population count has increased from 1995's count of 51 individuals, but according to New Zealand's DOC, only around 200 kākāpō remain. Along with organizations such as [Revive and Restore](#) and others, the department monitors many aspects of these birds' behavior, along with individual health and reproductive successes and failures. The efforts include using radiotelemetry to track them and providing food. Additional data comes from the Kākāpō125+ gene sequencing project that aims to sequence and assemble the genomes of all living kākāpō.

Scientists can apply to use data from the Kākāpō125+ project [online](#). Permission is given by the DOC and the Ngāi Tahu Māori, the main tribe of New Zealand's South Island. Pre-publication results must be shared with the tribe and are kept in confidence. Ngāi Tahu permission was required for a team that



There are only around 200 individual kākāpō left. To help nudge these animals away from the brink of extinction, it's important that "every single chick survives," says Lara Urban. Conservation genomics can help. Credit: L. Uddstrom

examined, among other aspects, reproductive success and failure in these birds and the possibilities of artificial insemination as a conservation tool in this and other species⁴.

Nights in the field

Ding dong. When Lara Urban's wake-up call sounds, it might be 10 p.m., it can be 3 a.m. She has brought Oxford Nanopore instruments for portable genomic sequencing to the island of Whenua Hou, which is to the south of New Zealand's South Island and reachable only by helicopter. There, she has been applying genomics approaches to help safeguard this critically endangered species. The alarm is part of a monitoring system connected to tiny cameras installed in all kākāpō nests, says Urban. When the mum leaves to forage for food for her chicks, "then we can actually approach the nest." They check on the chicks' health: she and others gently make some measurements, and may take cloacal or saliva swabs for analysis. When I spoke with Urban, she was just completing her time on Whenua Hou, where she had spent most of her fellowship. "We are alone on the island, it's just a beautiful island," she says.

The fruit the kākāpō mothers forage for is from the Rimu, a species of podocarp tree that only produces seeds every second to fourth year. "Only in these years do kākāpō breed and reproduce," she says. 2022 has been a breeding year, but seeds have not been ripening, "so the mums out here don't have enough food for the chicks," she says. The biologists brought food for the birds, and monitored every individual adult bird and all chicks. "Every single individual has its own name," says Urban. All family relationships are known. There's, for example, Nora, a kākāpō female, who is around 40 years old and has great-great-grandkids.

To help nudge these animals back from the precipice of extinction, it matters that "every single chick survives," she says. Among other tasks, the team checks for infectious diseases, such as aspergillosis, a respiratory tract infection. "If it affects a young chick that is immunocompromised or stressed, that can very easily lead to its death." This year, unfortunately, a number of kākāpō died and some eggs didn't hatch. In the last kākāpō breeding season, in 2019, some individuals died of aspergillosis, and that appears to be happening this season, too. The fungus occurs naturally: it's in the nest soil and in the animals. Analysis revealed the baseline abundance of this fungus, which is not harmful. "It's just the so-called mycobiome of the kākāpō saliva," she says. After swabbing the chicks, the scientists use nanopore sequencing to detect in real time when the fungus grows particularly quickly in the oral cavity, which could be when the danger of aspergillosis looms.

The birds are lek breeders, which refers to a mating pattern where males aggregate to attract females. Male kākāpō call out invitations to females to come to a little 'bowl' they dig in the ground. Says Urban, "You hear the males booming from everywhere and it's just a particular low-frequency sound that I've never heard before and it travels really far so you can hear so many kākāpō boom all over the island at night." After mating, male involvement in chick rearing is over: the mothers tend to the eggs and chicks.

Originally, Urban came to New Zealand to work on the takahē genome. Because the conservation biology teams focus on both species, she became involved in studying both the kākāpō and takahē. As a white European scientist, she made sure that she reached out to Māori as the guardians of these species

Some biodiversity numbers

There are approximately **8–12 million** eukaryotic species on the planet, **8.1 million** of which are plants and animals. There are around **1.84 million** taxonomically classified eukaryotic species. **More than 40,000** species are threatened with extinction. Planet Earth, by the end of the twenty-first century, might see its biodiversity reduced by **50%**.

Sources: Springer Nature, H. Lewin, University of California, Davis; the [International Union for Conservation of Nature Red List of Threatened Species](#).

who treasure these birds. Urban's next career stop is to launch her lab at the 'Pioneer Campus' of the Helmholtz Center's German National Center for Environmental Health in Munich, Germany. The focus there is on planetary health, a field in which researchers look at how ecosystems and human health are intertwined and use big data and artificial-intelligence-based analysis to assess and predict planetary health. She will also be on the faculty at the Technical University of Munich. In the virtual luggage she will be taking to her new venture, she is bringing large genomic datasets.

Finding a reference

An array of initiatives are underway to compile reference-grade genome assemblies of life on Earth. Such assemblies can shed light on many aspects of biodiversity. As Hogg says, a reference genome helps scientists determine if a sequence is a gene, to see what it encodes and assess if there is diversity at that gene. Conservation biologists might decide to move a population to improve gene flow. When one population clears a disease quicker than another, "we can move animals with the specific genetic variant that helps deal with disease." Unfortunately, most characteristics are polygenic, she says, but "in conservation we aim to maintain and promote as much genetic diversity as we can." Reference genomes, she says, provide a "blueprint of life" and help researchers understand how species interact with their often rapidly changing environment.

A consortium has assembled the kākāpō reference genome, and Urban has been part of the team compiling one for the takahē. It involves the Takahē Recovery team, the DOC, a team at Rockefeller University and Māori members. A high-quality takahē genome can inform all the downstream conservation efforts for this species, says Urban. It was challenging to get the right kind of samples in adequate quality, she says, "but it was totally worth it because it told us a lot about the actual genomic architecture of the takahē."

Takahē genomic information has been a crucial help in developing a computational method to assemble haplotype-resolved genomes when no parental data are available, which could prove helpful in many areas of biology. The quality of this phasing, says Urban, is comparable to that of one that involved parents' genomes. The method combines two types of genomic information: HiFi reads from Pacific Biosciences instruments and Hi-C chromatin interaction data. Pacific Biosciences introduced circular consensus sequencing a few years ago, which builds consensus reads, or HiFi reads, from multiple passes over a DNA molecule.

In developing this method, Heng Li at the Dana-Farber Cancer Institute, colleagues at University of Otago in New Zealand including Lara Urban and Neil Gemmel, and several teams from other US institutions such as Rockefeller University's Vertebrate Genome Project and the Center for Species Survival at the National Zoo, used data from the takahē and other animals, such as the critically endangered black rhinoceros.

When handling diploid and polyploid genomes, many long-read assembly tools collapse differing homologous haplotypes into a 'consensus assembly'. Some tools avoid erasing heterozygous differences and phase genomic regions with low levels of heterozygosity, and then build contiguous sequence by stitching these blocks together. The final assembly tends to include those phased blocks as an 'alternate assembly'.

With a method called trio-binning, which uses data from individuals and their parents, scientists can obtain a haplotype-resolved assembly with two sets of contiguous sequence: two haploid genomes. Other methods draw on additional data, such as chromatin interaction data from Hi-C or Strand-Seq, which applies single-cell sequencing and resolves homologs within a cell. In Strand-Seq, only the DNA template strand used during DNA replication is sequenced.

Li and colleagues developed the hifiasm algorithm⁵ to address complications they saw

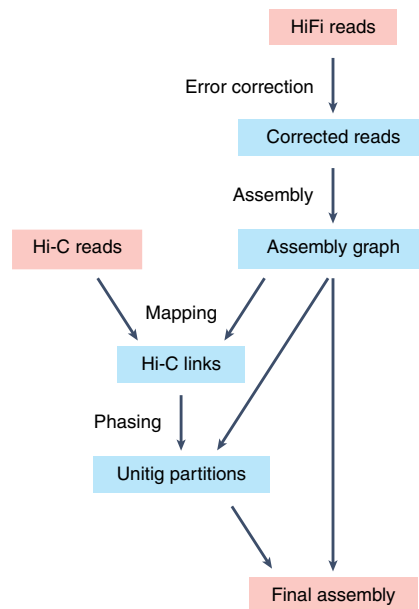


There are around 440 individual South Island takahē (*Porphyrio hochstetteri*) left. High-quality assemblies of the species' genome—parents and offspring—were used to benchmark a new computational tool. Credit: I. Warren

in this area, such as lengthy computational pipelines. Hifiasm applies string overlap graphs, which represent different paths along the assembled genomes. In a hifiasm graph, each node is a contiguous sequence put together from 'phased' HiFi reads. Li and colleagues have extended hifiasm to combine HiFi reads and Hi-C data⁶. First, hifiasm produces a phased assembly graph onto which Hi-C reads are mapped. The graph is made up of 'unitigs', contiguous sequence from heterozygous and from homozygous regions. Read coverage can be used to distinguish the two. Hifiasm further processes unitigs to build a haplotype-resolved assembly of a diploid organism.

The method avoids the traditional consensus assembly approach for a diploid sample, in which half of sequences are randomly discarded, and it mixes sequences from parents, which is clearly not ideal, says Li. With people, parental data can be hard to obtain and ethical approval is needed. Meanwhile, with samples obtained from animals in the wild, as in biodiversity studies, scientists usually have little or no way to locate parents. Methods exist for haplotype-resolved assembly without parent data, but they have only been tested on human samples, he says. "Making a haplotype-resolved assembler robust to various species is a lot more challenging," says Li. An algorithm designed for species of low sequence diversity, such as humans, may not work well for species of high diversity, such as insects. "Then there are species with mixed sequence diversity, which demands an algorithm can smoothly work with all these cases without users' intervention," he says. This motivated the team to extend hifiasm.

The takahē data from parents and chicks helped the researchers build a haplotype-resolved assembly that was a benchmark for their computational tool. "It is critical to have trio data as the ground truth," says Li.



The computational genome assembly method hifiasm has been extended. HiFi reads and Hi-C data are combined into a graph assembly that ultimately leads to haplotype-resolved assembly of diploid genomes for which parental data are lacking. Credit: Adapted with permission from ref. 5.

Instead of using human ‘trios’, they wanted to develop a robust algorithm that works for various diploid samples. Says Li, “Lara’s data is invaluable.”

The approach is applicable to many species, he says, but users should remember that the genomes of different species can vary dramatically in size, sequence diversity and repetitive sequence sections. “Although we have tried hard to make hifiasm work for various species, we may have overlooked cases or properties special to certain genomes,” he says. He recommends that researchers also evaluate their assemblies carefully based on what they know about the organisms they study. Users can raise a github issue or contact him and colleagues if they can’t resolve something on their own. “We are still learning how to build better assemblies,” he says, and assembly algorithms keep evolving as data quality improves.

Democracy how

Urban wants to see portable genomic approaches become globally and widely available, especially at locations where sequencing and bioinformatics infrastructure has not yet been built. Extracting DNA, getting it onto the nanopore sequencer and knowing how to analyze these data are not all automated. But the many rangers who work daily to keep the kākāpō safe don’t have time to learn all of today’s intricacies of sequencing and analysis, she says. “One of my goals in my career is to really make this more accessible to everyone so that you don’t require expert knowledge,” she says. Her fellowship has taught her that field-based sequencing is not yet at a point at which “everyone can use it for their own benefits



Whenua Hou, an island off New Zealand’s South Island, is a refuge for kākāpō, a critically endangered bird species. Credit: L. Urban

to in the end really democratize the usage of genomic sequencing approaches.”

Good funding mechanisms are needed in conservation biology and biodiversity research, which might also come from Western scientific funders, as hers does from the Alexander von Humboldt Foundation. Externally funded projects should respect local traditions, she says. She wants to get genomics tools into the hands of Indigenous peoples such as the Māori so they can explore how best to use such tools for their goals.

In *Aotearoa*, Māori for New Zealand, Māori can be rather wary of genomic approaches, “because DNA still reflects their genealogy, or *whakapapa*.” Western scientists have inadequately addressed the interests of Indigenous peoples and minorities in the past. For her work, she kept Māori tribal members in the loop. “You do have to consult with the Indigenous people here,” she says. “They can decide on if they

want this research to be conducted or not.” They also decide if a paper is to be sent to journals or not. Such interactions with Indigenous peoples about the ecosystems they know well have been crucial to her, and should be so for other researchers. “We also have this ethical, moral responsibility as scientists,” says Urban. Scientists should, for example, show Māori and other Indigenous peoples their intent and progress. “They are proper stakeholders in all of these scientific projects,” she says. The paper about the extension of hifiasm contains the phrase: “The South Island takahē reference genome was created in direct collaboration with the Takahē Recovery Team (Department of Conservation, New Zealand) and Ngāi Tahu, the Māori kaitiaki (‘guardians’) of this taonga (‘treasured’) species.”

Genetics, says Urban, is a path to understand the relationships of animals to one another, for instance to help determine how inbred they are. Genetics can reveal relationships within the population that are not yet apparent, and confirm parentage, too. With an endangered species, conservation becomes a numbers game, she says. With a population of 200 individuals, disease spread can lead to species extinction. “There’s so little genomic diversity left in the species because all of the individuals are sort of related to each other,” says Urban. Along with safeguarding these birds, researchers can use sequencing, observations and measurements to explore how to optimize reproduction rates by matching individuals that are as remotely related as possible. Urban’s work, and that of many others, shows how much time, effort and resources it takes to tend to every individual animal of a species threatened with extinction. With a species, “we should never let it go so far,” she says. In the future, “I want to focus on how we can basically also have a political and societal impact on that.” Instead of focusing resources on species safeguarding, “why not just safeguard our ecosystems, our environment from the very beginning and not let it go there and actually focus our energies somewhere else.” □

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Putting the e in eDNA

New Zealand is an island nation with two main landmasses, the North and South Islands, and hundreds of smaller islands, some of which are refugia where both birds have been taken to avoid predators. In her field work, Urban uses sequencing and environmental DNA (eDNA) analysis.

When analyzing eDNA from soil, water or air, “you have to be really careful with the assumptions that you make,” she says. Extracting DNA from a soil sample related to kākāpō lets her describe the biodiversity at that spot. But different animals shed different amounts of DNA through feces, skin, saliva or feathers: for example, larger animals will shed more DNA than smaller ones. Thus, finding more DNA at one location does not automatically translate to a higher species density there. Rarefaction is the statistical approach used to correct

for possible bias in field work, and it’s one Urban practices. She started her training in field biology, and later added data analysis and statistics while completing her PhD at the European Bioinformatics Institute.

Finding more eDNA at one location can indeed ease the detection of a rare species that might be not be present elsewhere. But its absence elsewhere can also mean “we didn’t have the statistical power to detect it,” she says. Instead of just giving a more DNA-laden sample more scientific weight, she applies a rarefaction curve. To make different samples from different locations comparable to one another, she might plot eDNA finds against the number of observations. It’s important to be “very, very careful” with all data from the wild, says Urban.