

# Current applications and future promise of genetic/genomic data for conservation in an Aotearoa New Zealand context

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# Executive summary

1. To achieve the vision outlined in the national strategy for biodiversity, Te Mana o te Taiao, we will need to unite diverse disciplines, including conservation genetics/genomics.
2. As conservation genetic/genomic data generated for—and associated with—taonga (treasured) species is also taonga, we highlight the need for collaborative research partnerships that centre the needs, aspirations and expertise of mana whenua.
3. As a team of predominantly early-career conservation genetics and genomics researchers working across institutions as Te Tiriti o Waitangi partners, each speaking to our own expertise, we review available and emerging tools in the conservation genetics/genomics toolbox.
4. To support practitioners in identifying appropriate and affordable tools from the toolbox, we present a table that encompasses resource requirements (including finances, time, and skill) to assist conservation practitioners in assessing the associated costs and benefits of these tools for informing conservation management.
5. To support researchers and practitioners in establishing long-lasting partnerships with mana whenua, we highlight key aspects of data management and data sovereignty for consideration.
6. Intended as a platform to initiate discussion within and among conservation practitioners and researchers, mana whenua, and local communities, the development of government policies is beyond the scope of this contribution.
7. To meet the vision of Te Mana o te Taiao, we conclude by calling for a transdisciplinary approach that includes conservation genetics/genomics.

# Part 1: Conducting conservation genetic/genomic research in Aotearoa New Zealand

## 1 Introduction

The recent national strategy for biodiversity, Te Mana o te Taiao, challenges Aotearoa New Zealand to restore and enhance biodiversity for future generations (Department of Conservation (DOC), 2020). Many taonga (treasured) animals and plants are threatened by habitat loss, disease, invasive species, incidental bycatch, direct hunting, and climate change. To achieve the vision of protection, restoration and sustainable use of biodiversity outlined in Te Mana o te Taiao, we must bring together diverse ways of knowing and seeing the world—each of which bring their own unique toolboxes, including the use of genetic/genomic data (Rayne et al., 2020).

Genetic and genomic data provides a lens for exploring the interconnections, histories and future of populations through DNA. Here, **genetic data**<sup>1</sup> refers to the use of a relatively small subset of variable loci assumed to be representative of the diversity present within the **genome** (the entire complement of DNA of an organism or species). **Genomic data** refers to that generated with high-throughput DNA sequencing methods to provide high-resolution data for characterising genome-wide variation across many thousands of loci. Both data types have benefits and challenges associated with development and analysis that make them suited to specific applications. Such knowledge is critical to better understanding the present state of our biodiversity, and to co-developing robust, evidence-based management strategies for threatened species. For example, many populations face challenges associated with inbreeding, loss of genetic diversity and ultimately, reduced capacity to

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<sup>1</sup> See

*Glossary* for definitions of technical terms in bold font.

respond to future change (i.e., **adaptive potential**; de Villemereuil et al., 2019). Strategies such as conservation breeding programmes and conservation translocations (i.e., moving plants or animals to promote gene flow and enhance diversity for existing or establish new populations; Seddon, 2010) can play an important role in minimising inbreeding, increasing genome-wide diversity and enhancing **adaptive potential** (Mable, 2019).

Recent technological advances and increased capacity and capability in the global genomics community further enable researchers and practitioners to ask new questions or revisit old concepts. For instance, the shift away from using a handful of neutral genetic markers toward whole-genome resequencing allows the investigation of adaptive variation and has reignited interest in the role of **structural variants**—large-scale rearrangements within the **genome** (Lamichhaney et al., 2016; Weissensteiner et al., 2020). Similarly, current research highlights the potential role of gene drives (a gene editing technology that increases the likelihood of an allele with a known beneficial or detrimental effect being inherited) in managing pest species or reintroducing critical genetic variation into threatened species that lack **adaptive potential** (Dearden et al., 2018; Phelps et al., 2020).

On the other hand, the rapid expansion of conservation genetics/genomics puts researchers and practitioners under pressure to keep up-to-date with an increasingly complex toolbox (Taylor et al., 2017). Beyond new tools (e.g., reduced-representation sequencing, whole-genome resequencing, gene-editing technologies), the resulting datasets and their potential applications can be numerous and confusing. Further, existing knowledge, capabilities and aspirations vary widely across both taxa and groups involved. For example, whereas kākāpō (*Strigops habroptilus*) recovery is informed by a dedicated species recovery group, an extensive pedigree, and world-leading **genomic data** ([www.nzgeo.com/stories/decoding-kakapo/](http://www.nzgeo.com/stories/decoding-kakapo/), [www.doc.govt.nz/our-work/kakapo-recovery/what-we-do/research-for-the-future/kakapo125-gene-sequencing/](http://www.doc.govt.nz/our-work/kakapo-recovery/what-we-do/research-for-the-future/kakapo125-gene-sequencing/)), recovery efforts for many other species—particularly

invertebrates and fish—are still in the preliminary stages of establishing distribution records and measuring genetic diversity (Nelson et al., 2019).

To navigate these technologies and co-develop approaches that (i) enhance biodiversity and (ii) empower all individuals and groups involved, scientists and practitioners must prioritise clear communication and genuine partnership (Jarvis et al., 2020). Namely, the framing, co-development and application of genetic/genomic approaches should be determined by the species' needs, as well as the needs, aspirations and expertise of those involved, especially mana whenua (Collier-Robinson et al., 2019). In Aotearoa New Zealand, Te Tiriti o Waitangi (1840) provides such a framework for partnership between Māori and non-Māori. Further, the WAI 262 claim and the subsequent Waitangi Tribunal report (Ko Aotearoa Tēnei) provide a clear mandate for mana whenua to maintain kaitiakitanga (stewardship) over data or resources arising from taonga species (Waitangi Tribunal, 2011).

We are aware that conservation practitioners may have limited opportunities to delve into the capabilities and promise of genetic/genomic tools for conservation, particularly when they may not encounter such tools in day-to-day operations. In this review, we—a cross-institutional team of predominantly early-career researchers using genetic/genomic tools for conservation applications—provide an overview of existing genetic/genomic methodologies, and the current and aspirational applications of such data for conservation. Many of these technologies may have been previously considered with regard to Aotearoa New Zealand's bioheritage (Inwood et al., 2020), but here we focus on those aspects of greatest relevance to Te Mana o te Taiao and a conservation management context. We present a table of attributes for existing genetic/genomic tools to assist conservation practitioners in identifying appropriate tools to inform conservation management that can be further discussed with conservation genetic/genomic researchers. We also highlight the importance of data sovereignty and data management considerations, identify future applications for these

data, and consider ways in which we can enhance conservation outcomes by better facilitating such research in Aotearoa New Zealand.

## 2 Considerations for taonga species

As conservation genetic/genomic researchers in Aotearoa New Zealand, our research primarily involves culturally significant species, many of which are taonga to iwi, hapū and whānau. Through whakapapa (genealogy), data associated with these species are taonga in their own right, including data obtained through genetic/genomic methods (Collier-Robinson et al. 2019). Thus, one of the most critical aspects in the application of genetic/genomic data to Aotearoa New Zealand conservation is ensuring that research is undertaken in a manner that upholds Te Tiriti o Waitangi, recognises the rangatiratanga (authority) of mana whenua, the mauri of the species and ecosystems in question, and prioritises Māori research leadership (Harmsworth & Awatere, 2013). Trusted research partnerships that centre the needs, aspirations and expertise of mana whenua will enhance the capacity of Māori and non-Māori research partners to restore and enhance biocultural diversity (Rayne et al., 2020; Wehi, Brownstein, et al., 2020). Through iterative engagement, clear data management plans can be co-created that realise Indigenous data sovereignty and ensure benefit-sharing (see *Part 1: 4.2 Metadata collection and management*). Although this contribution focusses on taonga species, we consider the research and data management practices described here to be broadly applicable across all Aotearoa New Zealand conservation genetic/genomic research.

While there is a clear impetus to engage with mana whenua when seeking to apply genetic/genomic tools to modern samples (Collier-Robinson et al. 2019, Hudson et al. 2020, Rayne et al. 2020), and when identifying the whakapapa of human remains (e.g., Knapp, Horsburgh, et al., 2012), there is as yet no standard practice for engagement relating to the use of historic samples originating from non-human organisms, whether held in museums or



private collections. In addition, the legal, ethical, and social ramifications associated with **environmental DNA** research in an Aotearoa New Zealand context are as yet unclear, with potential unforeseen impacts on Indigenous communities where such data may identify (or fail to identify) links between Indigenous communities and the land (see Handsley-Davis et al., 2020 for details). Further, emerging **microbiome** studies in conservation consider not just the **genome** of a focal taxon but also the **genomes** of its associated microbial communities (see *Part 2: 8 Microbiomes*), which also contribute to the mauri of species and ecosystems. We encourage researchers to apply the same process of iterative engagement for genetic/genomic research pertaining to modern samples to the use of historic specimens, fossils, and environmental and microbiome samples, whether the focal taxa are extinct or extant, as such data is taonga (Wehi, Whaanga, et al., 2020). It is important to ensure that mana whenua have kaitiakitanga and rangatiratanga over such taonga data outputs, and that benefit sharing is established.

### 3 Selecting an appropriate conservation genetics/genomics tool

As described in Part 2, there are a wide array of methodologies encompassing a variety of conservation applications which can make choosing the appropriate data type(s) a daunting task, and so researchers and practitioners must clearly define research questions to facilitate this decision-making. Where multiple data types may be available and appropriate for answering the research questions, additional factors in methodological decisions may include ethical considerations regarding sampling strategies, timeliness of the method to inform management decisions, and potential for future applications of the data to answer other research questions.

In terms of day-to-day management queries such as genetic sexing, existing partnerships with researchers may provide the platform for carrying out this work (e.g., University of Canterbury researchers in the Conservation, Systematics and Research Team provide

genetic sexing to recovery programmes free of charge when associated with active postgraduate research projects). There are also other opportunities for similar infrequent or intermittent projects to be carried out by service providers such as EcoGene<sup>®</sup>

([www.ecogene.co.nz](http://www.ecogene.co.nz)), that can provide genetic sexing, species identification, wildlife forensics, and conservation breeding services for clients including DOC.

In our experience as conservation genetic/genomic researchers, funding for conservation genetic/genomic projects is often obtained from external funding bodies (e.g., research grants on local, national, or international scales) in combination with DOC support. In these circumstances, excluding any in-kind contributions, DOC's primary contribution to research projects is not financial, but rather the logistical support and institutional knowledge supplied throughout the development and implementation of a given research project (e.g., assistance with obtaining samples and critical metadata). As such, the financial cost of such research may not be the primary determinant in DOC's decisions to establish a research project. However, a potential future challenge of the current funding system is that external research funding (particularly large national grants) is increasingly driven by novelty, and may become less accessible for routine conservation genetic applications using standard tools. Conservation genetic/genomic data should be considered a valuable investment as it represents a long-term resource for ongoing management.

With rapid technological advancements and broad overlap between many tools, there is no simple decision-making tool to help practitioners select appropriate methodologies that best fit the question at hand. In Table 1 we attempt to bridge this gap by providing estimates of costs, benefits, and considerations when implementing the various tools described in Part 2. This table can be used as a starting point for practitioners when considering implementation of genetic or genomic research to support conservation management. As sequencing costs continue to decline and new analytical methodologies are developed, many of the technical challenges associated with these tools will be reduced over time. Further, additional as yet

unforeseen benefits may arise. Other considerations for practitioners may include the extent to which routine management may need to be altered to incorporate sample or (meta)data collection, or the potential downstream impacts of results on current management practice. Currently genetic tools may appear most cost-effective across all measures described here, and may still be sufficient to answer the question of interest. However, these efficiencies must be weighed against the known limitations of **genetic data**, particularly as we move towards assessment of **functional** and **adaptive variation** (see *Part 1: 5.1.3 Functional variation*).

Table 1 Attributes associated with the establishment and implementation of various genetic/genomic tools for conservation applications, as summarised in Part 2. For each tool presented, we provide an example research question that could be answered. We then provide an estimation of the various costs (ranging from very light shade=very low to very dark shade=very high) associated with using the presented tool to answer the research question. Here we consider costs to include financial costs (including those associated with staff hours, laboratory consumables, computational requirements), time costs from outset of research through to conservation management actions based on research results, resources required to generate and analyse the data (encompassing technical skill, laboratory facilities (lab type and equipment), analysis software), sampling invasiveness (which may have ethical and permitting impacts), and computational and data storage requirements. Technical and computational requirements are predominantly provided by the research institute. With ongoing rapid developments within the field of conservation genetics/genomics, we recommend periodic review of this table (e.g., by the advisory group recommended in *Part 1: 5.2 Better facilitation of genetics/genomics research to enhance conservation outcomes*). SNPs = single-nucleotide polymorphisms, kB = kilobytes, MB = megabytes, GB = gigabytes, TB = terabytes. This table is available online at [TBD].

Tool	Pedigree	Genetic markers (e.g., microsatellite panel)	Reduced-representation genomic sequencing	Genome resequencing	Reference genome	eDNA	aDNA	Transcriptomes	Microbiomes
<b>Example research question</b>	Which individuals would make good pairings in a captive breeding programme?	Has neutral genetic diversity been maintained following population establishment at a new site?	What impact has inbreeding had on genome-wide diversity of a species?	What is the founder representation within a captive breeding population?	What is the genetic basis of disease susceptibility?	Is a particular cryptic species present at particular locations?	How genetically diverse was this species prior to human arrival?	How will a species respond to a climate change?	What are the microbial organisms associated with a species of interest?
<b>Invasiveness of sampling</b>									
<b>Time from initiation to implementation of management</b>									
<b>Financial cost</b>									
<b>Resource requirements for data generation and analysis</b>									
<b>Data analysis and storage requirements</b>									
<b>Additional considerations</b>	Pedigree validation may be required using genetic or genomic markers.	Estimates derived from neutral genetic markers may not reflect functional variation.	Typically used alongside a reference genome to improve accuracy of estimates.	Typically used alongside a reference genome to improve accuracy of estimates.	Typically used alongside population-level sequencing data to provide population-level estimates of conservation-relevant metrics.	Cannot be used to confirm species absence.	aDNA methods are often used in combination with genetic markers or SNPs generated from modern samples for comparison.	Typically used in combination with a reference genome.	Hologenomics are still in exploratory phase for conservation applications.
<b>Additional benefits</b>	Can readily integrate additional samples downstream.	Can readily integrate additional samples downstream.	Can readily integrate additional samples downstream.	Can readily integrate additional samples downstream and be applied to answer additional research questions.	Can readily be applied to answer additional research questions.	Can readily include additional sampling locations.	Provides a temporal aspect to genetic studies.	Potential to identify SNPs from transcriptomic data.	Can readily be applied to answer additional research question.
<b>Specific resources required</b>	Method to identify individuals (e.g., banding), spreadsheets and/or pedigree management software.	Genetics lab, including reagents and equipment; analysis software.	Genetics lab, including reagents and equipment; sequencing provider; high-performance computing system; analysis software; large data storage capacity.	Genetics lab, including reagents and equipment; sequencing provider; high-performance computing system; analysis software; large data storage capacity.	Genetics lab, including reagents and equipment; sequencing provider; high-performance computing system; analysis software; large data storage capacity.	eDNA clean lab, including reagents and equipment; sequencing provider; high-performance computing system; analysis software; reference databases; large data storage capacity.	aDNA clean lab, including reagents and equipment; sequencing provider; high-performance computing system; analysis software; large data storage capacity.	RNA clean lab, including reagents and equipment; sequencing provider; high-performance computing system; analysis software; large data storage capacity.	Genetics lab, including reagents and equipment; sequencing provider; high-performance computing system; analysis software; reference databases; large data storage capacity.

Table 1 Legend.

<b>Time from initiation to implementation of management</b>	
<1 year	
1-3 year	
>3 year	
<b>Financial cost</b>	
\$100s	
\$1,000s	
\$10,000s	
\$100,000s	
\$1,000,000s	
<b>Invasiveness of sampling</b>	
Observational sampling, environmental sampling or collection of pre-existing samples	
Blood or tissue sampling, skin swabs	
Lethal sampling (whole organism, organ collection, gut content)	
<b>Data analysis and storage requirements</b>	
Low (kB - MB)	
Moderate (GB)	
High (GB - TB)	
Very high (TB)	
<b>Resource requirements</b>	
Low	
Moderate	
High	
Very high	

One key technical challenge associated with the shift towards genomic methodologies is the computational requirements (and consequently additional financial costs) associated with analysis and storage of large genomic datasets. These requirements are variable depending on the scale of the data, with population-level genomic analyses requiring access to high-capacity computational systems that can process and analyse data on the scale of terabytes. While many institutions may provide local computing infrastructure, national and international services are also available (e.g., the New Zealand eScience Infrastructure, [www.nesi.org.nz](http://www.nesi.org.nz), cloud computing services such as Amazon Web Services [aws.amazon.com](http://aws.amazon.com), or more locally Catalyst Cloud [catalystcloud.nz](http://catalystcloud.nz)). Such services may incur additional expenses to projects and require specific data security considerations (see *Part 1: 4.3 Data sovereignty*).

## 4 Technical considerations for conservation genetic/genomic research

Following clear definition of the research questions, appropriate sampling protocols and data management are key components of any research project. Data management plans are essential and should encompass the short- and long-term management (including curation, storage, and access) of samples, raw data and associated metadata, and processed data and outputs. Such plans are particularly critical for large and/or long-term projects where many different people will be involved over the lifetime of the project.

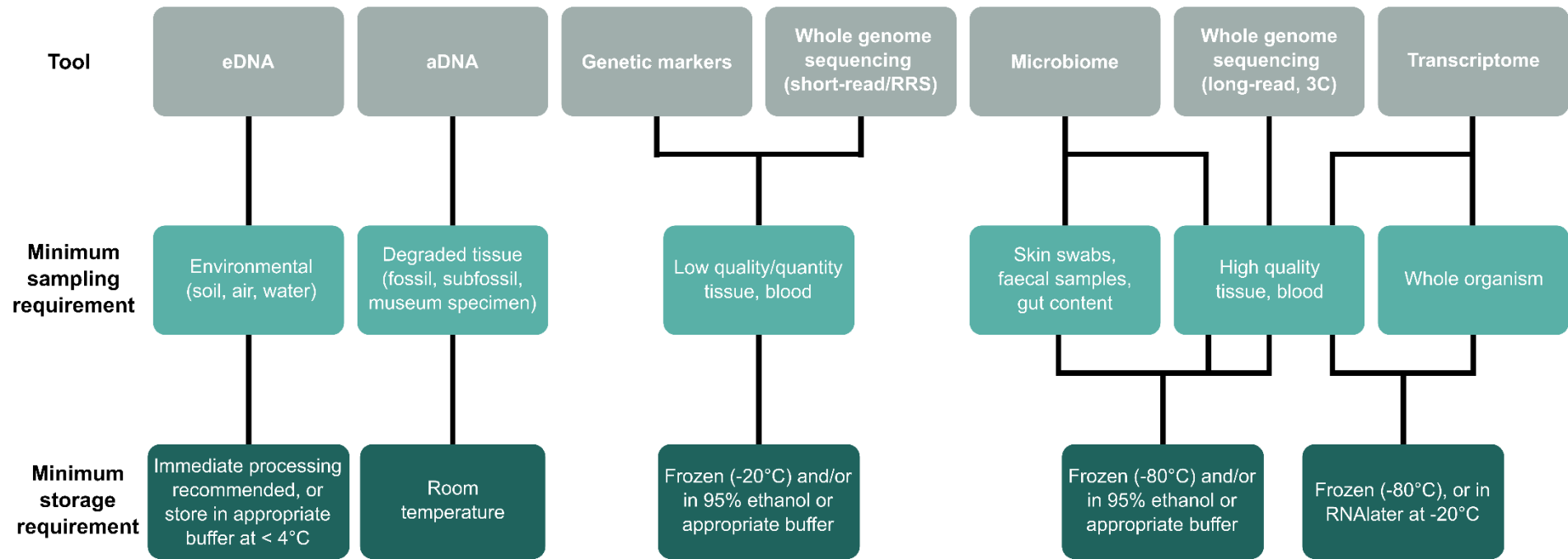
### 4.1 Best-practice sample collection and storage for genetic/genomic research

To ensure high quality sample collection and preservation for genetic/genomic purposes, where possible, we recommend that high-quality samples (e.g., blood, tissue) should be

collected once per individual and stored in a manner appropriate for the intended downstream application (Figure 1). While storage of samples in ethanol for downstream genetics/genomics is common, and may be the most practicable for population genomic research of widespread species, this impedes the use for **transcriptome** analysis. Collecting samples in this manner ensures usage for a range of potential downstream applications, reducing the need for resampling and minimising individual stress. These samples will act as a resource for future genetic or genomic applications, particularly those requiring high-molecular-weight DNA (e.g., **long-read sequencing** requires DNA of sufficient quality to sequence ultra-long reads; (Amarasinghe et al., 2020). Sampling, storage, and DNA extraction protocols differ for **eDNA** and **aDNA**, and relevant protocols should be followed (Hofreiter et al., 2001; Jarman et al., 2018).

In the absence of a centralised DOC repository, we encourage research institutes to follow best practice for collection and curation of samples. Samples should be recorded in laboratory databases to minimise the potential for unnecessary resampling, and to ensure sample usage can be tracked. Sample databases should capture individual identification, laboratory identification, type and quantity of raw material, and use of the material for analysis, along with all relevant metadata associated with the samples to provide the necessary context for downstream analyses (see *Part 1: 4.2 Metadata collection and management*). We encourage researchers and practitioners to collect other metadata (e.g., phenotypic, ecological data) simultaneously with sampling where possible, providing broader downstream data applications.

1



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3 Figure 1 Sampling and storage requirements for various genetic/genomic tools currently in use. These should be considered the minimum requirements, and are best  
4 considered in a broader context that includes an assessment of current and future use, as well as feasibility and cost. aDNA = ancient DNA, eDNA = environmental  
5 DNA, RRS = reduced-representation sequencing, 3C = 3C sequencing technologies.



## 4.2 Metadata collection and management

Here, we use metadata to refer to the 'data about the data'. For example, metadata associated with blood samples taken from individual birds may include such information as collection date, GPS location, species, individual identifiers (e.g., band/tag numbers), photographs of individuals and/or sampling locations, age/class, sex, pedigree (parents, siblings, offspring). Metadata associated with raw (unprocessed) genomic sequence data could include (but is not limited to) the genomic library preparation details, sequencing provider and platform, date of sequencing, individual or project barcode sequences, individual or location identifiers (where appropriate), and sequence quality scores. Metadata facilitates reproducibility of the research, and for processed data may include details of analysis tools and version numbers, **reference genomes** used for sequence alignment, or details of databases from which additional data was collected, or output data deposited.

Metadata may also encompass records of consultation with mana whenua, ethics approvals and sampling permit numbers, lists of collaborators and contributors, sources of funding, and publication outputs (e.g., student theses, journal articles, DOC internal reports). We recommend that relevant metadata are captured and stored alongside raw and processed data to ensure correct interpretation of the data (e.g., in README files alongside any **reference genomes**, spreadsheets capturing pedigree, phenotype or monitoring data). An example of metadatabasing in Aotearoa New Zealand is that of the Ira Moana Project ([sites.masse.ac.nz/iramoana/](https://sites.masse.ac.nz/iramoana/)), aiming to aggregate metadata associated with primarily marine genetic/genomic studies based on international metadatabasing standards (Riginos et al., 2020).

### 4.3 Data sovereignty

There is extensive scholarship regarding data sovereignty pertaining to human genetic/genomic data, growing scholarship pertaining to culturally significant species (e.g., Caron et al., 2020; Claw et al., 2018; Collier-Robinson et al., 2019; Handsley-Davis et al., 2020; Hudson et al., 2020; Koia & Shepherd, 2020; Potenski, 2020; Walter et al., 2020), and the establishment of initiatives such as Te Nohonga Kaitiaki ([www.genomics-aotearoa.org.nz/projects/te-nohonga-kaitiaki](http://www.genomics-aotearoa.org.nz/projects/te-nohonga-kaitiaki)), Traditional Knowledge Labels ([localcontexts.org/tk-labels/](http://localcontexts.org/tk-labels/); Anderson, 2012) and Biocultural Labels ([localcontexts.org/labels/biocultural-labels/](http://localcontexts.org/labels/biocultural-labels/); Anderson & Hudson, 2020). As such, we will not discuss these matters in detail here, but we strongly encourage researchers and practitioners to explore these existing resources, and to centre mana whenua needs, aspirations and expertise throughout all aspects of research and practice.

To date there have been disparities in the levels of protection given to different genetic/genomic data types, where **genomic data** are generally given greater protections than **genetic data**. Further, the rapid developments of the fields of **ancient DNA** and **environmental DNA** for conservation applications have not yet been given the same considerations for conservation purposes as similar human research (e.g., Knapp, Horsburgh, et al., 2012). However, we urge researchers and practitioners to engage with scholarship like Handsley-Davis et al. (2020) so they are better able to communicate the risks and benefits associated with these data to mana whenua.

Indigenous needs may conflict with the current global emphasis on open-access data publication to facilitate reproducibility of research, but Western scientists must be responsive to Indigenous views and values, and work to ensure Indigenous concerns are protected. Discussion of project-specific considerations regarding data security and accessibility during analysis and long-term storage should be initiated at the outset of the project (

Box 1). Many researchers will have access to centralised repositories maintained by their institutions (e.g., crown-research institutes and universities), where data can be securely held on behalf of mana whenua, as existing international genetic/genomic databases may not yet be capable of meeting data sovereignty requirements. Existing data repositories could adapt to meet these needs through the inclusion of Biocultural Labels ([localcontexts.org/labels/biocultural-labels/](https://localcontexts.org/labels/biocultural-labels/)) to indicate data provenance, associated ethics, permits, and expectations around appropriate use, and to connect data back to Indigenous communities (Anderson & Hudson, 2020).

Similarly, international research journals must also adapt to meet these needs. There are currently some concerns around the ability to publish research when data sovereignty agreements may include limited data access; however, international journals are beginning to recognise the need to adapt (Potenski, 2020). Indeed, genomic research relating to Aotearoa New Zealand taonga has been successfully published with data hosted on behalf of mana whenua on password-protected local repositories (e.g., Galla et al., 2019, 2020), and on the Genomics Aotearoa repository (e.g., Oliphant et al., 2020). Until clear frameworks for data sovereignty are better established, researchers and practitioners must continue to navigate open-access culture—particularly for methods such as **eDNA** which rely upon accessible databases—in ways that uphold the responsibilities mandated in Te Tiriti o Waitangi (Box 1).

Box 1 Key questions to consideration for iterative engagement with mana whenua when developing conservation genetic/genomic research.

To ensure preservation and protection of data associated with taonga species, a clear data management plan needs to be developed from the initiation of research that is responsive to the needs, aspirations and expertise of mana whenua. Key questions to consideration include:

**Engagement:** Which iwi/hapū/whānau should researchers engage with? What information and costs are required to support cultural expertise of mana whenua for any research to be considered? Western scientists should not presume that all iwi/hapū/whānau will have the same knowledge, priorities, and concerns, and careful engagement with all relevant parties will be key to reducing inequities relating to data management.

**Data generation:** What will the sampling strategy involve (e.g., sample type, number of individuals, locations)? Where will samples be processed? What method of data generation will be used (e.g., microsatellites, whole genome resequencing)? Where will data be generated (e.g., local/overseas sequencing)? Where will the data be analysed (e.g., by local/international researchers on local/overseas computing platforms)?

**Data security:** Where will the genetic/genomic data be stored—including samples, raw and processed data, and analysis outputs—before and after publication? How will genetic/genomic data be accessed by external researchers, before and after publication (e.g., on local servers, national/international repositories)? How will associated metadata be managed? Is there a need for metadata anonymisation (e.g., to limit sample identification, and protect sample locations and individual privacy), and how will this be implemented? If individuals, whānau, hapū or iwi contribute mātauranga, how will this be explicitly recognised and protected?

## 5 Future directions

In Part 2 of this contribution we have summarised the current state of play for genetics and genomics in Aotearoa New Zealand. We have highlighted the dynamic nature of the available tools for conservation applications, and we anticipate a growing number of conservation genetics and genomics research projects informing conservation management. Here we identify aspirational applications for the existing genetic/genomic tools and for those in development, and suggest potential strategies to further support this developing landscape.

### 5.1 Conservation genetic/genomic research toolbox horizon scan

Ongoing developments in genetics and genomics promise to expand the conservation toolbox, including several of the approaches described in Part 2 (e.g., **eDNA**, transcriptomics, **microbiomes**, gene editing). Some are also being combined to create new tools. For example, the combination of **aDNA** and **eDNA** for investigating ancient environmental DNA (aeDNA) is applicable to restoration ecology as **eDNA** binds to substrates and therefore can provide a record of past species presence (Buxton et al., 2017; Hofman & Rick, 2018; Wilmshurst et al., 2014). Here, we briefly summarise some of these developing approaches and how they can be implemented to support conservation, alongside the existing tools described in Part 2.

#### 5.1.1 Pangenomes

In the future, we also anticipate that genomics will be applied more broadly to capture population- or species-level diversity with **pangenomes**. **Pangenomes** incorporate multiple individual **reference genomes** to capture the entire complement of diversity within a species, and enables researchers to differentiate between 'core' (genes and gene regions fixed in all individuals) and 'accessory' (genes and gene regions that are variable) genomic

regions (Tettelin et al., 2005). This promises to be a significant advancement in the field of conservation genomics as highly complex and rare traits may be characterised in individuals of interest (Bayer et al., 2020; Gao et al., 2019), including those that hinder species recovery.

### 5.1.2 Chromosomics and structural variants

Other developing methodologies include **chromosomics** which integrates **cytogenetics** and whole-genome sequencing to study chromosomal diversity (Deakin et al., 2019; Potter & Deakin, 2018). This discipline goes beyond characterising single-nucleotide polymorphisms (**SNPs**) to include analysis of **structural variants (SVs)**. **SVs** represent a source of genomic variation likely to have large phenotype effects (Weissensteiner et al., 2020): **SVs** are large rearrangements (> 50 **base pairs, bp**) within the **genome** that impact the form and structure of chromosomes, and include regions of the **genome** that are inverted, translocated from one location to another, inserted, or even lost entirely. Recent genomic research from human clinical studies and primary industry indicates that **SVs** are a significant source of genomic variation, as they have been found to intersect with gene coding regions more often than **SNPs** and impact a greater proportion of the **genome** overall (Catanach et al., 2019; Chiang et al., 2017). As such, the high likelihood that complex traits (e.g., reproductive traits in birds; Huynh et al., 2011; Kim et al., 2017; Knief et al., 2017; Küpper et al., 2016) are determined by **SVs** make them of interest for conservation. The large and complex nature of **SVs** cannot be adequately captured by **short-read sequencing** (see *Part 2: 4 Whole-genome sequencing*), and so approaches that combine **cytogenetics** and **long-read sequencing** are currently being developed to reliably characterise **SVs** in threatened species. Moving forwards, we anticipate that combining pangenomic and transcriptomic approaches will facilitate research into adaptive and **functional variation** (see *Part 1: 5.1.3 Functional variation*; Alonge et al., 2020; Bayer et al., 2020; Golicz et al., 2020; Liu et al., 2020), with potential applications including modelling the ability of species to adapt to future climate change or novel diseases. Collaboration with

primary industry researchers has proven fruitful for conservation genomic research, due to the overlap in research interests pertaining to small populations (Galla et al., 2016). As much of the work regarding **pangenomes** and **SVs** to date has focused on crop species, continued collaborations with primary industry researchers will further enhance conservation research in these areas.

### 5.1.3 Functional variation

Traditional population genetics investigates **neutral variation** to answer demographic questions (e.g., population size, inbreeding, connectivity). However, the need to conserve and monitor functional genetic diversity is increasingly being emphasized (Hoelzel et al. 2019; Mable 2019; Teixeira & Huber, 2021). **Functional variation** refers to the genetic basis of phenotypic variation—traits that influence survival and **reproductive fitness** of individuals. While efforts have been made to characterize functional variation in model organisms, small sample sizes inherent in threatened populations, limited genomic resources (i.e., reference genomes and annotations) for non-model species, and the complexities of polygenic traits (those determined by the combination of multiple loci) remain limiting factors for broad application of these methods for species of conservation concern (but see Batley et al., 2019; Brandies et al., 2020). In a primary production context where specific traits are targeted in breeding programmes, pedigrees are used to inform a trait mapping approach to identify quantitative trait loci (QTLs), or genomic regions associated with specific phenotypic traits. Analytical advancements now facilitate statistical tests to characterise such loci (e.g., genome-wide association studies (GWAS); Mable 2019). Such pedigree-informed approaches could be applied to captive populations to identify maladaptive traits or those that convey a fitness advantage under particular environmental conditions.

A mechanistic understanding of how genetic diversity influences phenotype generally requires 'omics' data to understand how DNA methylation, gene expression, and protein

composition influence phenotype (Mable 2019). However, for the purposes of understanding the response of species of conservation concern to specific challenges (e.g., climate, disease), a statistical association between genetic and phenotypic evidence may be sufficient to guide management decisions (e.g., reintroduction of disease-resistant individuals to areas where disease has previously extirpated the species; Epstein et al., 2016; Hubert et al., 2018).

#### 5.1.4 Hologenomics

An extension of microbiomics relevant to conservation biology is **hologenomics** (Carthey et al., 2020). As evolution acts on both the host organism and its **microbiome** (the combined assemblage of which is termed the 'holobiont'), this causes changes in the 'hologenome', or genomic content of the entire holobiont (Morris, 2018). Thus **hologenomics** goes beyond analysing **genome** and **microbiome** data separately, explicitly considering the **genomes** of the complete assemblage (Rosenberg & Zilber-Rosenberg, 2018). Variation within the hologenome allows for more rapid adaptation to local environments than can be achieved through genomic changes alone (Rosenberg & Zilber-Rosenberg, 2018). **Hologenomics** could allow conservation-relevant issues such as disease susceptibility/resistance (Postler & Ghosh, 2017) and population sizes/connectivity (Wirth et al., 2005) to be assessed more broadly, and with greater resolution than investigating either the host **genome** or the **microbiome** alone. While the substantial analytical challenges of this approach have thus far limited research to model species (Snijders et al., 2016), techniques derived from **eDNA** sampling could be used to understand interactions between the host and the environment, along with the 'microbial appropriateness' of environments as potential translocation sites (Koskella & Bergelson, 2020). Similarly, metatranscriptomics approaches can be used to characterize gene regulation of the **microbiome** community and its potential application to host health and local adaptation (Knight et al., 2018).



### 5.1.5 Gene editing

Gene editing is the modification of an organism's DNA by adding, removing, or otherwise altering genetic material to produce targeted effects (Jinek et al., 2012; Mali et al., 2013). The development of the CRISPR-Cas9 genome engineering system is particularly relevant to conservation, as it provides a rapid, accurate, and efficient method for producing such targeted changes (Doudna & Charpentier, 2014). The primary biologically-feasible conservation application of gene editing in Aotearoa New Zealand is for pest control (Campbell, Beek et al., 2015; Dearden et al., 2018). Invasive species management currently employs direct culling, trapping and poisoning. Ecological variation, off-target impacts, and ethical concerns create challenges for existing invasive species management strategies (e.g., culling, trapping, and poisoning) and have prompted research into alternative pest control solutions (Latham et al., 2015; Russell, 2014; Kirk et al., 2020; MacDonald et al., 2020). Gene drives present one such method of non-lethal pest control, utilising the CRISPR-Cas9 gene editing system to target pest fertility (Esvelt et al., 2014). Gene drives alter inheritance mechanisms so that all offspring inherit the gene drive, 'driving' the technology through the target pest population (Figure 2). Gene drives can be used to disrupt the fertility of one sex, while the other sex continues to propagate the technology to subsequent generations by reproducing with wild, non-gene drive individuals (Prowse et al., 2017).

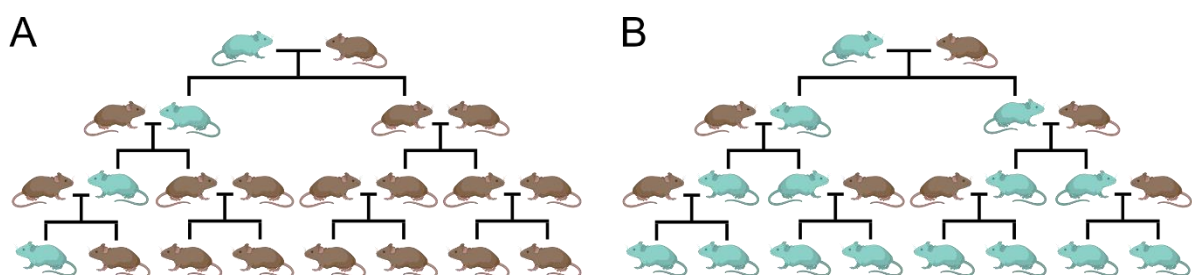


Figure 2 Comparison of (A) a typical inheritance pattern of an edited gene (blue rat) with (B) the enhanced inheritance pattern of an edited gene created by the gene drive, whereby all offspring inherit the edited gene. Harnessing the enhanced inheritance pattern, gene drives can be used to spread genes that bias offspring sex or reduce fertility to achieve targeted long-term pest control objectives. Credit: AC, created with BioRender.com.

Gene drives are also of significant international interest as an eradication technology for devastating human diseases like malaria (Hammond et al 2016), with malaria-vectoring species of *Anopheles* mosquitoes being crucial in the development and technical understanding of various gene drive systems in laboratory settings (Hammond and Galizi 2017). Further, gene drives have been proposed as a tool for engineering thermal tolerance in coral populations, to help mitigate widespread ecological impact of climate change (Anthony et al 2017). Other proposed conservation applications of gene editing include engineering endangered species **genomes** to build novel resistance to emerging pathological and environmental threats, or to restore lost genomic variation (Phelps et al., 2020). Its use for de-extinction of keystone historical species has also been proposed (Seddon et al., 2014). However, substantial technical advancements are still required, along with careful consideration of potential net benefit in biodiversity gain against the risks (e.g., redirection of funding away from existing threatened species conservation programmes; Bennett et al., 2017).

Technical challenges associated with gene editing include identification of target genes for gene drives, or genetic diversity that may act as a source of gene drive resistance impeding the population suppression or eradication capacity of the gene drive (Price et al., 2020). High-quality annotated **reference genomes** are required to identify target genes and enable comprehensive evaluation of the effects of existing variation on gene drive efficiency. These data can then inform predictive models assessing the effectiveness of specific gene drive systems in target populations under variable conditions (including environmental change and conservation management; Champer et al., 2020). Further, gene drive trials must be carefully designed to be representative of real-world impacts, as there may be differences in implementation and effects between captive laboratory populations and wild populations due to local behavioural adaptation or other indirect ecological effects (Mazza et al., 2020; Russell et al., 2009; Tompkins & Veltman, 2006).

In addition, social science research is required to assess social perspectives on gene editing and in particular those underpinning Indigenous values (Hudson et al., 2019). Such considerations are crucial as the fundamental impacts of gene drives concern inheritance, therefore strongly implicating whakapapa, and gene editing may have implications for the mauri of the species. Although many pest species have been introduced, some of these are considered taonga (e.g., kiore (Pacific rat, *Rattus exulans*); McClelland 2002). Furthermore, pest control has direct relevance to mana whenua as kaitiaki of taonga species that may benefit from these measures. Iterative engagement regarding the potential uses of gene drive technologies, both for pest control and to enhance biodiversity, will be required to encompass the broad range of values and perspectives (Hudson et al., 2019).

Alongside the technical and social challenges, legislative challenges have been exacerbated by rapid technological advances (Royal Society Te Apārangi, 2019). The current legal definition of gene editing in Aotearoa New Zealand limits the potential for research and funding. Consequently, in the absence of local knowledge, data evaluated in an international context may result in misinterpretation of data in local contexts, and may not adequately capture mana whenua values in data use and applications. Despite real-world application of gene editing technologies in Aotearoa New Zealand remaining distant, rapid advancements necessitate rigorous transdisciplinary evaluation integrating local social and cultural values before gene editing becomes feasible as a tool supporting ambitious projects such as Predator Free 2050 in Aotearoa New Zealand.

## 5.2 Better facilitation of genetics/genomics research to enhance conservation outcomes

To effectively implement the conservation genetics/genomics toolbox to inform management decisions in light of this developing landscape, we advocate for a well-resourced DOC

genetics/genomics advisory group comprising both internal and external practitioners and researchers across a range of career stages, that includes members of or exists in partnership with Kahui Kaupapa Atawhai. Such a group could centre the principles of Te Tiriti o Waitangi to facilitate the establishment of genetic/genomic research by advising on the most appropriate research strategies to meet conservation needs, while balancing current and future uses against feasibility and costs. This group could also be tasked with developing data management guidelines encompassing sample collection, curation, and management of data and metadata to support downstream research, and facilitating collaborations with institutes with the necessary skill, experience, and resources to implement specific research. Further, the establishment of such a group may facilitate connections—and mitigate conflicts of interest—among practitioners, researchers and mana whenua, and among species-specific research and conservation recovery groups, to enhance conservation outcomes across Aotearoa New Zealand.

In partnership with Kahui Kaupapa Atawhai, this group may also support researchers and practitioners in making connections with mana whenua, thus enabling appropriate engagement in accordance with Te Tiriti o Waitangi commitments. However, we stress that researchers and practitioners must be proactive in establishing and maintaining trusted relationships, which may then lead to larger and/or long-term collaborative research projects. Further, as early career researchers, we argue that senior researchers are best placed to maintain a clear and consistent line of communication between practitioners, mana whenua and their own research groups. In addition to better facilitating the permitting process, doing so will create the opportunities for early career researchers to gain an understanding of research best-practice within the limited time frames available to them (e.g., three years for a PhD).

Related to this, one primary concern identified by early career researchers relates to DOC's research permitting processes, including lengthy delays and inconsistencies among taxa,

type of data generated, and messaging around data management. With samples and data persisting beyond the timeframes of permits, and the increasing intergenerationality of research projects, further concerns arise from the absence of periodic reviews once permits are granted. These concerns are worth highlighting, although addressing potential solutions is beyond the scope of this contribution, but could well fall under the mandate of a DOC genetics/genomics advisory group.

Further, we foresee conservation genetic/genomic research becoming increasingly transdisciplinary in nature. Indeed, to achieve the vision outlined in Te Mana o te Taiao, both species-specific and ecosystem-based approaches will include genetic/genomic, microbial, ecological, physiological, and environmental data, alongside mātauranga Māori where mana whenua wish to contribute this. Thus, to produce more ‘winners’—including species, ecosystems, and people—benefitting from conservation actions (Nelson et al., 2019), we encourage researchers and practitioners to focus on developing collaborative, iterative, communicative practices incorporating a wide array of disciplines and perspectives.

## Part 2: Genetic/genomic data types and conservation applications

Here we describe the diversity of genetic/genomic tools—presented in loose chronological order of development—currently used to inform conservation management, and highlight challenges and limitations of these tools. Each subsection is intended as a stand-alone to be referred to on an as-needed basis by conservation researchers and practitioners, supporting Table 1. As this is a dynamic field, we recommend periodic review of these tools, their applications and limitations, along with those included in *Part 1: 5.1 Conservation genetic/genomic research toolbox horizon scan*.

### 1 Pedigree data

Pedigrees—family trees showing genealogical relationships between individuals—are a long-standing tool in biological sciences (Wright, 1922). While pedigrees are not a molecular tool as such, we classify them as a genetic tool as they are used to monitor and understand relationships and variation between individuals. Over the past 40 years, pedigrees have become a staple of conservation management, allowing practitioners to manage the genetics of small populations by strategically pairing or translocating individuals to minimise inbreeding and maximise **adaptive potential** (Ballou & Lacy, 1995; Ivy & Lacy, 2012; Ralls & Ballou, 1986; Rudnick & Lacy, 2008; Willoughby et al., 2015). Using genealogical relationships, pedigrees can be used to produce estimates of kinship (i.e., relatedness) and individual inbreeding. Conservation breeding programmes currently prioritise pairing individuals with low mean kinship (i.e., relatedness between an individual to all others in a population, including oneself). This paradigm minimises drift by maintaining the representation of individuals that started the population (i.e., founders); and in doing so, minimising the loss of genetic diversity, inbreeding, and adaptation to captivity (Frankham,

2008; Lacy, 2009). There are decades of empirical research and simulation studies that support this management approach to maximise **neutral variation** (e.g., Ballou & Lacy, 1995; Rudnick & Lacy, 2008), however no standardised approaches to date have incorporated **functional variation**, which has only recently been able to be quantified (see *Part 1: 5.1.3 Functional variation* 5.1.3 Functional ). Further, selection for/against individuals with adaptive/maladaptive functional traits (e.g., low hatching success or low immunocompetence) may inadvertently reduce the ability of populations to have sufficient evolutionary potential to adapt towards novel selection pressures in the future.

Pedigrees represent an accessible tool for conservation management, as collecting and analysing pedigree data can be readily incorporated into routine management practices for most captive populations. Researchers in the zoo and aquaria communities have developed tools for studbook management (SPARKS, PopLink, and ZIMS; Ballou et al., 2010; Faust et al., 2019, [www.species360.org](http://www.species360.org)) and pedigree analysis (PMx; Lacy et al., 2012), which has increased the uptake of this approach. While these management approaches have often been applied to captive or *ex situ* populations, their use is increasing for wild or semi-wild populations (Pemberton, 2008). A reliable pedigree is an asset for creating pairing or translocation recommendations, but can also be used to evaluate heritability of specific traits (Randolph et al., 1981), understand the fitness and contributions of individuals to the population over time (Hunter et al., 2019), and model population growth and viability (Lacy, 2000).

## 1.1 Best practice for pedigree establishment and maintenance

Four key practices when establishing an effective pedigree management system include: using a robust system to identify individuals to ensure correct assignment of relationships (Allen et al., 2019); collecting **genetic data** from founding individuals to evaluate relatedness (Bergner et al., 2014; Hogg et al., 2019); the use of monitoring technologies

(e.g., cameras, RFID tags; Bonter & Bridge, 2011) particularly when working with wild populations to ensure accurate breeding records; and collecting tissue or blood samples from all individuals for downstream genetic analyses in the case of any uncertainties or known errors (Frasier et al., 2009; Ryder & Feistner, 1995). This can also facilitate downstream pedigree evaluation to assist with error detection and correction.

## 1.2 Challenges of pedigrees and genetic solutions

While pedigrees are an intuitive tool with diverse applications in conservation, they also have limitations (Figure 3). First, pedigrees are unlikely to be developed for species other than those that are most threatened and/or geographically restricted, where individual identification and monitoring over multiple generations is both necessary and feasible in such small populations. For most pedigrees, founding individuals are assumed to be unrelated as their relationships are typically unknown. However, for threatened species that have experienced severe population bottlenecks, it is unlikely that the founders are completely unrelated (Bergner et al., 2014; Hogg et al., 2019). This ‘founder effect’ is exacerbated when pedigrees are shallow (< 5 generations recorded; Balloux et al., 2004; Pemberton, 2004; Rudnick & Lacy, 2008). In addition to the assumption regarding founder relatedness, pedigrees often struggle with missing data. This is particularly challenging for wild populations, where there may be difficulties in correctly identifying putative parents and offspring.

Estimates generated from pedigrees are only as accurate as the pedigree itself, so accurate individual identification and knowledge of the breeding system of the focal species are essential for creating a robust pedigree. Incorrect identification of relationships between individuals resulting from extra-pair paternity (Castro et al., 2004; Ewen et al., 1999; Forsdick et al., *In Press*) or nest parasitism (Overbeek et al., 2017) may impact conclusions drawn from pedigrees in the absence of molecular **genetic data** (Reid et al., 2014). Such



missing or erroneous data can be verified and corrected through the use of genetic or genomic markers (Overbeek et al., 2020).

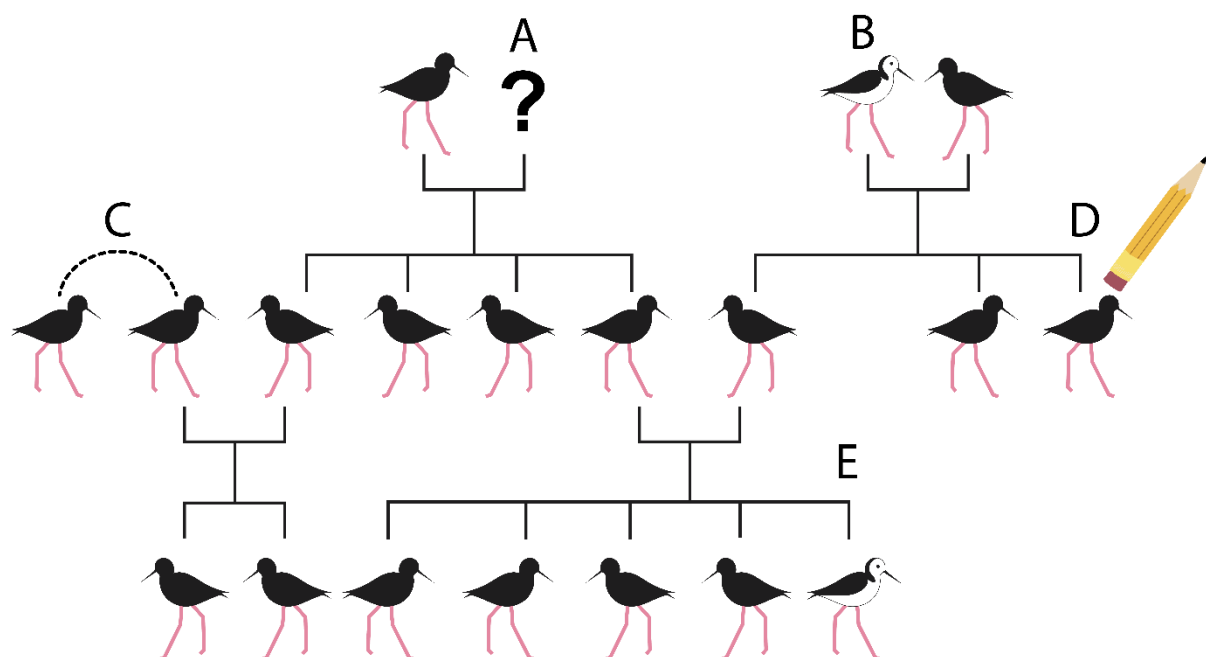


Figure 3 Visualisation of various challenges identified within the kakī (black stilt, *Himantopus novaeseelandiae*) pedigree, including (A) missing data, (B) interspecific hybridisation with poaka (Australian pied stilts, *H. h. leucocephalus*), (C) extra-pair paternity, (D) individual identification or data transcription errors, and (E) nest parasitism by poaka. Credit: SJG.

Relatedness estimates derived from pedigrees alone are probability-based, and may not capture the true extent of shared genomic variation, and so should be used with caution (Béréanos et al., 2014; but see also Nietlisbach et al., 2017). Pedigree data used in conjunction with **genomic data** may provide the most precise estimates of relatedness to support conservation breeding programmes (e.g, genomic data can be used to estimate relatedness among founders; Hogg et al., 2018; Wright et al., 2021).

Where long-term intensive population monitoring is not feasible, post-hoc pedigrees can be generated from **genetic** or **genomic data** (Flanagan & Jones, 2019). However, it should be noted that small numbers of markers (e.g., as for microsatellites) are often insufficient for estimating relatedness and/or inbreeding in genetically-depauperate species (Taylor, 2015),

and therefore genomic approaches yielding tens of thousands of genome-wide markers may be more useful (Galla et al., 2020). Further, relatedness estimates derived from pedigrees are probability-based, and may not capture the exact extent of shared genomic variation (Béréanos et al., 2014, but see also Nietlisbach et al., 2017). Pedigree data used in conjunction with **genomic data** may provide the most precise estimates of relatedness to support conservation breeding programmes (e.g, **genomic data** can be used to estimate relatedness among founders; Galla et al., 2020).

## 2 Genetic data

### 2.1 Nuclear genetic data

**Genetic data** typically comprise a small number of short, neutrally-inherited regions of the **genome**, and are assumed to be representative of the **neutral variation** of the whole **genome**. Common nuclear genetic markers include allozymes, AFLPs or RFLPs (amplified/restriction fragment length polymorphisms) and microsatellites. Such markers are usually derived from repetitive regions of DNA that can be highly variable between individuals, making them ideal for investigating diversity and differentiation within and between species (Forsdick et al., 2017), parentage (Castro et al., 2004; Overbeek et al., 2017; Taylor et al., 2008), relatedness (Carroll et al., 2012), and interspecific hybridisation (Cubrinovska et al., 2016; Steeves et al., 2010), and in assessing the outcomes of conservation management actions (e.g., translocation outcomes; Heber et al., 2013).

**Genetic data** also support wildlife forensics, tracking the illegal trade of wildlife and identifying the use of protected species in commercial products (Baker et al., 1996; Ferreira et al., 2015; Gentile et al., 2013).

**Genetic data** can also be used to assess functional gene regions that may be under selection, such as those associated with immune function and mate choice (Grueber et al.,

2015; Kamiya et al., 2014; Lillie et al., 2015; Miller & Lambert, 2004; Sutton et al., 2015). Functional loci (sites in the genome associated with genes) can be targeted and characterised through comparative genomics and species-specific primers (Alcaide & Edwards, 2011; Grueber et al., 2015). These data can help both with the management of detrimental alleles (Hedrick, 2001) and with the maintenance of adaptive variation at specific loci within a population (Amos & Balmford, 2001; Kohn et al., 2006).

## 2.2 Mitochondrial data

Mitochondrial DNA (**mtDNA**) differs from nuclear data in that it represents a short (15–20,000 **base pairs (bp)**), circular sequence of DNA (the mitogenome) that is maternally inherited, and found as 10–1000s of copies present in most cells of an individual (O’Hara et al., 2019). These properties make **mtDNA** useful independently or in combination with nuclear genetic markers for broad-scale comparisons of population-level diversity and differentiation (Alexander et al., 2016; Chapple et al., 2012; Mischler et al., 2018), taxonomic delimitation and phylogenetic inferences (Banker et al., 2017; Boon et al., 2000; Rosenbaum et al., 2017), and for investigating potential drivers of extinction, including in ancient DNA analysis (Allentoft et al., 2014; see *Part 2: 5 Ancient DNA*). Taxonomic delimitation is of particular importance for prioritisation of conservation efforts, and complete or partial **mtDNA** data may be sufficient for such assessments when combined with nuclear genetic/genomic, morphological and behavioural data (but see Rubinoff & Holland, 2005; Dincă et al., 2019; Pedraza-Marrón et al., 2019).

## 2.3 DNA profiling

DNA profiles consisting of genotypes constructed from multiple nuclear markers, sex-linked markers (those occurring on sex chromosomes that can be used to infer individual sex), and/or **mtDNA** markers (to confirm patterns of maternal relatedness) can also be used for

genetic monitoring of species including population demographic and genetic diversity estimates using repeated temporal samples (Carroll et al., 2018). Based on these samples, 'recaptures' of DNA profiles of individuals can be used with mark-recapture models to estimate population abundances (Taberlet et al., 1999). These approaches can also be combined with parentage analysis in a gametic-mark-recapture framework, where genotypes of individuals can be 'recaptured' in offspring to estimate both abundance and population connectivity (Carroll et al., 2012; Garrigue et al., 2004). These approaches are particularly useful for estimating the abundance of rare or cryptic species (i.e., from feathers, fur, or faeces; Bañuelos et al., 2019) and for species where photo-identification has limited applicability due to a low instance of natural markings and/or where tagging/banding is not possible (e.g., some cetacean species such as Hector's and Māui dolphins; Baker et al., 2013; Hamner, Constantine, et al., 2014; Hamner et al., 2017; Hamner, Wade, et al., 2014). Individual-based DNA profiles can additionally be used to identify rare immigration/emigration events (Hamner, Wade, et al., 2014), and for monitoring genetic erosion (Leroy et al., 2018).

## 2.4 Limitations of genetic data

Neutral genetic markers may be a poor proxy for **functional variation** (Grueber et al., 2015; Marsden et al., 2013), especially for highly variable genes like those of the major histocompatibility complex associated with immune function (Sommer, 2005). While **genetic data** has broad conservation application, genetic markers may lack the resolution required for accurate estimation of relatedness and inbreeding in populations that have experienced strong demographic bottlenecks and as a result have low genetic diversity, as is the case for many threatened species (Taylor, 2015). With technological advances in **genome** sequencing in the past decade, genome-wide analyses are now possible that in many cases can provide improved resolution and accuracy compared with genetic approaches (Galla et al., 2020; Supple & Shapiro, 2018).

Specific limitations associated with **mtDNA** arise from its nature as a single non-recombining, maternally-inherited genetic locus, meaning that it cannot be used to detect male-mediated gene flow, and may be impacted by incomplete lineage sorting resulting from rapid diversification events (Paijmans et al., 2013). Furthermore, even the use of complete mitogenomes lacks the power and resolution of multiple unlinked nuclear loci (e.g., microsatellites, **single nucleotide polymorphisms (SNPs)**; Teske et al., 2018). Thus, **mtDNA** is best applied in tandem with nuclear **genetic** or **genomic data** to inform species-specific conservation management.

### 3 Reduced-representation genomic data

Reduced-representation sequencing (**RRS**) involves sequencing a subset of the **genome** to identify a set of genomic variants (e.g., **SNPs**) across all sequenced individuals to facilitate estimation of conservation-relevant metrics. The two primary approaches are restriction site associated DNA sequencing (**RAD-seq**) and **microarrays**, although there are other approaches such as Genotyping-in-Thousands by sequencing (GT-seq; Campbell, Harmon et al., 2015; Schmidt et al., 2020). These approaches have similar conservation applications as genetic markers, but facilitate identification of thousands or millions of variable sites providing much greater power and resolution in analyses, increasing confidence in the accuracy of estimates (Lemopoulos et al., 2019).

#### 3.1 RAD-seq data

**RAD-seq** encompasses a range of approaches (including Genotyping By Sequencing (GBS), reduced representation libraries (RRL), and double-digest **RAD-seq** (ddRAD-seq)) that use restriction enzymes to target subsets of genome-wide loci across all sequenced individuals (Andrews et al., 2016). As a result, **RAD-seq** represents a cost-effective entry-

point for conservation genomics where resources (both genomic and economic) may be limited (Andrews et al., 2016). **RAD-seq** can therefore enable robust estimates of genetic diversity and population structure while requiring relatively low computational resources. This approach has particular advantages for species with large (> 3 **Gb**) or complex **genomes**, or where existing knowledge and/or resources are limited.

As such, **RAD-seq** approaches have been used to estimate genetic diversity (Zhang et al., 2019), population demographics (Kleinman-Ruiz et al., 2017; Marandel et al., 2020), parentage assignment and relatedness estimation (Thrasher et al., 2018), interspecific hybridisation (Colston-Nepali et al., 2019), and population structure and gene flow (Dierickx et al., 2015; Lavretsky et al., 2019; Rexer-Huber et al., 2019; Rick et al., 2019). Bioinformatic advances are enabling new and creative ways to leverage RRS to address a range of conservation questions (Dorant et al., 2020).

### 3.2 Microarrays

**Microarrays** are used to simultaneously genotype thousands of **SNPs** within and among populations at relatively low cost per individual. **SNP-chips** are one such **microarray** and are known for their low genotyping error rates and low rates of missing data. Development of a **SNP-chip** first requires identifying genome-wide variation. Most commonly, a **reference genome** is generated, against which resequencing data from 10-20 individuals is aligned for **SNP** detection (see *Part 2: 4 Whole-genome sequencing*). A subset of **SNPs** is then selected for inclusion on the **SNP-chip**. **SNP-chips** usually have a low density and hence only represent a small fraction of the **genome** (e.g., a **SNP-chip** comprising 50,000 SNPs from a bird with a 1.1 **Gb genome** represents < 0.01% of the **genome**). **Microarrays** typically require hundreds of samples for inclusion in each sequencing batch, and so may not be the most feasible method for genotyping individuals from threatened species. **Microarrays** have been employed to investigate genotype-phenotype associations, trait

heritability, population demographics and signatures of inbreeding in livestock and wild populations (Angeloni et al., 2012; Dutsch et al., 2020; Latch, 2020; Toro et al., 2014).

### 3.3 Limitations of reduced-representation approaches

It is important to consider that **RAD-seq** and **microarrays** remain reduced-representation approaches, whereby only a small fraction of the diversity of the **genome** can be explored. As such, **RRS** approaches will be superseded by genomic resequencing (see *Part 2: 4.2 Population-level resequencing*) for species with relatively small **genomes** (< 3 **Gb**) as sequencing costs continue to decline. **RRS** provides limited utility for characterising adaptive variation, and limited ability to detect other important types of diversity such as **structural variants**. While **RAD-seq** approaches are relatively cost-effective, initial high development costs and large minimum sample sizes (hundreds or even thousands of individuals) required for **microarrays** limit their use for conservation in the absence of consortia involvement or long-term interest, with more feasible applications for human health and commercially-significant species (e.g., sheep, Kijas et al., 2014; cattle, Harris & Johnson, 2010). Additional challenges arise from ascertainment bias with **microarrays** (McTavish & Hillis, 2015), or batch effects with **RAD-seq** approaches, whereby data generated from one sequencing batch may produce data of vastly different quality than that from another (Leigh et al., 2018).

## 4 Whole-genome sequencing

### 4.1 Reference genomes

Despite rapid developments in DNA sequencing technologies, it is not yet possible to sequence the entire complement of DNA of an organism in one piece. Thus, **genomes** must be assembled from many shorter sequences (analogous to puzzle pieces). Sequencing developments to date have increased the scale of sequencing to not only span more of the

**genome** (sequence **coverage**), but also to do so many times (sequence **depth**; Figure 4). Increased sequence **depth** increases the number of sequences produced that overlap with one another, allowing more accurate assembly of sequences into **genomes**. These high-quality assembled **genomes** can be used as **reference genomes** to guide alignments of population-level reduced-representation or resequencing data (see *Part 2: 4.2 Population-level resequencing*) for intraspecific comparisons of diversity and differentiation, or for direct interspecific comparisons.

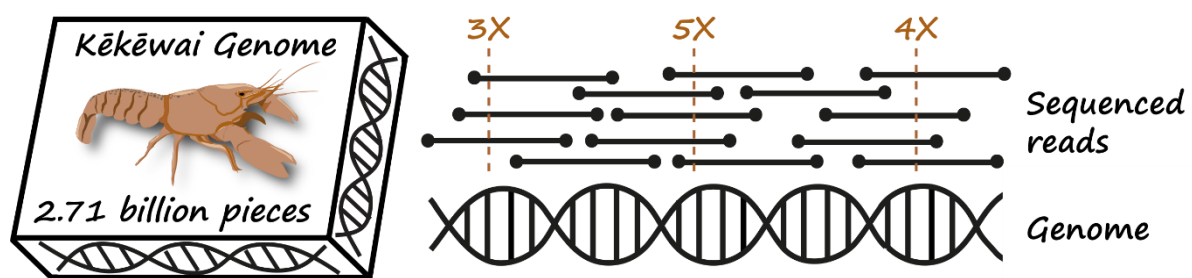


Figure 4 Visualisation of genome sequencing and assembly concepts using the analogy of a genome as a puzzle made of many puzzle pieces. For example, the size of the kēkēwai/freshwater crayfish (*Paranephrops zealandicus*) genome is 2.71 billion (2.71 Gb) base pairs, or puzzle pieces. To be confident about the arrangement of puzzle pieces, each is sequenced many times to create overlapping sequences (sequencing depth; here ranging from 3–5x, but typically ~ 40x for short-read sequencing for the purpose of genome assembly) from which sequencing and/or assembly errors can be identified and corrected. Coverage is the proportion of the genome that is sequenced. Credit: AR.

Initial conservation genomics research in Aotearoa New Zealand has been heavily biased towards birds. **Reference genomes** have been used to inform conservation management actions including breeding recommendations for kakī (black stilt, *Himantopus novaeseelandiae*) and kākāriki karaka (orange-fronted parakeets, *Cyanoramphus malherbi*; Galla et al., 2020), assessment of **adaptive potential** in hihi (*Notiomystis cincta*; de Villemereuil et al., 2019) and research currently in progress aims to characterise the underlying basis of inbreeding depression and improve breeding outcomes for kākāpō (Guhlin et al., *In Prep.*). **Reference genomes** are available for similar applications in other endemics including kea (*Nestor notabilis*), kiwi (*Apteryx* spp.), North Island kōkako (*Callaeas wilsoni*), mohua (yellowhead, *Mohoua ochrocephala*), titipounamu (rifleman; *Acanthisitta*



*chloris*), and tuatara (*Sphenodon punctatus*; Feng et al., 2020; Gemmell et al., 2020; Le Duc et al., 2015; Sackton et al., 2019; G. Zhang et al., 2014), and are coming online for kōwaro (Canterbury mudfish, *Neochanna burrowsius*), kēkēwai (freshwater crayfish, *Paranephrops zealandicus*), and wētāpunga (giant wētā, *Deinacrida heteracantha*), among others.

A **reference genome** acts as the foundation for population-level genomic analysis, and so the quality of a **reference genome** dictates its utility for downstream analyses. Substantial effort is required to produce high quality **reference genomes** that can be used to address a wide breadth of conservation challenges. A fundamental requirement for sequencing and assembling high-quality **genomes** is a high-quality sample, obtained through best-practice sample collection and storage (see *Part 1: 4.1 Best-practice sample collection and storage for genetic/genomic research*). Other requirements include a high level of technical knowledge and access to extensive computational resources, particularly when working with large **genomes** (> 3 Gb).

Although sequencing costs are decreasing, these combined costs remain high, and as a result, there will usually only be one high-quality **reference genome** produced per species. The increasing number of **reference genomes** available has been supported by the efforts of large consortia that aim to assemble **genomes** across a wide range of taxa and/or geographic locations (e.g., the Vertebrate Genomes Project, Genome 10K Community of Scientists, 2009; Koepfli et al., 2015; Bat 1K, Teeling et al., 2018; the Cetacean Genomes Project, Morin et al., 2020; the Earth BioGenome Project, Lewin et al., 2018).

The majority of **genomic data** in Aotearoa New Zealand to date has been generated with **short-read sequencing** approaches, producing millions of DNA sequences (“reads”) typically < 500 bp in length. High sequence **depth** (> 40-fold) is required to produce accurate **genome** assemblies from short-read data alone, but even high **depth** may not

provide accurate **coverage** of the **genome** due to complex repetitive regions that are difficult to accurately sequence and assemble using **short-read sequencing**.

With improving technologies and decreasing costs, **long-read sequencing** technologies are becoming more accessible. **Long-read sequencing** can produce reads tens of thousands of **base pairs** long, and is considered essential for assembly of large or complex **genomes**, such as those species with polyploid or highly repetitive **genomes** (Scott et al., 2020). Long reads act as a foundation for **genome** assembly to dramatically improve our ability to assemble high-quality **genomes** and ensure that a greater proportion of the **genome** can be assembled more accurately than with **short-read sequencing** alone (Figure 5; Morin et al., 2020). To further improve assembly accuracy, existing short-read data may be leveraged to 'polish' a long-read **genome** assembly.

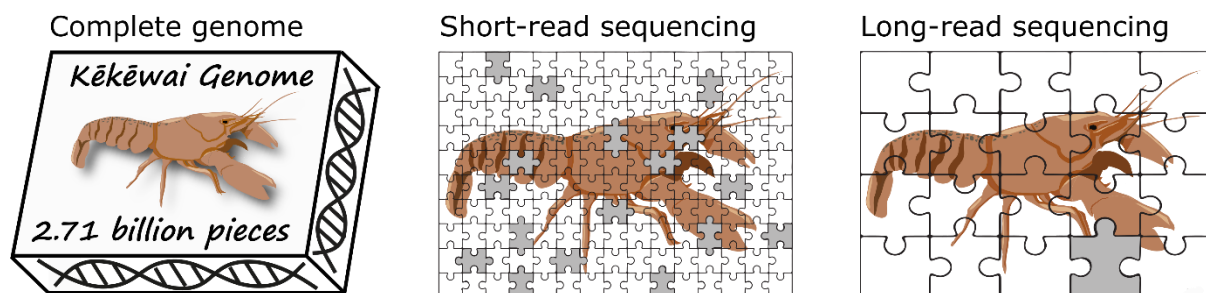


Figure 5 Visualisation of the difference between short- and long-read sequencing, using the 2.71 Gb kēkēwai (freshwater crayfish; *Paraneohrops zealandicus*) genome as an example. When using short-read sequencing, large numbers of reads (analogous to small puzzle pieces) are required to cover the kēkēwai genome. Such short-read genomes typically have many gaps (regions of unknown sequence), particularly due to sequencing challenges associated with repetitive DNA regions. In comparison, long-read sequencing (represented by large puzzle pieces) requires fewer reads to cover the same genome, and these reads are better able to span repetitive regions, resulting in fewer gaps. Credit: AR.

Once considered unattainable for species of conservation concern, gold- or platinum-quality **genome** assemblies (i.e., ultra-high-quality **genome** assemblies, such as that for the kākāpō **reference genome** from the individual known as Jane) that represent complete or near-complete chromosomes are becoming more common. Such high-quality **genome**

assemblies require not only **short-read sequencing** technologies but also **long-read sequencing**. However, with the wide range of sequencing platforms, read lengths, and computational pipelines for **genome** assembly, it is important to be aware that not all **reference genomes** are created equally.

More recent sequencing developments include the ability to capture the spatial structure of DNA within the nucleus (known as **3C sequencing**), providing additional context on the landscape of genes and regulatory elements within chromosomes, further enhancing assembly quality and completeness (Lieberman-Aiden et al., 2009). Stand-alone **reference genomes** can also be accompanied by a reference **transcriptome**, identifying the complement of genes encoded in the **genome** that characterise the phenotype of an organism, and enable assessment of responses to environmental change or disease (see *Part 2: 7 Transcriptomics*).

## 4.2 Population-level resequencing

Generating data to the level of resolution required for a **reference genome** at population-level scale remains prohibitively expensive. Resequencing data in combination with a **reference genome** can provide an affordable means to investigate diversity at the population level. In contrast with a **reference genome** where sequence data may be sourced from multiple platforms, resequencing data consists of **short-read sequencing** data individuals at low–moderate **coverage** (< 30-fold). These short reads act like puzzle pieces that can then be aligned against the complete picture that is the **reference genome**. Sequences can then be compared against the reference and between individuals to identify genomic variants (e.g., **SNPs**) throughout the **genome** with which conservation-relevant metrics can be estimated with much greater accuracy than that provided by low-resolution genetic markers (Galla et al., 2020). These **SNPs** can then be applied in a similar way to genetic markers (e.g., microsatellites) for comparisons of genomic diversity and relatedness

(Galla et al., 2020), population differentiation and structuring (Lado et al., 2020), and introgression resulting from interspecific hybridisation (Leroy et al., 2020), all of which can inform conservation management including translocations and conservation breeding programmes.

Additional applications of resequencing **genomes** include the ability to investigate **functional variation** (Brandies et al., 2019; see *Part 1:5.1.3 Functional variation*). This has broad and significant implications for understanding the genomic basis of traits important for management, such as those associated with adaptation (to the environment and/or captivity) and **reproductive fitness** (Angeloni et al., 2012; Hoelzel et al., 2019). To this end, leveraging genomic resequencing data beyond assessments of **neutral variation** requires that we consider genomic diversity beyond **SNPs** (see *Part 1: 5.1 Conservation genetic/genomic research toolbox horizon scan*).

## 5 Ancient DNA

Knowledge of the past can be used to inform the future. Ancient DNA (i.e., DNA isolated from old biological material, **aDNA**; Leonard, 2008) provides a window into the past via the retrieval of DNA from a variety of degraded sources including museum specimens, subfossils, sediment cores, and coprolites. **aDNA** can provide a useful tool for conservation managers as knowledge of past genetic diversity, geographic range expansions/contractions, and the factors that lead to population declines or extinctions is important for informing management decisions (Grealy et al., 2017; Leonard, 2008). Much of Aotearoa New Zealand's endemic biota has been driven to extinction or reduced to relictual distributions following human arrival, and so **aDNA** provides a means to examine the genetic composition of such species prior to anthropogenic impacts.

Examples of the use of **aDNA** to inform the conservation and management of endemic Aotearoa New Zealand species includes quantification of temporal declines in genetic diversity (Bergner et al., 2016; Dussex et al., 2015; Grueber & Jamieson, 2008; Tracy & Jamieson, 2011), determination of the origin of contemporary genetic structure (Tracy & Jamieson, 2011), clarification of the past distribution of species and populations/genetic lineages (Shepherd & Lambert, 2008; Verry et al., 2019; Wilmshurst et al., 2014), and assessment of the rate of harvesting at which a species may be driven to extinction (Rawlence et al., 2016).

## 5.1 Challenges and limitations associated with aDNA

The degraded nature of **aDNA** necessitates caution, and requires the use of dedicated clean laboratory spaces and specialised laboratory and bioinformatic techniques (Knapp, Clarke, et al., 2012). Care must be taken when generating and analysing these data as DNA degradation and/or modern DNA contamination can bias results and lead to erroneous conclusions. Furthermore, **aDNA** studies are often limited by small sample sizes, with available samples in museum collections/subfossil deposits unlikely to be representative of past populations. While much **aDNA** work to date has relied on mitogenome data due to its small size and high copy-number making it relatively easy to retrieve, decreasing sequencing costs mean it is becoming more feasible to generate nuclear **genomic data** from degraded samples. Primarily applicable to very well-preserved sources of **aDNA** (e.g., museum skins), sequencing of complete ancient nuclear **genomes** would enable the direct comparison of past and present genetic diversity within populations of threatened species. This could be directly applied to some of Aotearoa New Zealand's flagship conservation species (e.g., takahē (*Porphyrio hochstetteri*), kākāpō), with well-preserved specimens collected from the 1800s onward present in museum collections (Dussex et al., 2018; Grueber & Jamieson, 2008).

## 6 Environmental DNA

Environmental DNA (**eDNA**) has recently gained conservation interest due to the ability to detect rare, cryptic and invasive taxa on broad scales via non-invasive environmental sampling from water, soil, or air (Taberlet et al., 2012, 2018). Methodologies can be species-specific (targeted **eDNA**) or have broad multi-species applications (**eDNA** metabarcoding), with sensitivity equal to or greater than traditional species detection and monitoring methods using netting, electrofishing, or underwater videos (Evans et al., 2017; Goutte et al., 2020; Lacoursière-Roussel et al., 2016; Lodge et al., 2012; Olds et al., 2016; Stat et al., 2019). Such non-invasive sampling minimises disturbance and physical harm, which could be critical in studies of threatened species such as hoiho (yellow-eyed penguin, *Megadyptes antipodes*; Ellenberg et al., 2007, 2013; Young et al., 2020).

While **eDNA** methodologies can be as or more cost effective than traditional sampling and monitoring (Evans et al., 2017; Lugg et al., 2018; Shaw et al., 2016), patterns of **eDNA** dispersal in the environment must be understood in order to characterise the presence of taxa (Barnes & Turner, 2016; Jane et al., 2015). Studies of **eDNA** dispersal through water have shown macrofaunal signal does not travel far (< 1km) and may remain stratified within water layers, particularly within the marine space and other large, slow-moving bodies of water (Eichmiller et al., 2014; Jeunen et al., 2020). Thus **eDNA** can be applied to detect species presence or composition in specific sites within water bodies. In comparison, lotic bodies of water such as rivers may carry **eDNA** far downstream (> 10km), influencing species detection far from the source (Carraro et al., 2018; Deiner & Altermatt, 2014).

### 6.1 Conservation applications of environmental DNA

Conservation applications of **eDNA** for detecting species presence include biosecurity (Pochon et al., 2017), site occupancy modelling (Muha et al., 2017), and detection of cryptic pest species in managed areas (Ramón-Laca et al., 2014). Detecting species presence or

composition using **eDNA** methods may supersede current species monitoring methods, and may facilitate assessment of environmental stressors on ecosystems such as those resulting from primary production (Laroche et al., 2018; Macher et al., 2018).

**eDNA** can be used to assess wildlife and ecosystem health (Hall et al., 2016; Strand et al., 2019), and understand species interactions (Bleijswijk et al., 2014; Farrell et al., 2000; Nichols et al., 2015). Within Aotearoa New Zealand, analysis of faecal **eDNA** has been used to infer the diets of kekeno (New Zealand fur seal, *Arctocephalus forsteri*; Emami-Khoyi et al., 2016), kororā (little blue penguin, *Eudyptula minor*; Murray et al., 2011), hoiho (Young et al., 2020), and Bryde's whales (*Balaenoptera edeni brydei*; Carroll et al., 2019). **eDNA**-based diet analysis is especially useful for analysing soft-bodied prey, which are otherwise difficult to identify. For example, establishing the earthworm diet of the endangered endemic carnivorous land snail (*Powelliphanta augusta*) can inform habitat restoration and site suitability for translocations (Waterhouse et al., 2014).

**eDNA** also has potential as a tool for monitoring species abundance and measuring population genetic diversity and differentiation. There is increasing interest in the correlation between **eDNA** abundance and species biomass with applications for tracing migratory patterns or spawning activity (Bylemans et al., 2017; Doi et al., 2015; Laramie et al., 2015; Thalinger et al., 2019; Yates et al., 2019), although analytical challenges remain regarding the impacts of factors such as size, age and cell/DNA shedding rates (Iversen et al., 2015; Klymus et al., 2015; Vasselon et al., 2018). There is also growing evidence that **eDNA** can be used to estimate conservation-relevant metrics including genetic diversity and population structure in species that are challenging to sample (Adams et al., 2019; Parsons et al., 2018; Stepien et al., 2019; Tsuji et al., 2020).

## 6.2 Limitations of environmental DNA

**eDNA** is best used in tandem with traditional biodiversity surveying methods, as open-access sequence databases may be depauperate of target taxa due to regional biases or data-access limitations (Porter & Hajibabaei, 2018; Sato et al., 2018). Additional limitations arise from the high sensitivity of **eDNA** studies that can increase the risk of false positive or false negative results due to faecal deposits by mobile predators, extreme weather events, data-deficient databases, insufficient sampling, and field or laboratory contamination (Dickie et al., 2018; Furlan et al., 2020; Goldberg et al., 2016; Merkes et al., 2014; Staley et al., 2018). As with **aDNA** methodologies, dedicated clean laboratories are required to minimise the potential for contamination (Goldberg et al., 2016).

As **eDNA** tools are still in development in Aotearoa New Zealand and abroad, data is somewhat limited (e.g., Corfe-Tan et al., 2019). Widespread sampling will be required to generate baseline data and populate databases, which could be done through community-driven science initiatives, such as the Environmental Protection Agency's Wai Tūwhera o te Taiao - Open Waters Aotearoa programme ([www.epa.govt.nz/community-involvement/open-waters-aotearoa/](http://www.epa.govt.nz/community-involvement/open-waters-aotearoa/)). Nevertheless, **eDNA** methods hold great promise for species and ecosystem monitoring in Aotearoa.

## 7 Transcriptomics

The **transcriptome** represents the complete set of RNA transcripts that determine the expression of genes produced from the **genome** of a cell, tissue or organism at a specific development stage or physiological condition (Wang et al., 2009). The function and composition of these transcripts is essential for our understanding of an organism's phenotype. Two key aims of transcriptomics relevant to conservation are (i) to quantify changing gene expression during development or stress and (ii) to annotate a **genome** by cataloguing all transcripts. Various technologies have been developed for transcriptomic



research, with RNA sequencing (RNA-seq) propelling the field beyond clinical biology (Todd et al., 2016; Wang et al., 2009). Unlike whole-genome sequencing where reads are aligned to an assembled reference to improve confidence and resolution, RNA-seq does not require any prior knowledge of the **transcriptome**, making it particularly useful for the study of novel traits in non-model organisms (Alvarez et al., 2015; Todd et al., 2016).

Transcriptomics facilitates **genome** annotation, identifying features such as gene coding regions that enhance the utility of **reference genomes** (Yandell & Ence, 2012). Accurate **genome** annotation enables an understanding of gene expression changes, and identification of genes associated with disease and other functional traits that may be relevant for conservation (Connon et al., 2018; Videvall et al., 2015). Many of these annotations can be predicted using existing databases or inferred from closely-related species (Aken et al., 2016; Dominguez Del Angel et al., 2018; Ekblom & Wolf, 2014), but **transcriptome** sequencing may be required for accurate annotation of novel gene models in non-model species (Trapnell et al., 2010). **Transcriptome** sequencing for **genome** annotation often requires the sequencing of various tissues, sexes, and life stages to capture an accurate representation of the diversity of gene expression in a species.

Although every cell within an organism contains the same set of genes, not every gene is active in every cell. Furthermore, gene expression within a cell can be affected by stage of development, environment, or stress (Wang et al., 2009). Gene expression changes in response to stress or across development can be profiled in an individual cell, specific tissue or the whole organism (Kulkarni et al., 2019; Todd et al., 2016). These gene expression changes are frequently referred to as the up- or down-regulation of a gene (Costa-Silva et al., 2017). Historically gene expression changes were often assessed in individual genes but with the rise of RNA-seq whole **transcriptome** profiling has now become possible for almost any organism (Alvarez et al., 2015; Todd et al., 2016). Transcriptomic data generated through RNA-seq from across populations can also be used to identify genomic **SNPs**,

thereby enabling acquisition and comparison of two data types from one data set (Lopez-Maestre et al., 2016).

## 7.1 Conservation applications of transcriptomics

**Transcriptome** profiling is the most efficient way to acquire a comprehensive snapshot of an organism's physiological state, and has the potential to have an immense impact on understanding wildlife health (Figure 6), particularly for the understanding of the processes of and response to disease in non-model species (Campbell et al., 2018; Field et al., 2015; Videvall et al., 2015). **Transcriptome** profiling differs from **genome** annotation in that it is typically restricted to a single tissue type. Combining transcriptomics with other epidemiological data can explain why populations differ in their functional response to a disease. Although assessments of wild populations can be challenging, a recent study on lethal viral infections in amphibians successfully used infected and non-infected wild populations to understand how a history of disease alters a population's gene expression profile (Campbell et al., 2018). Greater understanding of disease history and population diversity can facilitate management of **functional variation** relating to immunity and development in wild populations.

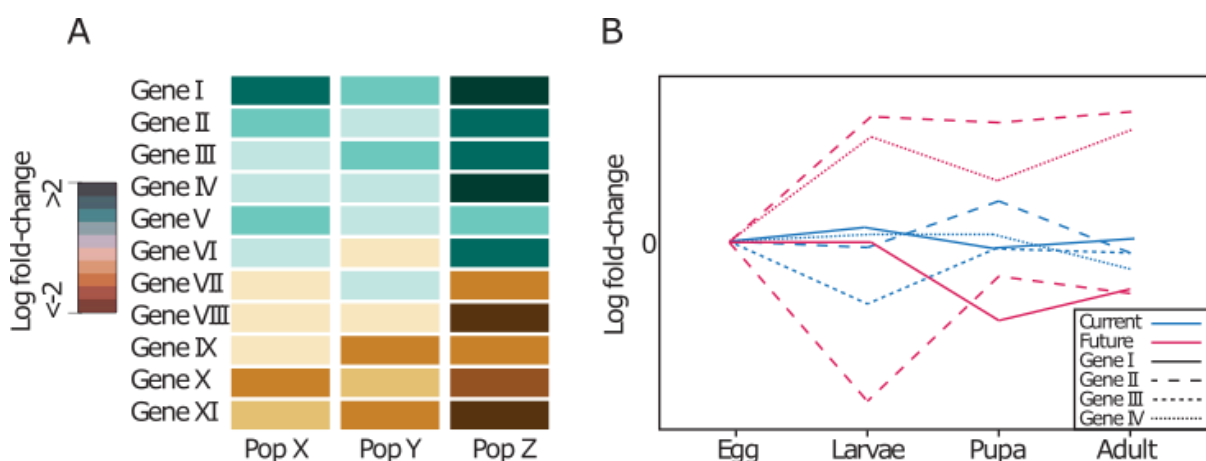


Figure 6 Two examples of the use of transcriptomics to inform conservation management. Example (A) compares hypothetical immune gene expression changes between healthy and diseased samples across populations. Gene expression changes are displayed as the log-fold change between

samples, where each box represents the log fold-change in expression of various genes between diseased and non-diseased samples from three populations (X, Y and Z). Genes that are up-regulated (green) have higher rates of expression in diseased samples. Due to connectivity between genes, up-regulation in one gene can result in down-regulation of others (brown), and visa versa. In this example a stronger immune response is seen in population Z. Example (B) compares hypothetical stress responses in an insect population reared in current and future climate conditions. Different genes display different levels of regulation across stages of development. In this example, log-fold changes are greater in populations reared in future conditions than in current conditions. Combined with ecological and physiological data these types of studies can inform on the adaptive potential of a species. Credit: EJD.

With an increasingly changing environment there is a growing need to understand organisms' resilience to change (Moritz & Agudo, 2013; Somero, 2010). When incorporated alongside ecological and physiological data, comparative transcriptomic assessments across environments can infer a population's physiological capacity to respond to changing environmental conditions (Anderson & Song, 2020; Kelly, 2019; Seebacher et al., 2015). Such studies typically involve moving wild individuals into controlled laboratory environments where they can be exposed to predicted future conditions (DeBiasse & Kelly, 2016; Narum & Campbell, 2015; Riddell et al., 2019). For example, research into the **adaptive potential** of an Afrotropical butterfly (*Bicyclus anynana*) has shown that despite expressing two distinct phenotypes in dry and wet seasons, this species has limited adaptability in the face of environmental change (Oostra et al., 2018). Research in this area can support conservation practitioners in making proactive management decisions regarding climate change.

## 7.2 Challenges and limitations for conservation transcriptomics

Transcriptomics has made significant contributions to our understanding of organismal physiology, evolutionary biology and ecology but is as yet under-utilised in conservation biology (Alvarez et al., 2015; Connon et al., 2018; Todd et al., 2016). This under-utilisation stems from the requirement for destructive sampling of individual tissues under tightly

controlled conditions to gain an understanding of responses to disease or stress. To detect meaningful gene expression differences requires the ability to distinguish true differential expression due to laboratory treatments from background noise (Todd et al., 2016). For example, to understand response to disease it would be beneficial to compare multiple samples of both infected and uninfected individuals from the same population (i.e., genetic background), sex, and developmental stage in identical environmental conditions. Such experimental protocols are often difficult to enact in wild populations of threatened species, and as a result conservation transcriptomic studies may remain limited (Connon et al., 2018). However, for species adversely impacted by disease or rapid environmental change, researchers and practitioners may need to consider whether the potential knowledge gained from transcriptomic approaches outweighs the costs of lethal sampling. Research into the adaptability of species to environmental change can facilitate proactive conservation in the face of climate change, and could be prioritised to focused on high-risk species identified via climate change vulnerability assessments (Wheatley et al., 2017), while considering species interactions (Hance et al., 2007; Memmott et al., 2007).

## 8 Microbiomes

So far our discussion of conservation genetics and genomics has been largely restricted to species-specific approaches. However, the **microbiome**—the microorganisms that reside on or within the tissues of a host species, including bacteria, fungi, and viruses—has the potential to offer insights into key conservation questions (West et al., 2019). Although initial research into host-associated **microbiomes** was largely based on human health and model organisms (Davidson et al., 2020; Gilbert et al., 2018), here we review some ways in which **microbiome** studies may inform conservation.

Just as conservation-relevant processes such as population bottlenecks leave an impact on the **genome**, the **microbiome** can also be affected. For example, Asian tiger mosquitoes

(*Aedes albopictus*) introduced to Italy show lower microbiomic diversity than mosquitos from within the native range (Rosso et al., 2018). Additional processes that can impact **microbiome** diversity include captivity (e.g., Tasmanian devils, *Sarcophilus harrisii*; Cheng et al., 2015); and other species as summarised by West et al. (2019)), poor physiological condition of individuals (e.g., fasting humpback whales, *Megaptera novaeangliae*; Vendl et al., 2020), and the presence of pathogens (Van Cise et al., 2020). Reductions in **microbiome** diversity are important as they are associated with negative health outcomes (Vangay et al., 2018). Many host organisms rely on symbionts for defence against pathogens (McLaren & Callahan, 2020; Vorburger & Perlman, 2018), and the **microbiome** can directly impact behaviour and memory in some species (Davidson et al., 2020). In addition, perturbation of the **microbiome** could have other consequences. The **microbiome** appears to have an important role in local adaptation (Suzuki et al., 2019) and adapting to a changing world (Cunning & Baker, 2020; Voolstra & Ziegler, 2020), which may be important to consider when planning translocations.

Aspects of the **microbiome** could also be useful for individual and/or population monitoring. In humans, the skin **microbiome** can predict the age of an individual to within ~4 years (Huang et al., 2020). When applied to species of conservation concern, similar analyses may allow for previously unknown individuals to be aged, which may be valuable when correcting pedigrees. Rapidly evolving microbes can help uncover patterns not evident in host **genomes**, especially when the host has low genetic diversity due to recent bottlenecks (Wirth et al., 2005). For example, genetic investigation of feline immunodeficiency virus revealed previously uncharacterized population structure and demography in cougars (*Puma concolor*; Biek et al., 2006). Similarly, extension of current genomic analyses for demographic inference to **microbiomes** could increase temporal resolution to conservation-relevant time scales. Finally, monitoring the **microbiome** of individuals released from captivity could be used as a measure of translocation success as the **microbiome** shifts towards that seen in wild conspecifics (e.g., Tasmanian devils; Chong et al., 2019). Despite

**microbiome** analyses for conservation being a relatively new field of interest, these methods have already been applied to threatened Aotearoa New Zealand species, including the critically endangered kākāpō (Perry et al., 2017; Waite et al., 2014). Such exploratory **microbiome** studies represent the first steps towards actively incorporating 'microbial rescue' into conservation strategies (Mueller et al., 2020; West et al., 2019).

## 8.1 Methodological considerations associated with microbiome analysis

Careful sampling design is essential when undertaking **microbiome** studies to inform conservation (Knight et al., 2018). Considerations should include which tissue types the **microbiome** will be sampled across (e.g., gut, faeces, skin, oral), and whether sampling will be sufficient to control for individual age and sex, seasonal differences, and other confounding effects. Metadata should be collected on all of the above, and any other factors that could influence **microbiome** composition as **microbiome** data is only as useful as the metadata that accompanies it (Goodrich et al., 2014; Knight et al., 2018).

Following the design of the **microbiome** sampling experiment, sample collection can commence. Because microorganisms are present in most environments, sampling blanks must also be collected at various stages to control for the presence of background environmental or laboratory contamination (Karstens et al., 2019; Knight et al., 2018). Sample storage is also key to ensure the extraction of ultra-high-quality DNA (as per the requirements for **reference genome** sequencing). Some microorganisms are resistant to standard DNA extraction techniques and may require specialised protocols. It is good practice to also extract DNA from a "mock community" with a known species composition to evaluate biases in the quantities of DNA extracted from different microorganism species.

Finally, after extracting DNA, **microbiome** profiling approaches must be determined. The most common current approach is to target small nuclear regions of **genomes** using PCR

amplification so that microbial species (e.g., bacteria, fungi) can be identified (Knight et al., 2018). The advantage of a PCR-based approach is that multiple samples can be included in a single sequencing run, reducing costs. However, this may be dependent on PCR primer choice, as not all species will be amplified equally, and so the results may not reflect the extracted DNA. In addition, PCR-based approaches can amplify background contamination (Karstens et al., 2019). An alternative is a more expensive metagenomic resequencing approach (similar to that of *Part 2: 4.2 Population-level resequencing*) to sequence the **genomes** of the microorganisms present in the sample. This approach can also be used to identify the entire microbial community and characterise the functional genes present in the sequenced DNA.

Additional challenges of **microbiome** analysis include preventing DNA of the host organism from overwhelming microbial DNA during sequencing (Knight et al., 2018). As downstream analyses generally include comparing microbial community richness and composition between groups of interest (e.g., captive versus wild animals), **microbiome** research is limited by the availability of microbial sequences in reference databases.

# Glossary

<b>Term</b>	<b>Definition</b>
<b>3C sequencing</b>	Chromosome conformation capture techniques such as Hi-C and Pore-C used to characterise the spatial structure of DNA to identify genomic interactions.
<b>adaptive potential</b>	The ability of a population to adapt to immediate environmental change, typically estimated by the extent of genomic diversity present, and quantified by relative reproductive fitness. Contributes to overall evolutionary potential, which refers to a species capacity to respond to environmental change through time.
<b>aDNA</b>	Ancient DNA; DNA extracted from historic museum skins, subfossils, or fossils.
<b>base pairs (bp)</b>	Individual nucleotides that code the DNA.
<b>chromosomics</b>	A discipline that integrates cytogenetics and whole-genome sequencing to study chromosome-level diversity.
<b>coverage</b>	Can have 2 distinct meanings, 1) when used in a reference genome context, can relate to the proportion of the genome represented by the reference; and 2) when used in a population-level sequencing context, refers to the amount of sequencing depth supporting a variant call.
<b>cytogenetics</b>	The study of the form and structure of DNA within the nucleus of a cell.
<b>depth</b>	The number of times the genome is sequenced.
<b>eDNA</b>	Environmental DNA; DNA extracted from environmental samples such as water, soil or air.
<b>functional variation</b>	Genomic variation that results in phenotypic variation and has some effect on individual fitness. Also known as functional diversity.
<b>Gb</b>	Gigabase pairs; one billion base pairs of DNA.
<b>genetic data</b>	Data representative of a subset of the genome, typically comprising tens of loci.
<b>genome</b>	The full complement of DNA characterising an individual.
<b>genomic data</b>	Data representative of the genome, comprising hundreds to millions of loci. These data are generated with high-throughput sequencing techniques.
<b>hologenomics</b>	The study of the genomic interactions between a host organism and its microbiome.
<b>long-read sequencing</b>	DNA sequencing using platforms such as Oxford Nanopore Technologies MinION or PacBio SMRT that can produce sequence reads in excess of 10,000 bp in length.



<b>microarray</b>	A genomic method used to genotype large numbers of loci at population-scale, simultaneously.
<b>microbiome</b>	The microorganisms that reside on and/or within the tissues of a host species, including bacteria, fungi, and viruses.
<b>mtDNA</b>	Mitochondrial DNA; the DNA specific to the mitochondrial organelle, with a short circular structure, and high copy number within an individual.
<b>neutral variation</b>	Genomic variation that does not impact fitness. Also known as neutral diversity
<b>pangenome</b>	Multiple high-quality genome assemblies that capture all of the genomic diversity within a species. Pangenomes may eventually supersede single individual genomes for reference purposes.
<b>RAD-seq</b>	Restriction-site associated DNA sequencing, a method using restriction enzymes to target subsets loci throughout the genomes of all sequenced individuals.
<b>RRS</b>	Reduced-representation sequencing.
<b>reference genome</b>	A representation of the genome of a species, that can be used alone for interspecific comparisons, or as a reference against which population-level resequencing or RRS data can be aligned for intraspecific comparisons.
<b>reproductive fitness</b>	Capacity of individuals in a population to propagate their genes to subsequent generations. Quantified through estimates of relative fertility, and mortality.
<b>short-read sequencing</b>	DNA sequencing conducted using platforms such as Illumina MiSeq, HiSeq and NovaSeq that produce short (< 500 bp) sequence reads.
<b>SNP-chip</b>	A type of genomic microarray used to generate single-nucleotide polymorphism data for large numbers of individuals within a species.
<b>SNPs</b>	Single-nucleotide polymorphisms; the most common form of variation in the genome. SNPs have low mutation rates, and are often biallelic, with known characteristics making analysis relatively straightforward.
<b>structural variants, SVs</b>	A diverse class of genomic variation impacting the form and structure of chromosomes. Common types of structural variants include copy number variants, deletions, duplications, insertions, inversions, and translocations > 50 bp.
<b>transcriptome</b>	The array of RNA transcripts that are expressed by an organism, determining individual phenotype.

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