Population and sexual genetics of *Phytophthora cinnamomi* in Australia using microsatellite markers

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This thesis is presented for the degree of

Doctor of Philosophy

of Murdoch University

School of Biological Sciences and Biotechnology

Murdoch University,

Perth, Western Australia,

December, 1999

Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for degree at any tertiary education institution. Work that I did not perform is acknowledged (see p xvii).

Mark Paul Dobrowolski

Abstract

Phytophthora cinnamomi is a plant pathogen that causes dieback disease in southern Australia. It threatens the biodiversity of many natural ecosystems due to the susceptibility of the native vegetation. If methods of control are to be successful then we must appreciate the genetic variation in the pathogen and the ways in which this variation is generated. Previously, the only genetic markers available to study *P. cinnamomi* were isozymes, which showed that isolates in Australia were one of three isozyme types.

In this thesis I describe the development of microsatellite DNA markers for *P. cinnamomi*. Five microsatellites were successfully developed into markers for the nuclear genome and protocols for their use were established. Research into microsatellites for the mitochondrial genome is also presented though this was unsuccessful in providing markers useful for population genetic studies.

The developed microsatellite markers were used to study inheritance in sexual progeny of four *P. cinnamomi* crosses. All but one of 201 progeny germinated were outcrosses. A large amount of non-Mendelian inheritance of the microsatellite alleles was observed. This could be explained by a high frequency of imperfect meiosis (e.g., nondisjunction, unequal crossing over) leading to additions and deletions in the chromosome complement of the sexually derived progeny.

A population genetic study of three intensively sampled *P. cinnamomi* disease fronts located in southwest Australia is also presented. A total of 647 isolates were analysed from these hierarchically sampled sites with the microsatellite markers along

with 133 culture collection isolates from across Australia. This analysis revealed that *P. cinnamomi* in Australia consists of three clonal lineages, with no sexual reproduction evident, even though both mating types co-occur. However, within these clonal lineages I found evidence for frequent mitotic recombination (mitotic crossing over). This mechanism for producing genetic variation may explain phenotypic variation known to occur within the identified clonal lineages.

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Abbreviations

A	deoxyadenine nucleotide
ACT	Australian Capital Territory
ATP	adenosine triphosphate
bp	base pair
C	deoxycytidine nucleotide
CALM	Department of Conservation and Land Management (WA)
cDNA	complementary DNA
cm	centimetres
cM	centimorgans (genetic recombination)
cpDNA	chloroplast DNA
cpm	counts per minute (radioactivity)
CSIRO	Commonwealth Scientific and Industrial Research Organisation
dCTP	deoxycytidine triphosphate
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
EDTA	disodium ethylenediaminetetraacetate
g	acceleration due to gravity
G	deoxyguanine nucleotide
g	gram
h	hour
kbp	kilobase pair

km	kilometres
L	litre
m	metre
M	molar (mol L^{-1})
mg	milligram
min	minute
mJ	millijoule
mL	millilitre
mm	millimetre
mM	millimolar (mmol L^{-1})
mmol	millimoles
mol	moles
mtDNA	mitochondrial DNA
ng	nanogram
nmol	nanomoles
NSW	New South Wales
NT	Northern Territory
nuDNA	nuclear DNA
PCR	polymerase chain reaction
pmol	picomoles
PNG	Papua New Guinea
QLD	Queensland
rDNA	ribosomal DNA

RNase A	ribonuclease A
s	second
SA	South Australia
SDS	sodium dodecyl sulphate
SSC	standard saline citrate (NaCl-citrate)
T	deoxythymine nucleotide
TAS	Tasmania
T_m	melting temperature of a DNA duplex
Tris	Tris(hydroxymethyl)aminomethane
V	volt
v/v	volume per volume
VIC	Victoria
vol	volumes
w/v	weight per volume
WA	Western Australia
°C	degrees Celcius
µg	micrograms
µL	microlitres
µM	micromolar (µmol L^{-1})

Acknowledgements

My gratitude must first go to my supervisors, Dr. Phil O'Brien and Dr. Inez Tommerup, who through there support, enthusiasm and friendship, made my life as a PhD student very rewarding. Inez must be thanked especially for providing literature, and for her fruitful discussions on genetics of *P. cinnamomi* while assisting with their culturing.

I would like to thank the following people for their contributions:

Dr. Ken Old and Mark Dudzinski (CSIRO, Canberra) for supplying isolates and additional information;

Dr. Clive Brasier for supplying isolates;

Janine Catchpole (CSIRO, Perth) for producing sexual progeny and their mating type testing (in concert with Dr. I.C. Tommerup), making media, culturing isolates, and assistance with sampling and isolation of field isolates;

Dr. Bryan L. Shearer (CALM) for his collaboration in selecting and sampling *P. cinnamomi* disease fronts and supplying additional isolates;

Aaron Maxwell for his technical assistance with sampling, and isolation and culture of field isolates;

N. Pipe, A. Hardham and B. Grant for supplying *P. infestans* isolates;

B. F. Lang for permission to use the unpublished *P. infestans* complete mtDNA sequence <http://megasun.bch.umontreal.ca/People/lang/species/phyti/phyti.html> for primer design;

Judy Smith and Francis Brigg for processing my sequencing reactions.

I would like to thank Land and Water Resources Research and Development Corporation, Alcoa of Australia Ltd. and Murdoch University for their financial support for this project. I am also grateful for the Murdoch University Research Studentship I received.

For there personal support, camaraderie, and friendship I thank my fellow students and colleagues, Morag Glen, Letitia Quay, Nui Milton, Denis Erceg, Vidyani Manatunga, Jo Edmondston, Christa Nicholson, Audrey Ah Fong, Panomporn (Tik) Panutat, Gordon MacNish, Mohammed Zamani, Mark Farbey, and other members of the Biotechnology Programme and School of Biological Sciences and Biotechnology.

And finally for there love and patience, I thank my parents, Val and Czeslaw Dobrowolski, and a special thanks to Sue Richmond.