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Opportunities for modern genetic technologies to maintain and enhance Aotearoa New Zealand's bioheritage

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Abstract: In the past few years genetic technologies springing from advances in DNA sequencing (so-called high-throughput sequencing), and/or from CRISPR/Cas9 gene editing, have been proposed as being useful in bioheritage research. The potential scope for the use of these genetic technologies in bioheritage is vast, including enabling the recovery of threatened species, engineering proxies of extinct species and genetically controlling pests. While these technologies are often complex, they provide new opportunities that may help support New Zealand's beleaguered flora and fauna, and thus warrant scientific examination. Here we discuss these genetic technologies, focussing on scientific benefits and risks of each. We also acknowledge the social, cultural, ethical and regulatory constraints on their use, with emphasis on the importance of partnership with tangata whenua to determine when, whether or how these technologies should be used in enhancing New Zealand's bioheritage. We hope this will provide source material to support future decision making around the use of new genetic technologies in bioheritage.

Keywords: Conservation, de-extinction, gene editing, genetics, genomics, metagenomics, pest management.

Introduction

Biological systems the world over are under strain with many showing signs of a biodiversity crisis. Extinction rates are 100–1000 times higher than pre-human levels in many taxonomic groups (Pimm et al. 1995) and the biological heritage, herein bioheritage, of New Zealand is not immune. With 1002 threatened and 3096 at-risk species identified (New Zealand Threat Classication System), conservation of bioheritage in Aotearoa New Zealand is a priority. High-throughput DNA sequencing and gene editing technologies present us with opportunities to understand and thus better protect and restore NZ's bioheritage. Here we discuss technologies that allow us to better understand the genomes of individuals and species and assess biological diversity from environmental DNA samples, as well as technologies that might be used to understand the functions of genetic variations, control pests and carry out 'de-extinction' of lost species. Such technologies have evident applications in New Zealand but these warrant careful scientific consideration.

While focusing on the logistics of such genetic technologies, we acknowledge the social, cultural and ethical dimensions regarding their use; that is, the imperative need for robust dialogue among the scientific community, indigenous partners, involved stakeholders and the wider public to determine when, whether or how these technologies should be employed (Bennett et al. 2017; Moffat & Zhang 2014). A comprehensive review of the social, cultural and ethical challenges, and the most effective approaches for engaging with diverse communities, are beyond the scope of this paper and the expertise of its authors. Instead, we direct readers to Pretty and Smith (2004), Gupta et al. (2012), Braverman

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(2017), Kaebnick & Jennings (2017) and Bioheritage NSC Bioethics panel (2019), which contain substantial reviews of this area and cite relevant literature.

We also acknowledge that all research using genetic technologies on taonga (treasured) species, must be carried out in partnership with tangata whenua (Gavin et al. 2015; Moorhouse 2017; Ruru et al. 2018), especially if the eventual intent is to release genetically modified organisms in New Zealand. Indeed, beyond gaining social acceptance and passing regulatory checks for controversial research, the future of all bioheritage research in New Zealand must endeavour to build capability in Māori iwi and hapū and ensure benefit flows back to them. These principles are also specified in the Hazardous Substances and New Organisms Act (HSNO) 1996, which regulates many of the technologies discussed here, and requires that "the maintenance and the capacity of people and communities to provide for their own economic, social, and cultural well-being" must be recognised and provided for. With this broader context in mind, here we set out a range of genetic technologies that may have potential to improve our understanding, and thus better protect and restore the bioheritage of New Zealand.

Understanding species, ecosystems and communities using genomic techniques

Genome sequencing of native species

Genome sequencing is a common starting point for investigating the genetics of an organism. Sequencing the genomes of native New Zealand species has the potential to provide information that will enhance conservation and customary outcomes, yield new commercial opportunities and provide answers to fundamental scientific questions. Such sequences are also vital to support effective population genomics (below) which can inform management practice. New Zealand is well known for its unusually high number of phylogenetically isolated species, including vertebrates (Tennyson 2010), invertebrates (Buckley et al. 2015) and plants (Timewell 2015), and these species with no phylogenetically close relatives will require de novo assembly of their genomes, rather than relying on a closely related species as a reference scaffold. To date only a few genome assemblies of New Zealand native animals and plants have been published including a few birds (Le Duc et al. 2015; Galla et al. 2016; Cloutier et al. 2018; Galla et al. 2018; Galla et al. 2019), insects (Wu et al. 2017; Wu et al. 2017), and trees (Timewell 2015). There are now many local genome sequencing projects underway, with genuine indigenous partnerships, that target New Zealand vertebrates, invertebrates and plants (see Collier-Robinson et al. 2019). With the cost of short read sequencing declining and advancement in long read sequencing technologies and chromosome interaction mapping, the next few years will see a dramatic increase in the number of New Zealand native species with high-quality assembled genomes.

The sequencing and assembly of eukaryote genomes is becoming an increasingly routine exercise, particularly for diploid species with relatively small genomes (< 1 billion base pairs, or approximately a third of the size of the human genome). Many recent published assemblies are generated using high-coverage (≈ 100-fold), short read sequences of small inserts (< 1000 base pairs), with or without the help of larger inserts (> 2000 base pairs) (Li et al. 2010). These assemblies represent the spatial ordering of genes in a genome, but are usually fragmented, typically in tens of thousands of pieces, largely because highly repetitive regions and regions of high genetic diversity often cannot be assembled (Alkan et al. 2011).

Despite this, such 'low-quality' or 'draft' genomes are useful for bioheritage research, including the characterisation of single nucleotide polymorphisms (SNPs) (Nielsen et al. 2011) and development of conservation strategies based on genome-wide estimates of genetic diversity (Nielsen et al. 2011; Zhang et al. 2014; Galla et al. 2016). However, the fragmented nature of these assemblies limits downstream biological inferences. Other genome sequencing technologies, for example long-read technologies (Jung et al. 2019; Midha et al. 2019), are becoming increasingly available and affordable and promise 'high-quality' genome assemblies (Phillippy 2017).

While obtaining a high-quality genome is more expensive than generating low-quality drafts, the biological insights gained from these assemblies are striking, including the study of introgression informed by the phasing of alleles, genomic rearrangements and repeat structures (Han et al. 2017; Weisenfeld et al. 2017). Studies of gene family diversification and tandemly duplicated genes are more accurate because recent paralogues, duplications and alleles can all be differentiated (Miller et al. 2017).

Many native species of high conservation, customary, or commercial interest have large genomes, e.g. kauri *Agathis australis* ≈ 15 billion base pairs Davies et al. (1997). Given the cost and computational challenge of sequencing and assembling these large genomes, and the breadth of New Zealand's bioheritage, prioritisation of species for genome sequencing is needed. Such prioritisation must consider a wide range of factors, such as the natural and cultural value of a species, its threat status, how its loss might impact their ecosystem, and the extent to which its recovery could benefit from genome sequencing. New Zealand researchers have a special responsibility to focus on genomes from native, phylogenetically isolated species, as these will provide genomic information for effective conservation management (e.g. Galla, et al. 2016; Galla, et al. 2019) and understanding of New Zealand's unique biota. Beyond informing conservation, many native species also display unusual traits that have evolved in response to the unique and isolated New Zealand environment, for example the nocturnal lifestyle of kiwi. A focus on the genomes of species with these traits will lead to a deeper understanding of their evolution (Le Duc et al. 2015), which in turn may better inform conservation prioritisation.

In this context, a crucial issue is the protection of indigenous rights in regard to genomic data generated from taonga species. This has become even more pressing as DNA sequencing technology has expanded the ability of researchers to sequence reference genomes, as well as genomes from multiple individuals or populations for single species (see below). Overseas museums hold many samples of New Zealand native/endemic specimens, many of which are now accessible to genome sequencing, making protection of indigenous rights challenging when sequencing can be done with no reference to New Zealand. Such issues can also occur with New Zealand based collections. Hand in hand with technological advances is the expectation for genome data to be open access to enable replication and further study (Foster & Sharp 2007), which restricts the ability for indigenous peoples to protect information derived from genomes or exclusively benefit from it. In New Zealand, these and related issues, were encapsulated in the WAI 262 claim to the Waitangi Tribunal, which resulted

in the report Ko Aotearoa Tēnei (This is New Zealand), which seeks to exercise rights guaranteed under the Treaty of Waitangi over natural resources and other cultural treasures (Waitangi Tribunal 2012). Despite calling for the recognition of rights held by Māori over native species to be balanced against other rights and agendas, WAI 262 has not yet been resolved in a constitutional or legal framework. Regardless, we acknowledge that it is vital to build meaningful relationships with relevant Māori iwi and hapū, even if sequencing museum or zoological specimens held overseas, to co-develop effective and timely research that is responsive to their needs and expectations. In doing so, the likelihood of striking a balance between the rights of Māori and the expectation of open access research will be higher.

While guidelines are currently being developed, the single best option for researchers conducting New Zealand bioheritage work using genetic technologies is to engage early and often with relevant Māori iwi and hapū (see Collier-Robinson et al. 2019). In the absence of existing relationships, this is something that could be facilitated by Ngā Koiora Tuku Iho Biological Heritage National Science Challenge, Te Tira Whakamātaki Māori Biosecurity Network or Genomics Aotearoa. The Te Mata Ira guidelines (Hudson et al. 2016), developed for Māori health research, provides indication of some of the approaches that should be considered.

Genomics for measuring population genetic diversity

While sequencing a genome is a critical first step towards understanding the genetics of any threatened New Zealand native species, the genome assembly alone is insufficient for addressing most issues of conservation concern, as it is often generated from a single individual. Such sequences give us no idea of population level variation or structure (Brandies et al. 2019; Wright et al. 2019). To adequately address these issues, it is necessary to characterise genetic diversity across the genome and among multiple individuals. Such population-level studies are providing the foundation for investigating the genetic vulnerability and genetic resilience, of New Zealand's threatened species. These investigations will ultimately enable more targeted, and more specific, conservation efforts.

Genotyping by sequencing (GBS) (Elshire et al. 2011) is currently being used to measure genome-wide diversity at the population level for numerous New Zealand native species. This method, and related ones such as Andrews et al. (2016), use restriction enzymes to cut at homologous regions of the genome. Ligation of sequencing adaptors at these cut sites then allows sequencing across homologous regions among large numbers of individuals. Single nucleotide polymorphisms can then be genotyped and used for population genetic analyses. However, for species with relatively small, diploid genomes, it is now becoming feasible to 'leapfrog' this technology and move directly to genome sequencing. Indeed, genomes have already been sequenced for most living kākāpō *Strigops habroptilus*. Small populations and declining sequencing costs are increasingly making this kind of study possible for similar threatened species such as kakī black stilt *Himantopus novaezelandiae*, (Galla et al. 2016; Galla et al. 2019) and kōwaro Canterbury mudfish *Neochanna burrowsius* (Collier-Robinson et al. 2019). It is yet to be determined if these whole species approaches provide more useful data for conservation management (Fig. 1).

As many high-throughput sequencing based approaches are more developed in human genomics, it is possible to use this field as an exemplar for future directions of genomic scale data with threatened New Zealand native species. Given the recent trajectory of human genomics research, it seems

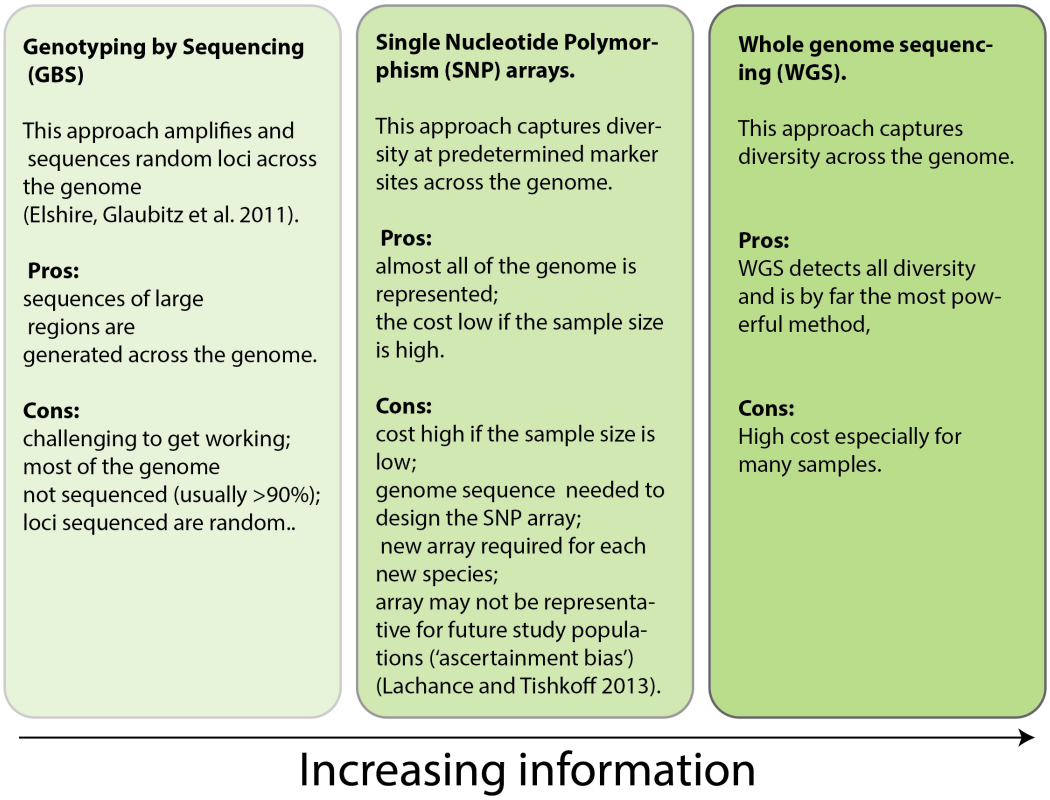

Figure 1. Benefits and pitfalls of various methods of large scale genotyping to understand population structure.

likely that whole genome sequences will rapidly become de rigueur, although delay is anticipated for species with large, complex genomes. Human genome sequencing projects are already pushing the limits of scale, for example the Genome Asia Project aims to sequence one hundred thousand Asian genomes within three years, with half of these already obtained. While the context of efforts such as these may not be directly relevant to a conservation setting, they will (1) drive reductions in population-level sequencing costs, (2) provide a framework for implementing the kinds of large infrastructure required for big genomic studies, and (3) stimulate the development of computational frameworks, algorithms and software to analyse data at a much larger scale. These advances will be hugely beneficial for sequencing whole populations of New Zealand native species in the future.

For extinct New Zealand native species, obtaining genome sequences for multiple individuals is challenging. However, population-level ancient DNA (aDNA) studies using complete mitochondrial genomes (mitogenomes) provides an opportunity to improve our understanding of past ecological and evolutionary processes (Cole & Wood 2018). For example, recent bioheritage research that combines mitogenomes from pre- and post-decline populations has revealed rapid biotic shifts in the distribution of *Phocarctos* sea lions (Rawlence et al. 2016) and rapid loss of genetic diversity in kākāpō (Dussex et al. 2018). Moreover, mitogenomes recovered from the remains and substrates of extinct species (e.g. bone, sediment, coprolite, egg shell, feathers) provide the opportunity to address key questions in bioheritage, such as determining species, alterations to previous species distributions, and changes in diversity on an ecosystem scale (Cole & Wood 2018).

Finally, the focus of geneticists will increasingly return to translating genome sequence data collection into information about function. Although recent decades have seen geneticists measuring diversity largely as an end in itself, the original purpose was to determine how genetic change leads to differences in phenotype (see below).

DNA metabarcoding and metagenomics for measuring bioheritage

Biodiversity patterns from metabarcoding

DNA metabarcoding requires PCR amplification of DNA fragments (amplicons) that are found in all of the species in question, but which contain enough genetic diversity to provide taxonomic information. Sample-specific DNA tags are then attached to these amplicons so different samples can be pooled together for high-throughput sequencing, while still allowing sequences to be correctly assigned to samples afterwards. Using high-throughput sequencing to monitor change in these DNA fragments allows for investigation of community structure or composition over time or between states (Caporaso et al. 2012). Metabarcoding is promising for broad biodiversity assessments to simultaneously capture the biodiversity of multiple taxa, such as microbes, fungi, protists, invertebrates in environmental samples. This approach, when applied to New Zealand soils, has yielded comparable results to traditional non-molecular methods (Drummond et al. 2015).

This amplicon-based approach has been used to identify diversity trends on both national, e.g. in catchments across New Zealand (Lear et al. 2017), and global scales (Thompson et al. 2017), at a level of taxonomic range and spatial scale that has not previously been possible. The sequencing depth per sample afforded by high-throughput amplicon sequencing enables insights into the rare biosphere (Sogin et al. 2006) and microbial seed banks that persist in the environment (Caporaso et al. 2012). Metabarcoding for water and soil quality monitoring can give insights into the responsiveness of communities to disturbance and stress (Shade et al. 2012a) and recovery post-disturbance (Shade et al. 2012b), providing a direct measure of ecosystem impacts, e.g. on bacterial, foraminifera, and macro-fauna communities associated with offshore oil extraction in New Zealand (Laroche et al. 2018).

Metabarcoding provides a relatively cheap and effective method for species richness, including rare species, in environmental samples. It is relatively easy to analyse the data, and this, combined with its relatively low cost, makes it very suitable for surveying many samples. However, metabarcoding suffers from an inability to accurately quantify the absolute numbers of specific organisms in a sample and, because the process uses PCR, it is biased to sequences that amplify well, and will miss any where the primers used for amplification do not bind a target.

Metagenomics for biodiversity and gene set assessments

Metagenomics, where DNA extracted from environmental samples is sequenced without amplifying a diagnostic amplicon, does not carry such limitations. Consequently, the approach can provide a broad assessment of sample attributes by recovering diverse taxonomic and functional information. There are two primary methodological approaches for handling metagenomics data: (1) direct annotation of read data, and (2) assembly. Direct annotation of read data is ideal for relatively rapid identification of changes in functional gene abundance across conditions, e.g. hydrocarbon degradation gene abundances in oil contaminated sediment (Mason et al. 2014), and has been used to correlate functional traits with abundant taxa in soil (Fierer et al. 2013). Where direct read annotation is the goal, shallow sequencing can be employed to increase sample sizes (Fierer et al. 2013) to partially offset the higher costs of metagenome sequencing. Shallow metagenomic sequencing has been shown to provide greater taxonomic resolution and species recovery than 16S rRNA gene based metabarcoding (Hillmann et al. 2018). This approach is effective where non-target DNA concentration is not prohibitively high, such as in some host microbiome systems.

The second approach involves de novo sequence assembly with contig binning to recover metagenome-assembled genomes (MAGs). Metagenome-assembled genomes are considered environmental 'population' or 'composite' genomes representing one or more strains (Tyson et al. 2004). This method is more labour-intensive than metabarcoding or unassembled read analysis, and requires deep sequencing of samples, which places constraints on sample numbers. However, the MAG approach generates a clear link between taxonomy and function, providing insights into the lifestyles of culture-resistant prokaryotes (Wrighton et al. 2014), prokaryotic auxotrophies (Kantor et al. 2013; Brewer et al. 2016), biogeochemical cycles (Baker et al. 2015; Anantharaman et al. 2016), contaminant response (Tyson, et al. 2004; Daly et al. 2016; Handley et al. 2017), and viral diversity and biology (Roux et al. 2016). The vast majority of MAGs recovered to date are from prokaryotes or viruses, owing to their relatively small and simple genomes. There are considerably greater challenges associated with assembling large and repetitive eukaryotic genomes from environmental DNA. Despite this, the genomes of uncultivated microbial eukaryotes have been assembled from complex communities (West et al. 2018), or after pre-sorting cells by flow cytometry into simplified

communities (Cuvelier et al. 2010). Such studies can achieve insights into the evolution and physiology of these small, but complex organisms (Cuvelier et al. 2010). However, researchers argue that these insights are limited by the relatively high importance of morphology and behavior in eukaryote function (Keeling & del Campo 2017).

Metagenomics, by avoiding the use of PCR, also avoids the problems of PCR bias described for metabarcoding; this also means it is not as effective at identifying rare species in environmental samples. The great strength of metagenomics is the ability to describe the diversity of an environment in much more than just what species are there, but also what metabolism or biology those organisms encode in that environment. However, metagenomics is very data intensive and requires great skill in interpretation and understanding.

Genomics for Biosecurity and Diagnostics

Genomics in the context of bioheritage, should not focus solely on threatened native species, but also those that threaten native or production species. Increased movement of people and trade has amplified the risk of introduction of threats that undermine ecosystems (see Ramsfield et al. 2016), and it is unlikely that this will ease, nor can we quarantine or inspect everything. Consequently, trade movement presents a major threat to New Zealand's bioheritage. The use of sequencing technologies allows for rapid development of diagnostic methods and characterisation of causal agents in response to new biosecurity incursions (see Grünwald 2012).

Biosecurity systems are built on a foundation of scientific and indigenous knowledge, which enables alien introductions to be quickly identified. In New Zealand, surveillance systems have been in place since the 1950s with the current focus on forests and high-risk locations such as ports of entry. This system works via traditional diagnostic methods, referring to taxonomic records and specimen collections within national herbaria, insect and fungal collections, arboreta and botanic gardens. These are vital national resources for species that can be visually identified, readily collectable and/or cultured. However, accurate identification is often challenging for species as many are microscopic, or difficult to culture, or lack morphological distinction.

Genomic tools such as metabarcoding and metagenomics have great power in biosecurity, particularly for species that cannot be easily identified by non-molecular methods. Such tools can provide rapid identification of key biosecurity pests (Hodgetts et al. 2016) and enable broad-scale screening for pathogens (Abdelfattah et al. 2017). Given the amount of sequence data that can be generated, it is important that national benchmarks are set describing endemic and naturalised microbial and microscopic diversity, against which new introductions can be identified.

While the uptake of genomic techniques, such as metabarcoding, for plant disease diagnostics in New Zealand has been slow, their value has been proven overseas. This approach has been successful in determining the causal agents of new plant diseases, such as the identification of an unculturable *Phytophthora* pathogen associated with oak decline, and subsequent development of a diagnostic qPCR assay (Català et al. 2017). Such identifications enable hygiene procedures to be implemented and the pathogen managed.

Genomic analyses (metabarcoding and genome sequencing) are also instrumental in characterising the genetic diversity and taxonomy of introduced pathogens (Brar et al. 2017). The taxonomic confusion that occurred with myrtle rust during the Australian incursion (Carnegie & Cooper 2011), as well as the diversity of species in the Myrtaceae that could be threatened by myrtle rust in New Zealand, demonstrated a need to prepare for what was considered to be an inevitable incursion. Molecular diagnostics and DNA-barcoding projects initiated in preparation have assisted in the rapid identification of the myrtle rust pathogen and host species affected during the 2017 New Zealand myrtle rust response (Baskarathevan et al. 2016; Buys et al. 2016).

Pest/pathogen management and response

The identification of pathogenicity genes and their roles in infection, using genomic and transcriptomic studies, is an active research area (Plissonneau et al. 2017). In New Zealand, genome sequences have been produced for several *Phytophthora* pathogens from different ecosystems (Studholme et al. 2016) alongside transcriptomic analysis of susceptible and resistant host genotypes.

Meta-barcoding, genome sequencing and metagenomics all have potential to provide information that supports the maintenance of ecosystem genetic resources in the context of biosecurity threats. For instance, in the selection of individuals that are resistant to pathogen challenges, such as myrtle rust and kauri dieback, a negative repercussion might be the narrowing of the genetic pool of vulnerable host species. Understanding genomic variation in populations provides the tools to inform these selections, allowing one to select for desirable traits whilst maintaining diversity and localised ecotypes over time to avoid genetic bottlenecks (see Toczydlowski & Waller 2019).

The genetic technologies we describe here, including metabarcoding, metagenomics and genome sequencing can all allow us to better understand host-pathogen interactions, especially in response to environmental, microbial and chemical stimuli. Genomics can better inform these complex interactions, developing an understanding of the selection pressures on pest and pathogen species under agronomic and ecological selection. Key areas of microbiome analyses in plant pathology include improved understanding of pathogen suppressive soils (Schlatter et al. 2017), litter diversity (Christian et al. 2017) and the role of plant endophytes (Gdanetz & Trail 2017) in plant establishment and health. Genomics thus has considerable ability to assist in pest/pathogen detection and management. In the future it will be critical to ensure that New Zealand's bioheritage preservation strategies include not only conservation of threatened native species, but genomic research into the pests/pathogens which threaten the recovery of these species.

Manipulating genomes to understand and improve our biological heritage

Functional genomics – moving beyond genome assembly

The technologies discussed thus far are screening technologies used to investigate varying levels of genetic diversity, be it ecosystem wide or species-specific. While these technologies are important, they often also require downstream functional genomics research to fully understand the consequences of any observed variation. Understanding the consequence of particular variants on phenotype will allow us to identify variation that is beneficial and variation that is not. The field of functional genomics is rapidly gaining attention, due in part to advances in techniques that can be used following

genome assembly. To gain information about the biology of organisms, it is useful to connect traits to individual genes or gene pathways.

Less direct methods of associating gene function with phenotype include gene expression analysis. Here changes in phenotype can be induced through experimental manipulation and corresponding changes in gene expression can be measured. This method typically relies on sequencing messenger RNA abundance and is known as RNA-seq (Todd et al. 2016). RNA-seq has been successfully applied to a number of New Zealand native species; for example, *Veronica* shrubs (Mayland-Quellhorst et al. 2016), stick insects (Dennis et al. 2015), and glow worms (Sharpe et al. 2015). These studies can identify candidate genes and pathways underlying adaptation to the New Zealand environment. More and more of these approaches are also being applied to better understand the functional diversity and resilience of complex ecosystems. One increasingly popular application is microbiome studies, which have been carried out on highly diverse systems including the human gut and skin, soil, rhizosphere, and in association with plant pathogens. In addition to whole genome sequencing, associated methods including RNA-seq (interrogating gene expression), micro-RNA sequencing (identifying microRNA regulators of gene networks) and bisulphite sequencing (examining DNA methylation (Duncan et al. 2014)), will be increasingly applied to native species. Collectively, these methods will reveal the genomic basis of traits in New Zealand species as well as the processes regulating genomic function.

More direct functional studies are laborious, and usually rely on the expression of genes in cell cultures or other vectors. Functional genomics often employs gene-silencing methods such as RNA interference (RNAi). RNA interference uses double stranded RNA to disrupt gene expression (Fire et al. 1991), and this generally leads to an abnormal phenotype, providing clues about the function of a gene.

In more recent years, gene editing has been used for functional genomic techniques (Hsu et al. 2014; Piaggio et al. 2017). CRISPR-Cas9 is an enzyme/RNA complex (Cong et al. 2013; Mali et al. 2013; Shalem et al. 2015), which allows the targeting of a double stranded DNA cut to almost any sequence in the genome (Figure 2a). This very specific targeting allows precise editing of genes, because when DNA is cut, cells repair that cut by copying a similar piece of DNA found in the cell (Jasin & Rothstein 2013), or by an error-prone mechanism of linking the two cut ends back together (Figure 2b) (Carroll 2014). By providing a synthetic piece of DNA mostly identical to the regions around the cut site, but with changes or an insertion, those changes or insertions will be copied into the genome, allowing edits to be made (Figure 2b) (Carroll 2014). This technology allows the apparently precise modification of DNA within an organism, providing the potential to modify the genome of a species. Reasons for doing such are broad; modification could be to better understand functional consequences of diversity information or could be used to increase genetic diversity in a threatened species to enhance its recovery in the wild (see below).

Regulatory status of gene editing technologies in New Zealand

The use of gene editing technologies, such as CRISPR/Cas9, involves genetic engineering and under current legislation must be closely regulated regardless of whether it will be contained in a laboratory setting or done with the eventual intent to release genetically modified organisms. The requirements of a range of Acts must be taken into account when considering gene editing techniques. The main legislation that covers such work is the HSNO Act 1996. As its name implies, the HSNO Act regulates organisms that are considered to be new to New Zealand, including genetically modified organisms (GMOs). Other relevant legislation in the conservation context includes the Biosecurity Act (1993), the Wildlife Act (1953), the Animal Welfare Act (1999), the Agricultural Compounds and Veterinary Medicines Act (1997), the Medicines Act (1981), and the Conservation Act (1987). The definition of a new organism to New Zealand is found in section 2A of the HSNO Act, contains several clauses that affect various genetic

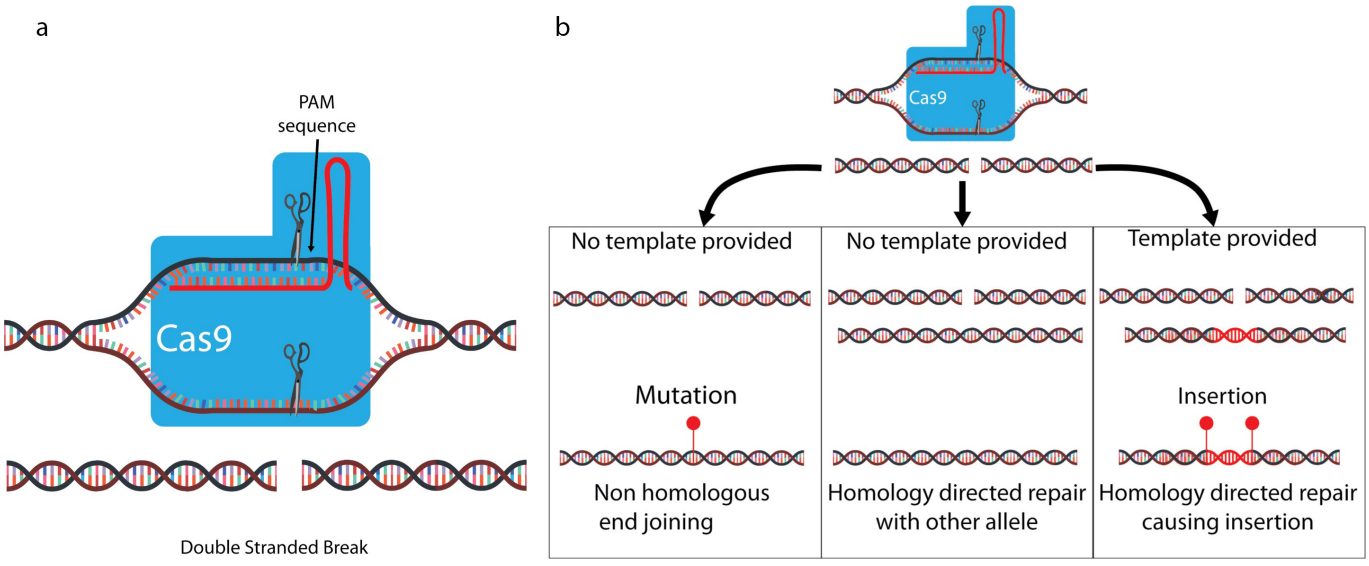

Figure 2. CRISPR/cas9 gene editing. a) Guide RNAs (shown in red) guide the Cas9 enzyme to any sequence adjacent to a PAM sequence, where-upon it makes a double stranded break. b) Double stranded breaks are repaired in cells via either non-homologous end joining (which can be error prone causing mutations), or homology directed repair (which can repair without error), or be manipulated to insert a piece of DNA.

technologies that can be used in bioheritage applications. Specifically, a new organism is:

"(a) an organism belonging to a species that was not present in New Zealand immediately before 29 July 1998

(b) an organism belonging to a species, subspecies, infrasubspecies, variety, strain, or cultivar prescribed as a risk species, where that organism was not present in New Zealand at the time of promulgation of the relevant regulation

(c) an organism for which a containment approval has been given under this Act

(ca) an organism for which a conditional release approval has been given:

(cb) a qualifying organism approved for release with controls

(d) a genetically modified organism

(e) an organism that belongs to a species, subspecies, infrasubspecies, variety, strain, or cultivar that has been eradicated from New Zealand."

A genetically modified organism is specifically defined in section 2 of the HSNO Act as:

"unless expressly provided otherwise by regulations, any organism in which any of the genes or other genetic material—

(a) have been modified by in vitro techniques; or

(b) are inherited or otherwise derived, through any number of replications, from any genes or other genetic material which has been modified by in vitro techniques"

As noted above, exemptions to this definition are specified in regulation in the HSNO (Organisms Not Genetically Modified) Regulations 1998. Recent amendments to these regulations clarified that the gene technologies discussed in this paper create organisms that are considered to be GMOs in New Zealand.

Under the HSNO Act, all gene-edited organisms would be considered to be genetically modified organisms (GMOs). The HSNO Act also specifies that any new organism requires some form of approval from the Environmental Protection Authority (EPA; www.epa.govt.nz) before it can be imported, developed, or released in New Zealand. The EPA is responsible for all decisions regarding GMOs, including research in containment laboratories and release into the environment. All applications regarding new organisms, whether for importation into containment, for development (including the development of GMOs), or for release, undergo a thorough assessment of potential risk, risk mitigation, and potential benefit. Public consultation is an aspect of many of these decision-making pathways.

CRISPR/Cas9 for returning lost diversity

A potential opportunity for CRISPR/Cas9 technology is to use genome editing to return lost genetic diversity to enhance the recovery of genetically depauperate threatened species (Frankham 1995; Kennedy et al. 2014; White et al. 2015; Piaggio, et al. 2017). The idea is that genetic variation could be added back to a species to improve its long-term survival. An understanding of the genetic variation in the original population before population decline could be gained from examining the genomes of museum specimens or sub-fossil remains (Orlando et al. 2015), and, with genome sequencing of the current population, missing genetic variation could be identified. CRISPR/Cas9 gene editing techniques could then be used to reintroduce the lost genetic diversity into the remaining individuals (Piaggio et al. 2017).

To date, this idea has largely been restricted to editing genes of known function to facilitate pathogen resistance (e.g. sylvatic plague in black-footed ferrets; Novak et al. 2018). However, recent discourse in New Zealand has shifted to the possibility of editing genes or gene pathways underlying complex detrimental traits associated with inbreeding depression or high genetic load (Taylor et al. 2017).

This idea is problematic for a number of reasons. The first problem is that while high levels of genetic variation are typically beneficial in populations, how do we distinguish standing neutral or beneficial variation from deleterious mutations? Given limited samples of historical variation, it is possible that even variants found at high frequency might not be advantageous (Bodmer & Bonilla 2008). Given that we have little understanding, in most conservation species, of the function of genes, let alone regulatory sequences, it seems reckless to return random variation to the genome. For these technologies to work, the epigenetic and genetic bases of the traits in question must first be identified, and our ability to do so is still in its infancy, particularly for threatened species (Galla et al. 2016).

A second problem arises from the technical aspects of gene editing. Gene editing is not 100% successful in the most tractable of species: developing the technology, and then carrying out multiple gene edits on a single individual, will require a large number of oocytes or eggs (100–1000s). For example, successful gene editing of a genetically depauperate species such as kākāpō (White et al. 2015; Dussex et al. 2018), would require the production, and potential wastage, of hundreds of eggs; an impossibility at this point (Houston et al. 2007).

Finally, organisms gene edited using CRISPR/Cas9 would be regulated as genetically modified "new organisms" under the HSNO Act; acceptance from all relevant public groups including Māori iwi and hapū, would be paramount before the genome of any taonga species could be gene edited. Given the ethical, cultural and social considerations, combined with a high risk of scientific failure, it seems unlikely that genetic technologies will be used in this particular situation (but see Taylor et al. 2017).

De-extinction

Beyond the reintroduction of extinct genetic variation into an existing population, is the idea of de-extinction, or the engineering of functional proxies for extinct species (Seddon et al. 2014; Shapiro 2015). A key step in de-extinction was demonstrated by an experiment in which an entirely synthetic genome of a bacterium was made, inserted into a bacterial cell from which the DNA had been removed, and shown to be able to keep that cell alive (Gibson et al. 2010). This experiment raised the possibility that the genomes of extinct organisms could be synthesised, inserted into an oocyte, or one-cell embryo, and left to develop into the missing organism (Figure 3).

The idea of using genetic technologies to engineer proxies of extinct species that could restore ecological function presents a number of technological hurdles. Some might argue that the first challenge, the acquisition of the genome of extinct species, has been solved by improved sequencing and ancient DNA technology (Orlando et al. 2015). Indeed, we now have the genomes of a range of extinct organisms; however, only a select few are of high quality (Shapiro 2017).

The second challenge is synthesising the genome of an extinct species. The technology used in the bacterial example is not able to make the amounts of DNA required to produce

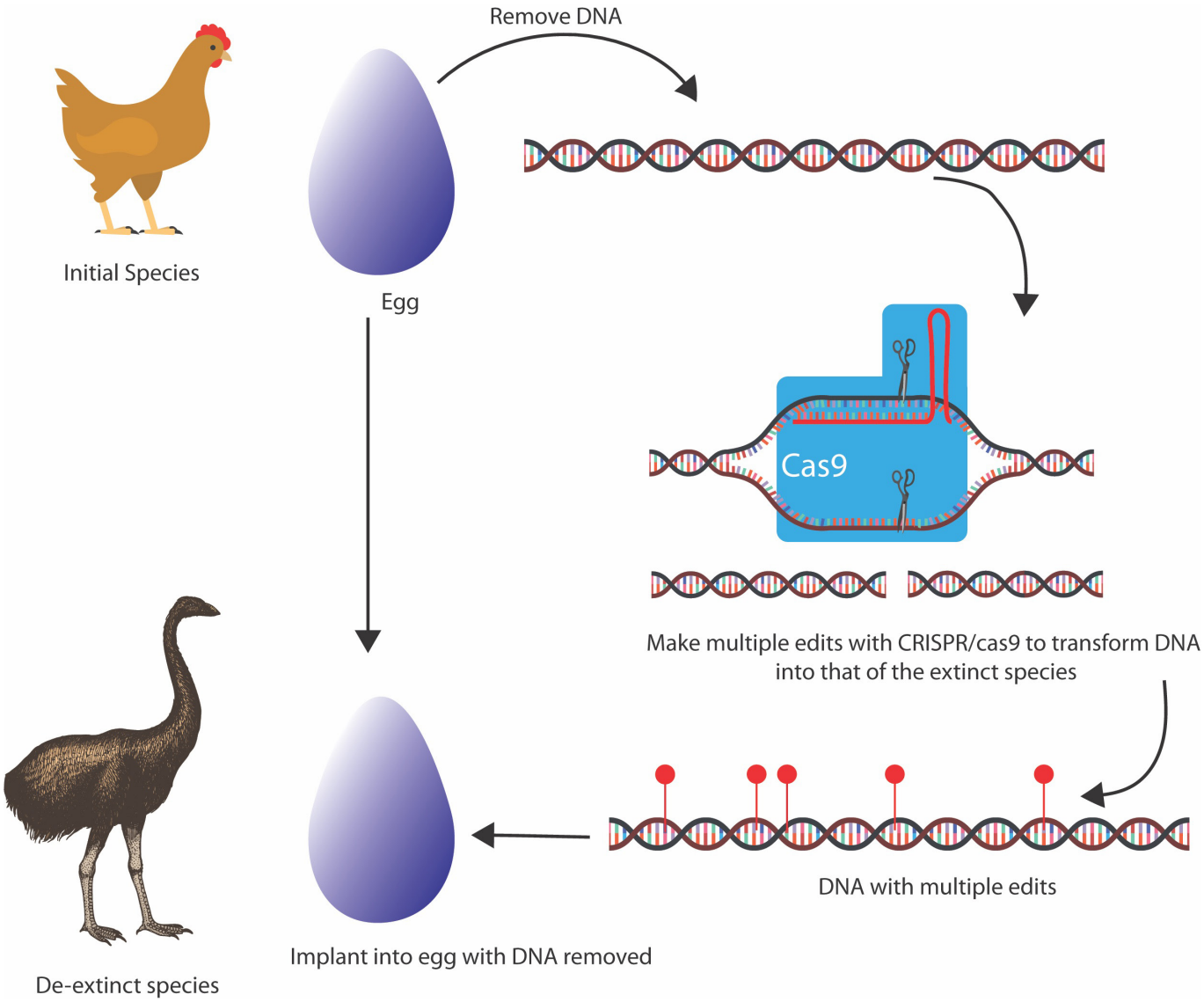

Figure 3. De-extinction. Using CRISPR/Cas9 gene editing it is theoretically possible to edit the genome of a related species into that of an extinct species. By placing that editing DNA into a donor egg or embryo, it may be possible for that DNA to drive the development of that egg/embryo into a functional proxy of the extinct species.

a eukaryotic genome, so the idea has arisen to instead edit the genome of a closely related organism to resemble that of the extinct organism (Shapiro 2015). This is technically difficult, but the advent of CRISPR/Cas9 gene editing technology has made it plausible (Cong et al. 2013). For example, there are estimated 1.5 million nucleotide differences between the genome of the woolly mammoth and its closest living relative, the Asian elephant (Lynch et al. 2015), meaning all those sites would have to be individually changed to produce a 'mammoth-like' genome.

The next challenge is to identify which cell to insert the now modified genome into. In animals, the obvious solution is an egg cell that has had its normal DNA removed. This is routinely carried out in cloning procedures (Campbell et al. 1996), but it is challenging for extinct animals. It has been proposed, for example, that a synthesised mammoth-like genome could be inserted into an elephant oocyte. This seems like a reasonable proposition, except that oocytes are not featureless balls of cytoplasm waiting for the DNA to direct them. Maternal provision of proteins and RNA into oocytes means that they are patterned, and that patterning directs the expression of genes and drives early development (King et al. 1999; Li et al. 2010). While the patterning cues in an elephant oocyte might support the development of a mammoth-like organism, we know that in many systems such maternal patterning factors evolve relatively rapidly, meaning we should not assume that an egg from a closely related species will work (Palmer 2004).

Alongside this, DNA is not placed as a naked structure into oocytes in nature. DNA is wrapped around nucleosomes, resulting in a 3D structure that affects gene expression, and in each cell-type of an organism, this 3D structure of the DNA differs (Cavalli 2006). Oocyte DNA is structured differently to DNA from somatic cells, and those differing structures are required to ensure proper gene expression and development (Cavalli 2006). While we can sequence the genomes of extinct animals, it seems unlikely that we can determine the proper 3D structure of the genome in the oocyte (Orlando et al. 2015).

The next question is if we can de-extinct a species, what

could we do with it? Like species gene-edited to restore lost diversity, engineered organisms that replace an extinct species would also be regulated as GMOs under the HSNO Act. Setting this point aside, such organisms would still require EPA approval for release as new organisms because, as formerly extinct organisms, they would be considered to have been "eradicated" from New Zealand (see definition above). Therefore, release into the environment is not likely under existing regulatory frameworks (Richmond et al. 2016). Given the costs of creating and then managing such species, the possibility that they may be maladapted to current environmental conditions (Seddon 2017b), and the risk of their re-extinction (Steeves et al. 2017), many have asked if the necessary resources would be better spent on conserving threatened species, rather than resurrecting those we have already lost (Ehrenfeld 2013; Bennett et al. 2017). At this early stage, the value in improving biodiversity through resurrection of a single species appears to be outweighed by how many different threatened species could be conserved with the same resources (Bennett et al. 2017; Iacona et al. 2017). Currently, although de-extinction is not a zero-sum game (Seddon 2017a), bioheritage is best maintained through conservation of at-risk species, a process that should be informed by genetic technologies, but not yet enhanced through species de-extinction.

Gene Drives for Pest Control

Pest control presents another aspect of bioheritage that has the potential to be assisted by genetic technologies. Our unique, treasured ecosystems, and agricultural systems, are damaged greatly by introduced pests ranging from vespine wasps (Donovan 1983), through a slew of mammalian predators, to sea squirts that damage our marine ecosystems (Fletcher et al. 2013). These pests are widely distributed, prolific and are currently controlled in some areas by costly poisoning and trapping campaigns, often met with much debate.

In 2016, the government announced the 2050 Predator Free New Zealand Project (Kirk 2016), which aspires to eliminate mammalian predators, particularly rats, stoats and possums, from New Zealand. The announcement triggered a great deal of speculation in the media about the use of gene drive technologies (Knight 2016), a way of causing the local eradication of a pest population. Gene drives rose to prominence as a potential solution to mosquito-borne diseases (Achenbach 2016), particularly Zika virus. While gene-drive technologies have been proposed for a few years, the advent of CRISPR/Cas9 genome editing technologies (Hsu et al. 2014) recently provided an effective tool. Gene drives have been developed in the lab for yeast *Saccharomyces* (DiCarlo et al. 2015), *Drosophila* (Gantz & Bier 2015), *Anopheles* mosquitos (Gantz et al. 2015; Hammond et al. 2016) and mice (Grunwald et al. 2019). Gene drive technologies for pest control in New Zealand have recently been reviewed extensively (Dearden et al. 2017) and so we refer readers to that publication for details of gene drive technologies, their risks and benefits and their potential deployment against a range of NZ pests.

Most of the pests that beset New Zealand's ecosystems have not been examined for potential gene drive systems. Those gene drive systems that have been developed have benefitted from over 100 years of research into yeast and *Drosophila* genetics (informing the biology of mosquitos), which allowed the identification of targets, and the development of the gene systems that might cause population decline. Few of the pests we have in New Zealand have been subjected to any genetic study and even fewer have had their genomes sequenced, a prerequisite for the development of effective gene drive systems. In all meiotic gene drive systems, the development of genetically modified organisms is required. None of the pest species affecting New Zealand, except mice (Doetschman et al. 1987) and rats (Geurts et al. 2009), have been genetically modified.

Furthermore, while gene editing technology has allowed the simplified development of gene drive systems to collapse pest populations, in turn making it a more plausible opportunity to improve national bioheritage (Esvelt et al. 2014), few of these systems have been developed (DiCarlo et al. 2015; Hammond et al. 2016; Windbichler et al. 2011) and none deployed. When used in research situations many gene drive systems have not been effective, and a number of technical issues must be overcome before gene drives can become usable (Champer et al. 2017). Due to the lack of fundamental knowledge required to genetically modify many of our pest species or identify key genes that could be modified to engineer population collapse, gene drives will not be used in a bioheritage context in New Zealand in the near future without extensive research beforehand. We still have much to learn about the pests that beset New Zealand ecosystems, and the technology needed if we are to develop effective gene drives.

Conclusions

New Zealand has many opportunities to use genetic and genomic technologies for the enhancement of bioheritage. While we do not necessarily advocate for the use of these technologies, and acknowledge the social, cultural, ethical and regulatory issues raised by each, we also contend that it is critical to initiate a public discourse about the technologies that have the greatest capacity and potential to assist in our battle to preserve New Zealand's bioheritage. Indeed, such technologies will only continue to develop further, so we must ensure that New Zealand, a country celebrated for bioheritage and nature, is not left behind. It is vital, therefore, that research into these technologies, in containment where necessary, is carried out in New Zealand, to ensure we have the capability and capacity to understand them and deploy them if needed.

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