New approaches to explore the past and present diversity of Australian sandalwood species—from palaeobotany to next generation sequencing

Patricia Fuentes-Cross
(M.A. Ethnobotany)

A thesis submitted to the

School of Biological Sciences
The University of Adelaide
AUSTRALIA

In fulfilment of the requirements for the degree of Doctor of Philosophy

July, 2015

Dedication

To Hugh

Thank you for believing in me.

Maringka Baker "Kuru Ala" 2007. Synthetic polymer paint on canvas 153.5 x 200 cm
National Gallery of Australia, Canberra. Purchased 2007
© Maringka Baker, Tjungu Palya
Reproduced with permission.

"This is *Kuru Ala*. These are creeks and rocks holes everywhere, and many trees. There is *puli* (rocks) and *apu* (rocky hills). This is *Minyma Tjuta Tjukurrpa* (Seven Sisters Creation Story). This area is close to *Tjuntjuntjarra*" (In Western Australia, near the South Australian border) -Maringka Barker.

TABLE OF CONTENTS

ABSTRACT

The effects of climate change on plant natural resources is becoming an urgent problem that requires effective action to preserve what is left of remnant native and wild species. Native plant species can hold important genetic information that can be applied to sustainable management efforts. The main theme of this thesis concerns the genomics of the genus *Santalum*, also known as the sandalwoods, a highly exploited genus of trees, extending from Australasia into the Pacific. The sandalwoods are much sought after for their fragrant timber and oil, and have been wild harvested for centuries; to such an extent that one of the species is now extinct.

Using next generation sequencing techniques (NGS) with an Ion Torrent platform, this thesis involves an extensive genomic survey of the genus to realise several evolutionary questions. The central approach is to obtain genome-wide genetic markers using NGS, and then use these markers to first, infer the genetic diversity of populations of *Santalum acuminatum*, a sister species of *Santalum spicatum* or Australian sandalwood, across South Australia, Western Australia and New South Wales; second, to explore the feasibility of this approach in reconstructing phylogenetic relationships between species, some of which exhibit morphological phenotypes that make their identification difficult. Included in this work is an exploration of the utility of NGS platforms for ‘deep’ sequencing traditional markers (ccmp2, *trn*L, nrITS) and a consideration of their applications for various areas of research. To give a time perspective to the work, an isotope and macrofossil analysis of cave sediments, to infer past plant vegetation and climate scenarios, closes the data chapters.

Among the results inferred from markers obtained from the Ion Torrent are that the genetic diversity of *S. acuminatum* appeared to be low and a population structure analysis reveals a mixing of genotypes across the geographic range of the populations assessed. The phylogenomics of *Santalum* using NGS markers revealed a similar path of dispersals out of Australia and into the Pacific as reported in previous studies, but with fewer events than previously thought. It was also found that there appear to have been hybridisation between species in some populations of *Santalum* in the dry lands of South Australia. As well, it was found to be quite feasible to deep sequence traditional markers, such as chloroplast locus ccmp2, proving that they can be applied in new ways for research in metabarcoding of bulk samples. This novel application of traditional markers can then be applied in studies of soil sediments, as another proxy to sedimentary studies alongside isotope and macrofossil analyses.

Together, the set of studies presented in this thesis represent an interdisciplinary approach to a botanical study and begins to answer questions concerning the relationship between humans and plants. It is possible to integrate a battery of methods to study a highly exploited plant genus such as *Santalum* in its long historical trajectory with our species, and obtain results that can help in proposing potential solutions for its future sustainable management.

STATEMENT OF ORIGINALITY

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Patricia Fuentes-Cross
Ås, Norway
July, 2014

ACKNOWLEDGMENTS

Thank you to the University of Adelaide, School of Biological Sciences, the Australian Research Council (ARC), the Department of Environment, Water and Natural Resources (DEWNR) and the University of Adelaide Graduate Centre for their support with my PhD scholarship. I am thankful to the following funding agencies: the Nature Foundation of South Australia, Inc., the Sir Mark Mitchell Research Foundation and the Australian Flora Foundation for the research grants awarded to fund my field and laboratory work, and to the National Science Foundation (NSF), via the Open Science Network (PSN) in the USA, for their travel grants.

I am grateful to the staff of the State Herbarium of South Australia for their help with inter-institution voucher loans, with sample identification and for support with the production of my thesis. Also thank you to the staff of the following herbaria institutions: the National Herbarium of Victoria, the National Herbarium of New South Wales, the Western Australia Herbarium and the Bishop Museum Herbarium in Hawaii, USA. I would like to thank the staff of the Australian Genomic Resources Facility (AGRF) Adelaide node, for all their help with my sampling sequencing, and to the University of Adelaide (SARFMEE) School of Biological Sciences, for infrastructure support.

I am thankful to Hugh Cross, together we travelled the wonderful Australian landscapes in search of quandongs, sandalwoods and other Australian native plants, marvelling at the beauty and diversity of country—the bush. Hugh helped me all along with the laboratory work, trained me in laboratory techniques and always encouraged me to keep going to get results. His input with analysis and interpretation was crucial. The next generation sequencing was daunting at the beginning, but his passion for science, his ingenuity, innovation and unflappable optimism—can-do attitude—made it possible for me to begin to grasp and appreciate this world of genomics.

Thank you to my family for their support: Olivia, Tatiana, Everardo, Carlos and Fernando. To my parents (RIP) for instilling in us, their kids, the importance of education and love for science and academia.

I am very grateful to my academic advising panel. Circumstances facilitated an exposure to a diversity of expertise and this was, in the end, something positive: by harnessing the strengths that each of them would bring to my work. Thank you to Michelle Waycott, for her support during stressful and difficult times, for sharing knowledge and resources. Thank you to Liz Reed, always supporting anything Naracoorte related, and for being there during the ups and downs. To Mike Gardner and Maarten Ryder thank you for your support. As well, thank you to Ed Biffin and Kor-jent van Dijk, for taking time to give me a hand in the lab, and with analysis and drafting. To Andrew Lowe, thank you for helping with support for my scholarship.

I would like to acknowledge many other researchers, who supported this work in many ways: Mike Bunce from Curtin University and his lab group, James Haile, Daithi Murray, Nicole White and Silvana Tridico, and Margaret Byrne from DEC, for their help and for making my collecting of quandongs in Western Australia a fun and memorable experience. To Hope Jahren, Bill Hagopian and Caleb King, from the University of Hawaii, and to Brian Schubert from the University of Louisiana, for supporting the isotope work component and for their enthusiasm and friendship. I am especially thankful to Siri Fjellheim, for making me feel welcomed from day one at my new home in these northern lands.

I am super thankful to Jenny Templeton for her friendship and support, her kindness and positive attitude are some of the fondest memories I have from Adelaide. Thank you to Christina Adler, Janette Edson, Fran MacGillivray and Guido Valverde for their friendship. Across the research groups, thank you to Kym Abrams, Steve Richards, Kor-jent van Dijk, Ainsley Calladine, Wolfgang Haak, Bastien Llamas, Kyle Armstrong, Yuki Konichi, Emmanuelle Souzeu and Gary Templeton, for the words of support, the coffees, parties, camping and hiking, to help us all to come down to earth, to bond across research groups. To Amy Macken and Steve Bourne, for their friendship and for their help with excavation and macrofossil sampling. As well, thank you to Chris Brodie and Duncan Jardine for their help with field work.

Without the help of an amazing staff at university, I do not think I would have been able to get this work to completion: Thank you to Jenny Reiners and Sheryl Jurgens for your help in handing this thesis from afar. Thanks to Terri Finn, Jane Turner Goldsmith and Jane Copeland, who were so kind and supportive during the difficult time of my father's passing. Professor Rick Russell, thank you for coming to think out of the box and providing the opportunity to finish.

Thank you to David Symon (RIP), who had faith in and supported my project. His vibrancy and tremendous support in the field, at the age of 91 years young, was just amazing to me. The field trip to the Eyre Peninsula with him was the foundation of my *Santalum* project. I know that he would be very happy to know that we got results from those collections!

And finally, and with profound respect, thank you to Aboriginal Australians, to country, to the amazing Australian landscapes and to the quandongs and their relatives, who inspired much of this work and with whom I feel truly connected. You will always have a special place in my heart.

SCOPE AND CONTEXTUAL RATIONALE

The body of work presented in this thesis, has been conceptualised and arranged as a set of chapters written for publication, currently as drafts or ‘in preparation’. The overall theme of the thesis concerns native Australian flora and impacts of changes to their landscape mediated by our species. At core of the study is the pressing issue of conservation of native flora in the face of global warming created by human activities, presented here in case studies.

The chosen species for the main part of the study is *Santalum acuminatum*, or quandong. The rationale behind selecting this species lies in the fact that *Santalum* is a charismatic, but also highly exploited genus across its natural range. The *Santalum* genus has species that produce precious fragrant timber and oils. According to the current inventory on the IUCN Red List, one of its species is now extinct in South America and three of them, in the Pacific islands, are now listed as vulnerable or endangered. Species of *Santalum* in Australia have not been listed yet as under threat by the IUCN, but as the genus is economically relevant, it is important to continue to assess its conservation status. Illegal logging has also been on the increase in Australia due to higher sandalwood prices in the last couple of years.

The thesis includes four data chapters, two of them report on molecular work undertaken on the genus *Santalum*, using next generation sequencing (NGS) to identify single nucleotide polymorphisms (SNPs) that were then used to infer the genetic diversity of populations of quandong and construct phylogenetic trees. Next generation sequencing techniques were also applied to explore their potential for deep sequencing of traditional markers to reassess current markers and their potential for continued application on different platforms. The fourth core chapter reports on a fine-scale study on sedimentary deposits of the Naracoorte Cave system to infer native taxa on site in a time scale with plant macrofossils and isotope analysis of tree rings and sediments.