Population and sexual genetics of

*Phytophthora cinnamomi*

in Australia using microsatellite markers

Mark Paul Dobrowolski

BSc(Hons), The University of Western Australia

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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

<table>
<thead>
<tr>
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<th>Full Form</th>
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<tbody>
<tr>
<td>A</td>
<td>deoxyadenine nucleotide</td>
</tr>
<tr>
<td>ACT</td>
<td>Australian Capital Territory</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<tr>
<td>bp</td>
<td>base pair</td>
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<tr>
<td>C</td>
<td>deoxycytidine nucleotide</td>
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<td>CALM</td>
<td>Department of Conservation and Land Management (WA)</td>
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<tr>
<td>cDNA</td>
<td>complementary DNA</td>
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<tr>
<td>cm</td>
<td>centimetres</td>
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<tr>
<td>cM</td>
<td>centimorgans (genetic recombination)</td>
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<tr>
<td>cpDNA</td>
<td>chloroplast DNA</td>
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<tr>
<td>cpm</td>
<td>counts per minute (radioactivity)</td>
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<tr>
<td>CSIRO</td>
<td>Commonwealth Scientific and Industrial Research Organisation</td>
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<td>dCTP</td>
<td>deoxycytidine triphosphate</td>
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<td>ribosomal DNA</td>
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RNase A  ribonuclease A  
s  second  
SA  South Australia  
SDS  sodium dodecyl sulphate  
SSC  standard saline citrate (NaCl-citrate)  
T  deoxystyminucleotide  
TAS  Tasmania  
$T_m$  melting temperature of a DNA duplex  
Tris  Tris(hydroxymethyl)aminomethane  
V  volt  
$\nu/\nu$  volume per volume  
VIC  Victoria  
vol  volumes  
$w/\nu$  weight per volume  
WA  Western Australia  
$^{\circ}\text{C}$  degrees Celsius  
$\mu\text{g}$  micrograms  
$\mu\text{L}$  microlitres  
$\mu\text{M}$  micromolar ($\mu\text{mol L}^{-1}$)
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