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Ancient DNA studies of dental calculus

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Thesis submitted in fulfilment of the requirements for the degree of Doctor of
Philosophy

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Thesis Declaration

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Andrew G. Farrer

Date

“But what if in future one should tell such people that there are living more animals in the unclean matter on the teeth in one's mouth than there are men in a whole Kingdom?”

Antoni van Leeuwenhoek, “Letter to the Royal Society”
28th December 1683.

Translation: Digitale Bibliotheek voor de Nederlandse Letteren, 1952

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Thesis Abstract

Over the last 10 years, the human microbiota has been identified as a major force in human health and disease. Microbiota are the bacterial communities that live on the internal and external surfaces of the body, and comprise ~50% of the total cell count of a human individual. Recent studies have indicted the role of cultural and environmental factors on shaping these bacterial communities, including diet, interaction with people and animals, and medical treatments. However, the majority of microbiota studies are in modern human populations or animal models. Consequently, there is limited knowledge on the diversity of microbiota in the past, and how this diversity has been altered through time.

Ancient DNA analyses of the oral microbiota preserved in dental calculus (calcified dental plaque) offer a way to examine historical microbiota composition. Thus, microbiota alterations through time can be mapped, and, with use of detailed archaeological and historical records, the cultural and environmental factors that trigger change identified. Further, elucidating fine scale population structure may be possible due to the rapid response of microbiota to changing environments. Results from ancient DNA studies are critical in understanding historical microbiota composition and population substructure, examining how microbiota change and adapt through time, defining the health status of historical and modern populations, and indicating routes of investigation for medical manipulation of microbiota in disease prevention.

This thesis provides the most detailed analysis of historical microbiota to date, complemented with comprehensive metadata. Initially, I explored methods to minimize the impact of environmental contamination on analyses of ancient dental calculus collected from museums and archaeological sites. This allowed me to identify the optimum decontamination protocol and prepare over 250 British dental calculus samples from the Pre-Roman period to the Early Victorian period (~ 2,000 years). Utilizing high-throughput shotgun sequencing, I identified distinct, unique bacterial community

structures present throughout history that are driven by the diets of different socio-economic classes and are not evident in the modern oral cavity. I then focused on an ~800-year period of London history (1066 – 1853) and identified the first associations of microbiota and disease in an ancient population. Ultimately, these studies alter our understanding of the modern oral microbiota and help define and calibrate ancient microbiota analysis as a powerful new tool for studying human history.

Finally, I provided a framework for science communication within a research group that provides benefits and training to each member. I highlighted and demonstrated that science communication is a powerful tool for informing and engaging the public, and can provide direct research benefits. Moving forward, this framework should be utilized to disseminate research results to peers and the public with the aim to stimulate collaborations, and inform and engage public in new understandings of human biology, history, and medicine.

Publications

Journals

Weyrich *et al.* 2016 Reconstructing Neandertal behavior, diet, and disease using ancient DNA from dental calculus, *Nature*, 2016

- Included in this thesis (Appendix III)

Cooper, A.; Weyrich, L.S.; Farrer, A.G. 2015, The relevance of ancient DNA to contemporary disease, *Pathology*, 47, S28-S28

Weyrich, L.S.; Dixit, S.; Farrer, A.G.; Cooper, A.J.; Cooper, A.J. 2015, The skin microbiome: Associations between altered microbial communities and disease, *Australasian Journal of Dermatology*, 56, 4, 268-274

Conference Items

Farrer, A.G.; Dobney, K., Weyrich, L.S.; Cooper, A. 2016, "Unappreciated ecosystem diversity of oral microbiota detected in ancient Britain", 7th Annual International Symposium for Biomolecular Archaeology (ISBA⁷), Oxford, United Kingdom

Farrer, A.G.; Lekis, M.; Weyrich, L.S.; Cooper, A. 2016, "Training and Inspiring University Students: Incorporating Science Communication into Research Groups", Festival of Learning and Teaching, University of Adelaide, South Australia

Farrer, A.G.; Weyrich, L.S.; Cooper, A. 2015, "Ancient DNA studies provide a basis for medical research", Australian Society for Medical Research (ASMR) SA Scientific Meeting, South Australia

Grants and Funding

2015: \$1,250 – Royal Society of South Australia Small Research Grants Scheme: "The changing function of the microbiota throughout Medieval and Post-Medieval London, U.K."

2014: \$15,000 – L. F. and D. Denholm Scholarship, University of Adelaide

Community engagement

"Extracting DNA: separating the wheat from the chaff"

- Blog Post: Explaining the basis of DNA, *ACAD August 2016*

"ScienceAlive! Career Ambassador"

- Event: Speaking to high school students about science careers and university, National Science Week *August 2016*

"The life on us: Fossils of a microscopic world"

- Article: Outreach to high schools on why ancient dental calculus can be used to study impacts of changing culture, *eScience Magazine May 2016*

"LabARTory"

- Blog Post: Artistic elements of an ancient DNA lab, *ACAD December 2015*

"Thank you to our generous supporters"

- Video: Non-speaking role to thank University supporters, specifically scholarship donor Mrs Denholm *December 2015*

"Science says! - Adelaide"

- Event: Panel show organised by Science Nation, hosted by RiAus *November 2015*

"Revealing a skeleton's history by cleaning its teeth"

- Article: Letter to Mrs. Denholm in the Lumen (University of Adelaide Magazine) *Winter 2015*

"Andrew Farrer – Ancient DNA and the life on us"

- Interview: Discussing the 3 Minute Thesis competition and PhD research, Radio Adelaide *October 2015*

" Ancient DNA and the life on us"

- Talk: TransTasman 3 Minute Thesis: Final 10 of the Asia-Pacific competition, University of Queensland *October 2015*

"Ancient DNA and the life on us"

- Talk: Winner and People's Choice, University of Adelaide *September 2015*

"OAGR: a brand new genome database"

- Blog Post: Showcasing a new ancient DNA database, Co-author: Jimmy Breen, *ACAD August 2015*

"The Good, the Bad, and the Ugly: Bacteria in your Body"

- Children's University, extra-curricular science for primary school children,
Co-presenter: Laura Weyrich *July 2015*

"Cleaning the teeth of skeletons: Ancient DNA studies of the oral microbiota"

- Talk: ADOHTA Professional Development Evening *July 2015*

"Cleaning the teeth of skeletons: Ancient DNA studies of dental calculus"

- Talk: Community presentation, Boroughbridge, UK *March 2015*

"I hope you remembered to shower"

- Blog Post: Insight into sterile ancient DNA protocols, ACAD *February 2014*

Acknowledgements

This all began among the collections of the Natural History Museum, London, where I first met Alan Cooper and Laura Weyrich (and apparently didn't alarm them enough that they quietly disappeared back to Australia and stopped returning my emails). In the three years since that meeting I have amassed a long list of people who need thanking for their guidance and support. Firstly, to my supervisory team, Alan Cooper, Laura S. Weyrich, Neville Gully, and Keith Dobney, who provided stimulating discussion and challenges, listened to, supported, and helped develop my ideas, and have allowed me to develop professionally and personally. Particular thanks to Alan Cooper, who supported my application, pushed my scientific thinking, and supported my interest in science communication.

The Australian Centre for Ancient DNA has been an incredibly supportive and enjoyable place to work and learn. To all members who have shared time with me in the offices and labs, thank you for making the opposite side of the world feel like home. The dental calculus team, which has expanded dramatically since I started three years ago, it has been rewarding to work with you all, and to enjoy a few social events together as well.

Special thanks go to Laura Weyrich and Maria Lekis. Laura, you were a supervisor and a friend throughout. Seemingly endless patience and always able to restore my confidence, it would not have been the same without you. Maria, academia is a bubble world, and time spent with you has made me aware of the world outside the lab and the importance of working as a team to accomplish the most. I will keep "Finder, Minder, Grinder" in mind wherever I end up. To both of you, your friendship has been and is the highlight of Australia.

To friends and family. I've been terrible at keeping in touch but have enjoyed every catch-up and visit. Finally, to my girlfriend: Cadda you were unbelievably supportive for so long. You put up with my flaws and my dark moments, and were there to cheer me up. For this, I will be forever grateful.

Author's Note



“I would like to introduce you all to Georgina. Georgina, as I've called her, lived in Medieval London during some of the worst disease epidemics to ever strike the city, beginning with the Black Death in 1348. Sadly, she died before her 26th birthday, and was buried in an abbey cemetery close to the Tower of London.

But today, I have recruited her, and over 200 other men and women from the last one and a half thousand years of British history, for a medical study. A study to look at how their life experiences impacted the bacteria that lived on their bodies.”

I opened my 3-minute thesis presentation with these lines. They serve to impress upon the audience, and myself, that beyond the literature, protocols, contamination, bioinformatics, taxonomy, and functional pathways; that behind the samples, this is a human story.



Image credits:

Top – Museum of London, UK.

“Georgina”, who provided a dental calculus sample during my visit to the Museum, March 2015.

Bottom –University of Queensland, Australia.

Presenting my research at the TransTasman 3MT final, October 2015.