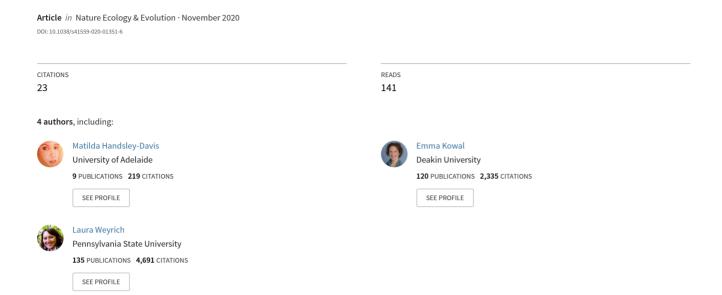
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The study of environmental DNA can reveal information about the history and presence of Indigenous communities on their lands — potentially even inadvertently. Better engagement with the ethical aspects of environmental DNA research is required in the field as a whole, and especially for researchers working on Indigenous lands.

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nalysis of environmental DNA (eDNA) is a rapidly developing research area with broad applications for ecology and conservation biology¹⁻⁴. Extraction of genetic material from environmental samples, such as water, soil, air or sediments, can provide current and historical information about people, their environment and their interactions with other species. Many environmental samples are legally collected from sediments, water and other materials on Indigenous lands, without extensive regard as to how the findings may affect Indigenous traditional owners and knowledge holders. Such considerations may be especially critical when considering that eDNA can be preserved in some environmental samples for thousands of years⁵⁻⁷, which could be used to directly link certain human populations to specific locations in the past^{4,8}. Indigenous peoples have often been excluded from genetic research, in part because of a history of ethical transgressions and a lack of trusting relationships with researchers⁹⁻¹¹. Although collaborations between Indigenous communities and geneticists are now developing with enhanced ethical oversight, this has not been extended to the novel ethical, legal and social implications that arise from the use of eDNA.

Because environmental samples may contain DNA from many different organisms, it falls to researchers to choose how narrowly or broadly to target their sequencing efforts. Increasingly, eDNA research is moving away from metabarcoding and amplicon studies that target specific organismal groups and towards whole-genome or shotgun sequencing approaches that can reveal the total diversity of DNA present in a sample8. This indiscriminate approach raises key ethical questions, especially in Indigenous contexts. In settler-colonial contexts, such as Australia, Indigenous people have been marginalized, dispossessed and



Fig. 1 | **Birthing trees.** Gum trees, such as the one shown here, are integrated into Aboriginal Australian knowledge and practice. For example, an 800-year-old Djab Wurrung birthing tree (not pictured) in Victoria, Australia, was the site of perhaps 10,000 births of Aboriginal children and holds profound cultural and spiritual significance. The tree was slated for demolition in 2018 as part of a highway reconstruction project. After more than a year of on-site protesting by Djab Wurrung traditional owners and their supporters, protection for the birthing tree was secured in an agreement with the Victorian government. Efforts to secure protection for other culturally significant trees are ongoing yet not always successful, and the 350-year-old sacred Djab Wurrung Directions Tree was cut down in October 2020. Credit: THPStock.

disadvantaged. Throughout this, however, many have maintained connection to culture and country, and exercise their cultural rights, albeit within a colonial framework which contains and constrains. Intergenerational trauma, and in particular the removal of children, has resulted in deep suspicion of and alienation from academic and scientific research. As such, it is critical that ethical questions arising from new approaches to genetic research, such as eDNA analysis, be carefully considered. For example, what are the potential risks and benefits for Indigenous peoples engaging

with eDNA research? How should such research be classified, regulated and governed? How can the potential of this new technical approach be communicated without 'hype' or over-promising results?

Some of these questions can be illustrated using the example of the birthing trees that were regularly used by Aboriginal women in southeastern Australia before the expansion of European settlement in the mid-nineteenth century (Fig. 1). Women typically gave birth and buried placentas under a birthing tree, and contemporary Aboriginal researchers have used archival

records and oral testimony to show that the trees were associated with labour techniques, midwifery and ceremonies surrounding labour and birth¹². Birthing trees also facilitated connections to land for the mother and baby, and aspects of the traditional practice are still known and used by Aboriginal groups in southeastern Australia. Acknowledging this historical and ongoing importance of birthing trees for Aboriginal women, we approach this topic as a team of Indigenous and non-Indigenous female researchers drawing collectively on several decades of engagement with Indigenous communities.

Environmental DNA research could provide insight into birthing trees via several mechanisms, investigating questions of mutual interest to researchers and Indigenous communities. For example, human DNA preserved in soil or sediment adjacent to a birthing tree could be revealed, potentially confirming a known birthing site, identifying a previously unknown birthing tree, or demonstrating a connection between a specific family or group and a specific tree. Further, identification of ancient human-associated microbes could provide information on past infections or birth complications. However, such research also presents risks for Indigenous peoples. For example, analysis of soil or sediment samples may not identify human DNA at a birthing tree site, which could potentially be used to argue against land rights claims or the protection of cultural heritage, or eDNA may identify microorganisms associated with potentially stigmatizing diseases, such as sexually transmitted infections. A further risk relates to the use of a Western scientific lens to assess eDNA use. In this example, we see Western science as complementary to Indigenous knowledge, although some may interpret the use of eDNA technology as an implication that Indigenous knowledge is insufficient to inform decision-making about the preservation of birthing trees.

Another potential discrepancy between Western and Indigenous viewpoints might arise from the way human and non-human research is distinguished. Human research is typically subject to more stringent ethical requirements and approval from institutional committees, but it is currently unclear if analysis of human DNA isolated from an environmental source would constitute human research. In Australia, for example, the National Statement on Ethical Conduct in Human Research (2015) states that "human research is... conducted with or about people, or their data or tissue"13. Our interpretation of these guidelines is that eDNA may not be considered human tissue, but human genome sequence data

produced from eDNA may be, particularly if it is intended or possible to compare this with other human DNA to identify genetic relationships. If so, this would imply a requirement for human ethical review of eDNA research. The National Statement continues that any human research involving Aboriginal and Torres Strait Islander peoples must undergo ethical review. However, human ethical review is currently not typically required for the study of environmental samples, such as soil. We argue that the potential for eDNA analysis to intersect with human research means that, in certain circumstances, scientists working with environmental samples may need to engage with human research ethics.

It is also possible that relying on existing ethical frameworks is insufficient to regulate eDNA research in Indigenous contexts. Even if no human DNA is isolated from an environmental sample, or if researchers agree to disregard any human DNA detected, Indigenous stakeholders may still have ethical concerns. For example, the cultural significance of non-human animals, water, landscapes and natural phenomena differs between Indigenous and Western contexts^{14–16}, which may complicate existing distinctions between human and non-human research. Because the study of non-human eDNA, including that of animals or plants, could have culturally important implications for Indigenous people, ethical review of such research should implement Indigenous oversight from communities with connections to the land from where eDNA originates.

Like human genetics and human microbiome research before it, eDNA research faces a challenge in how to communicate its potential benefits without over-promising^{17,18}. Research using eDNA offers a new technique for producing valuable knowledge about historical and current Indigenous practices, which may be particularly important to Indigenous groups who have experienced cultural dislocation. eDNA evidence could also be useful in influencing decisions on cultural preservation made by non-Indigenous government officials - for example, in obtaining protection for sacred trees (Fig. 1). However, the limitations of this technology must be appreciated and discussed in any consultation process. eDNA research is not infallible; technical limitations. such as DNA degradation due to age and environmental conditions¹⁹, leaching of DNA between soil or sediment layers^{20,21}, or the difficulty of detecting false negative results, could all hamper eDNA research. This means, for example, that if the DNA of a particular species cannot be found

in an eDNA dataset, its presence cannot be ruled out, as it may be in unsampled parts of the region or be a simple failure of DNA preservation in that environment. Furthermore, even if the technical work of DNA extraction, sequencing and analysis is successful, mistakes or uncertainties in the interpretation of genomic data are still possible. Engaging in multidisciplinary research that combines eDNA analysis with other lines of evidence represents the best opportunity to understand the past. Researchers have an ethical responsibility to avoid hype; hence, the limitations of eDNA research need to be clearly communicated to Indigenous groups who may have particular expectations of or interests in an eDNA research project.

The complex issues surrounding eDNA research illustrate how applying new technologies in novel contexts may modify our understanding of what counts as human research, and of what types of research need to recognize Indigenous interests. In addition to institutional mechanisms for human ethical review and Indigenous research governance, such as consultation with ethics review committees or Indigenous Advisory Committees, it is critical to include Indigenous communities and researchers in all stages of an eDNA study (design, sample collection, analysis and interpretation) to help minimize risks and maximize benefits of research. Ideally, this includes the establishment of long-term partnerships between researchers and Indigenous communities and the creation of Indigenous-led research governance structures. In the absence of dedicated structures, researchers should look to local Indigenous communities, regional and national Indigenous organizations, Indigenous scholars and/or regional and national guidelines for research with Indigenous people in the country in which they conduct their research. In the future, it may be beneficial to revisit current regulatory frameworks to formally incorporate these principles into ethical standards of conduct for eDNA research. Provided that these challenges can be adequately addressed, eDNA analysis offers an exciting new prospect for researchers and Indigenous communities to work together to generate knowledge.

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Published online: 12 November 2020 https://doi.org/10.1038/s41559-020-01351-6

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Acknowledgements

We, especially L.R., acknowledge the many Aboriginal women they have discussed these matters with and from whom they have learned a great deal. Funding for this project was provided by the Australian Research Council Centre of Excellence in Australian Biodiversity and Heritage (CE170100015). E.K. and L.S.W. are also supported by Australian Research Council Future Fellowships (FT160100093 and FT180100407, respectively). M.H.-D. is supported by a Westpac Future Leaders scholarship. We also thank the participants in the Penn State Bioethics Colloquium for their feedback on an earlier draft of this manuscript.

Competing interests

The authors declare no competing interests.