

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Origins and Evolution of the New Zealand Forest Flora

a Molecular Phylogenetic Approach

A thesis presented in partial fulfilment of the
requirements for the degree of

Doctor of Philosophy
in
Plant Biology

at Massey University, Palmerston North,
New Zealand.

Karen Stöckler
2001

ABSTRACT

The origins and evolution of the New Zealand flora have puzzled the imagination of botanists world-wide. Competing hypotheses have sought to explain the floristic relationships between New Zealand and other Southern Hemisphere landmasses. Scientific approaches have involved geology, plant morphology, palynology and palaeobotany in investigations of the distribution patterns of these floras. Analyses presented in the current thesis use molecular data to investigate phylogenetic relationships of plant lineages native to the New Zealand forest flora.

In the present thesis, molecular work included amplification and sequencing of standard DNA markers such as nuclear ribosomal DNA, *ndhF* and *rbcL* gene sequence. These data were obtained for New Zealand and overseas species of Myrsinaceae, Nothofagaceae and genus *Agathis* (Araucariaceae). Analyses of these data have been presented alongside results and re-analyses of genetic data for Podocarpaceae, Proteaceae, Winteraceae and genus *Metrosideros* (Myrtaceae). These analyses aimed to synthesise recent work and provide a framework for further molecular investigations into the origins of the New Zealand woody forest flora.

Amplified fragment length polymorphism (AFLP) was used to locate polymorphic genome regions that were converted into sequence specific DNA markers. Information from AFLP and AFLP derived markers was used to elucidate evolutionary processes as well as interspecific and intraspecific relationships between closely related taxa of *Myrsine* and *Nothofagus*.

DNA analyses showed that the New Zealand forest hosts plants with very different origins and evolutionary histories. Results presented in the current thesis support hypotheses of vicariance and long-distance dispersal across Southern Hemisphere lands.

Molecular data are consistent with a continuous presence of *Agathis* (Araucariaceae), *Dacrydium* (Podocarpaceae) and *Pseudowintera* (Winteraceae) on the New Zealand archipelago since the break-up of the Gondwanan supercontinent. It is proposed that extant species of these lineages have evolved from ancestors that arrived on the New Zealand landmass during the Cretaceous. In contrast, divergence time estimates on *Nothofagus* suggest that relationships between extant *Fuscaspora* and *Lophozonia* beeches date back to the Mid Tertiary and are not explained by vicariance and continental drift.

Phylogenetic analyses substantiate fossil evidence of a Tertiary arrival of *Metrosideros* (Myrtaceae), *Myrsine* (Myrsinaceae), *Knightia* and *Toronia* (both Proteaceae). Similarly, dispersal from New Zealand to other southern lands has been inferred for *Metrosideros* and *Myrsine*. These findings and those reported earlier for

alpine plant groups suggest that trans-oceanic dispersal is likely to be an important explanation of floristic relationships between New Zealand and other distant landmasses.

Molecular studies on New Zealand *Myrsine* suggest recent speciation events, geographic differentiation and ongoing hybridisation between some morphologically and ecologically distinct species. Intraspecific analyses on *Myrsine divaricata* and *Nothofagus menziesii* show that extant distribution patterns within New Zealand are relatively recent and may have developed during the Quaternary. Although both lineages have an ancient history on the New Zealand archipelago, it is concluded that species and their distributions – including that of the monotypic genera *Elingamita* - are of recent origin.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Pete Lockhart, who gave me the chance to work in this exciting field of research: thank you for your scientific experience, for countless discussions and for your support. Thank you for your motivation and your friendship!

I would like to thank my co-supervisor Prof. Dr. Bruce Christie for his efforts in providing ideas and plant material for this study.

I would like to thank my co-supervisor Prof. Dr. Uwe Maier for his encouragement, for providing a lab space in Marburg and for never shutting his door on me.

I would like to thank Prof. Dr. David Penny and Dr. Mark Large for discussions and for their friendly encouragement. It was very much appreciated!

Special thanks go to Trish McLenachan for her help in countless occasions: thank you for your advise in the lab, for listening and talking, for proof-reading my thesis, and..., and..., and.....

Thanks to all the people in the DEB Lab and the Boffin Lounge: to Richard, Carmel, Leon, Dan, Matt, Ant, Abbey, Nicole....

I really appreciated the support of the Institute of molecular BioSciences that gave me the opportunity to attend an international conference and provided possibilities for scientific exchange.

I gratefully acknowledge the Deutsche Akademische Austauschdienst, the New Zealand Marsden Fund and Massey University, without whose financial support this thesis work would not have been possible.

Last but not least, I would like to thank my husband Dr. Volker Sieber: thank you for standing by me after my decision to go to New Zealand, thank you for listening and supporting my efforts. Thank you for your time, patience, and encouragement. Thank you for giving helpful advise while I was writing up - without you I wouldn't have completed this work.

ABBREVIATIONS

A	Adenine
AFLP	Amplified Fragment Length Polymorphism
bp	base pairs
C	Cytosine
DNA	Deoxyribonucleic acid
G	Guanine
Indel	Insertion or deletion
ITS	Internal Transcribed Spacer
ISSR	Inter Simple Sequence Repeats
ML	maximum likelihood
MP	maximum parsimony
m.y.	million years
m.y.a.	million years ago
PAA	polyacrylamide
PCR	Polymerase Chain Reaction
QP	quartet puzzling
rDNA	ribosomal DNA
T	Thymine
RAPD	Random Amplified Polymorphic DNA markers
RFLP	Restriction Fragment Length Polymorphism
SSR	Simple Sequence Repeats

TABLE OF CONTENTS

Chapter 1

Introduction	1
1.1 Plate Tectonics and the Geological History of the Southern Continents...	1
1.1.1 The Plate Tectonic Theory	1
1.1.2 The Geological History of Southern Hemisphere Lands.....	2
1.2 Biogeographic Relationships between Southern Hemisphere Lands.....	2
1.3 The History of the New Zealand Landmass.....	4
1.3.1 The Geological History of New Zealand	4
1.3.2 New Zealand Palaeoclimates.....	6
1.3.3 Effects of Pleistocene Glaciation on the Landscape	7
1.4 The Flora of New Zealand	8
1.4.1 Biogeographic Elements.....	9
1.4.2 Vegetation Types	10
1.4.3 History of the New Zealand Flora: Evidence from the Fossil Record.....	13
1.4.4 The Importance of Trans-Oceanic Dispersal.....	15
1.4.5 Effects of Past Environmental Changes on the New Zealand Flora	16
1.4.6 Distribution Patterns within the New Zealand Flora	17
1.4.6.1 Glacial Refugia: A Hypothesis on the Survival of Species	18
1.4.6.2 Tectonic Movements: A Hypothesis on the Disjunction of Populations	19
1.5 Outline of the Thesis	20

Chapter 2

Material and Methods	22
2.1 Material	22
2.1.1 Laboratory Equipment	22
2.1.2 Plastic and Glass Ware	22
2.1.3 Consumables	22
2.1.4 Chemicals and Reagents	22
2.1.5 Kits and Ready-to-Use Products.....	23
2.1.6 Enzymes.....	23
2.1.7 DNA markers.....	24
2.1.8 Software	24
2.1.9 Standard Molecular Markers Used for Phylogenetic Analyses	24
2.1.10 Oligonucleotides	24
2.1.10.1 Oligonucleotides Used for PCR and DNA Sequencing	24
2.1.10.2 Oligonucleotides Used for AFLP.....	26

2.3 Methods	26
2.3.1 DNA Extraction from Plant Material	26
2.3.2 Gel Electrophoresis and Visualisation of DNA	28
2.3.2.1 Agarose Gel Electrophoresis	28
2.3.2.2 Denaturing Polyacrylamide Gel Electrophoresis.....	29
2.3.3 Polymerase Chain Reaction (PCR)	32
2.3.3.1 Amplification of DNA Regions Using a One-Step PCR Protocol	32
2.3.3.2 'Nested' and 'Semi-Nested' PCR	33
2.3.4 Purification of PCR Products and Plasmid DNA	34
2.3.5 Cloning Procedures	34
2.3.5.1 Ligation of PCR Products into a Plasmid Vector.....	34
2.3.5.2 Transformation of Ca ²⁺ Competent Cells.....	35
2.3.5.3 Selection of Transformed Cells.....	35
2.3.5.5 Isolation of Plasmid DNA from <i>E. coli</i>	36
2.3.5.6 Analytical Restriction of Plasmid Vectors.....	36
2.3.5.7 Characterisation of Cloned Inserts by PCR	37
2.3.6 Amplified Fragment Length Polymorphism (AFLP).....	37
2.3.6.1 AFLP Step 1: Restriction of Genomic DNA.....	39
2.3.6.2 AFLP Step 2: Ligation of Adapter Sequences	39
2.3.6.3 AFLP Step 3: Preamplification	40
2.3.6.4 AFLP Step 4: Selective Amplification	40
2.3.7 Conversion of AFLP Bands into Sequence Specific DNA Markers.....	41
2.3.7.1 Isolation of DNA Fragments from Polyacrylamide Gels	41
2.3.7.2 Reamplification and Cloning of Excised AFLP Fragments.....	41
2.3.7.3 Primer Design	42
2.3.8 DNA Sequencing	42
2.3.9 Collection and Processing of Sequence Data	43
2.4 Phylogenetic Analyses of DNA Sequence.....	43
2.4.1 The Data Alignment.....	43
2.4.2 Pair Wise Distance Analyses	44
2.4.3 Models of Sequence Evolution.....	44
2.4.3.1 Objective Distance Models.....	45
2.4.3.2 Substitution Models and Likelihood Ratio Tests	47
2.4.3.3 A General Model for Additive Paths	47
2.4.4 Tree Building Methods.....	48
2.4.4.1 Maximum Likelihood and Maximum Parsimony.....	48
2.4.4.2 UPGMA and Neighbor Joining.....	49
2.4.4.3 Quartet Puzzling	50
2.4.4.4 Spectral Analysis and Split Decomposition.....	50
2.4.5 Measures for the Evaluation of Phylogenetic Trees:	51
2.4.5.1 Bootstrap and Jackknife Analyses.....	52
2.4.5.2 Quartet Puzzling Values.....	52
2.4.5.3 Global Fit Statistics on Graphs and Trees.....	53
2.4.5.4 Comparing Evolutionary Trees Using Global Scores	54

2.4.5.5 Kishino Hasegawa (KH) Test.....	53
2.4.5.6 Shimodaira Hasegawa (SH) Test.....	54
2.4.6 Molecular Clock Analysis.....	54
2.5 Approaches in Population Genetics.....	55
2.5.1 Alleles and Allele Frequencies.....	55
2.5.1.1 The Hardy-Weinberg Theorem.....	56
2.5.2 Wright's Statistics and Gene Flow.....	56
2.5.2.1 The Fixation Index.....	56
2.5.2.1 Genetic Exchange among Populations.....	57

Chapter 3

Origins and Evolution of the New Zealand Forest Flora.....	58
3.1 Introduction.....	58
3.1.1 Aims and Expectations.....	59
3.1.2 Data and Analyses.....	60
3.2 Araucariaceae Henkel & W. Hochst. 1865.....	61
3.2.1 Introduction.....	61
3.2.1.1 New Zealand Araucariaceae:.....	62
3.2.2 Phylogenetic Analyses of Araucariaceae <i>rbcL</i> - Sequence.....	63
3.2.2.1 Split Decomposition.....	63
3.2.2.2 Maximum Likelihood Analyses.....	65
3.2.2.3 Molecular Clock Tests and <i>rbcL</i> -substitution Rate Estimates.....	67
3.2.2.4 Estimating the Age of the <i>A. australis</i> Lineage.....	68
3.2.3 Discussion: <i>Agathis australis</i> - a Living Fossil?.....	69
3.3 Podocarpaceae Endlicher 1847.....	70
3.3.1 Introduction.....	70
3.3.1.1 New Zealand Podocarpaceae:.....	71
3.3.2 Phylogenetic Analyses of Podocarpaceae 18S Sequence.....	72
3.3.2.1 Split Decomposition:.....	72
3.3.2.2 Maximum Likelihood Analyses:.....	74
3.3.2.3 Molecular Clock Tests.....	76
3.3.3 Discussion: Are New Zealand Podocarps Gondwanan Relicts?.....	76
3.4 <i>Metrosideros</i> Banks ex Gaertner 1788 (<i>Myrtaceae</i> de Jussieu 1789).....	78
3.4.1 Introduction:.....	78
3.4.1.1 New Zealand <i>Metrosideros</i> :.....	79
3.4.2 Phylogenetic Analyses of <i>Metrosideros</i> nuclear rDNA Sequence.....	79
3.4.2.1 Split Decomposition.....	79
3.4.2.2 Maximum Likelihood Analyses.....	82
3.4.2.3 Molecular Clock Tests and Substitution Rate Estimates.....	84
3.4.3 Discussion: <i>Metrosideros</i> in New Zealand.....	84

3.5 Myrsinaceae R. Brown 1810.....	86
3.5.1 Introduction	86
3.5.1.1 New Zealand Myrsinaceae.....	86
3.5.2 Phylogenetic Analyses of <i>Myrsine</i> Nuclear rDNA Sequence	87
3.5.2.1 Split Decomposition.....	88
3.5.2.2 Maximum Likelihood Analyses.....	90
3.5.2.3 Molecular Clock Tests and Substitution Rate Estimates.....	92
3.5.3 Discussion: Ancient Origins and Recent Speciation?	93
3.6 Nothofagaceae Kuprianova 1962.....	95
3.6.1 Introduction	95
3.6.1.1 Hypotheses on the Phylogeography of <i>Nothofagus</i>	96
3.6.1.2 New Zealand Nothofagaceae	97
3.6.2 Phylogenetic analyses of <i>Nothofagus</i> species.....	98
3.6.2.1 Split Decomposition on Nuclear rDNA Sequence.....	98
3.6.2.2 Maximum Likelihood Analyses on Nuclear rDNA Sequence	99
3.6.2.3 Split Decomposition on Plastid Sequence Data.....	100
3.6.2.4 Maximum Likelihood Analyses on Plastid Sequence Data	101
3.6.2.5 Molecular Clock Tests and Substitution Rate Estimates.....	103
3.6.2.6 Dating the Divergence Time for New Zealand <i>Nothofagus</i> Species.....	104
3.6.3 Discussion: Is <i>Nothofagus</i> Dispersed or Vicariant?	105
3.7 Proteaceae Juss., nom. cons. 1789	107
3.7.1 Introduction	107
3.7.1.1 New Zealand Proteaceae.....	108
3.7.2 Phylogenetic Analyses of Proteaceae <i>atpB</i> Sequence.....	108
3.7.2.1 Split Decomposition.....	109
3.7.2.2 Maximum Likelihood Analyses.....	110
3.7.2.3 Molecular Clock Tests and Substitution Rate Estimates.....	112
3.7.3 Discussion: <i>AtpB</i> and the Trans-Tasman Connections of New Zealand's Proteaceae.....	112
3.8 Winteraceae Lindl., 1830	113
3.8.1 Introduction	113
3.8.1.1 New Zealand Winteraceae.....	114
3.8.2 Phylogenetic Analyses of Winteraceae Nuclear rDNA Sequence	114
3.8.2.1 Split Decomposition.....	114
3.8.2.2 Maximum Likelihood Analyses.....	115
3.8.2.3 Molecular Clock Tests and Substitution Rate Estimates.....	116
3.8.3 Discussion: Pseudowintera	117
3.9 Discussion.....	118
3.9.1 Data and Analyses.....	118
3.9.1.1 Sampling and General Sequence Substitution Properties	118
3.9.1.2 Rates of Sequence Evolution.....	120
3.9.2 Biogeographic History of New Zealand Forest Plants	121

Chapter 4

Molecular Markers for Studying Closely Related Taxa 124

4.1 Introduction 124

4.1.1 Amplified Fragment Length Polymorphism.....125

4.1.2 Aims and Expectations 127

4.2 AFLP Derived DNA Markers for *Myrsine* 127

4.2.1 Characterisation of Ma-af1130

4.2.2 Characterisation of Md-af9132

4.2.3 Genetic Distances between Taxa133

4.2.4 Split Decomposition and Spectral Analyses.....134

4.2.5 Molecular Clock Like Properties of the Data136

4.2.6 Summary: The AFLP Derived Markers Ma-af1 and Md-af9137

4.2.6.1 The Ma-af1 Region.....137

4.2.6.2 The Md-af9 Region.....138

4.3 AFLP Derived DNA Markers for *Nothofagus* 139

4.3.1 Characterisation of Nm-af2141

4.3.2 Genetic Distances between Taxa142

4.3.3 Split Decomposition and Spectral Analyses.....144

4.3.4 Molecular Clock-Like Properties of the Data.....145

4.3.5 Summary: the AFLP Derived Marker Nm-af2.....146

4.4 Discussion..... 147

4.4.1 How efficient is the Approach Used to Derive Sequence Specific Markers?..... 148

4.4.2 Are the New Molecular Markers Identical to the isolated AFLP Bands?..... 148

4.4.3 What Types of Markers Were Isolated from AFLP Profiles? 149

4.4.4 What is the Potential of AFLP Derived Markers?..... 149

Chapter 5

Phylogenetic Analyses on Closely Related Species of *Myrsine* with Particular Reference to *M. divaricata* 151

5.1 Introduction 151

5.1.1 *Myrsine divaricata* A. Cunn. 1839 151

5.1.2 *Myrsine argentea* Heenan and de Lange 1998..... 152

5.1.3 Aims and Expectations..... 152

5.2 Phylogenetic Analysis of Multiple Plant Loci..... 153

5.2.1 *NdhF* Gene Sequence..... 154

5.2.2 Nuclear rDNA Sequence 155

5.2.3 AFLP Derived Marker Ma-af1 157

5.2.4 AFLP Derived Marker Md-af9 158

5.3 Phylogeographic Analysis of <i>Myrsine divaricata</i> and <i>M. argentea</i>	159
5.3.1 Investigating Geographical Distribution of <i>M. divaricata</i> Genotypes	159
5.3.1.1 <i>NdhF</i> Sequence Type	159
5.3.1.2 Nuclear rDNA Sequence	161
5.3.1.3 AFLP: 'genetic fingerprints'	162
5.3.2 Investigating Interspecific Geneflow at Sympatric Sites	164
5.3.2.1 <i>NdhF</i> Sequence Type	164
5.3.2.2 Nuclear rDNA Sequence	165
5.3.2.3 AFLP Derived Marker Ma-af1	166
5.4 Discussion	167
5.4.1 Interspecific Relationships within genus <i>Myrsine</i>	167
5.4.2 Intraspecific Relationships for <i>M. divaricata</i> and <i>M. argentea</i>	168
5.4.3 Geographic Structure in Populations of <i>Myrsine divaricata</i>	169
5.4.4 Hybridisation	170
5.4.5 Evolution of Morphological Features	171

Chapter 6

Intraspecific DNA Studies on *Nothofagus menziesii* and *N. moorei* .. 173

6.1 Introduction	173
6.1.1 Southern Beech in New Zealand	173
6.1.1.1 <i>Nothofagus menziesii</i>	174
6.1.2 Southern Beech in Australia	175
6.1.2.1 <i>Nothofagus moorei</i>	176
6.1.3 Aims and Expectations	176
6.2. <i>Nothofagus menziesii</i>	177
6.2.1 Sequence Analyses of Nuclear DNA	177
6.2.2 Sequence Analyses of Plastid Sequence	179
6.2.3 Nm-af2 Allele Frequencies and Wright's Statistics	181
6.3. <i>Nothofagus moorei</i>	185
6.3.1 Sequence Analyses of Nuclear DNA	185
6.3.2 Sequence Analyses of Plastid Sequence	187
6.4 Discussion	187
6.4.1 How Much Geneflow Occurs between Disjunct Beech Populations?	188
6.4.1.1 <i>Nothofagus menziesii</i>	188
6.4.1.2 <i>Nothofagus moorei</i>	188
6.4.2 Inferring the Age of Beech Population Disjunctions	189
6.4.2.1 <i>Nothofagus menziesii</i>	189
6.4.2.2 <i>Nothofagus moorei</i>	190
6.4.2.3 Obtaining Further Information about the Nature of the Disjunctions	191

Chapter 7

General Discussion.....	192
7.1 Origins of the New Zealand forest flora.....	192
7.1.1 Gondwanan Origin of New Zealand Forest Plants.....	193
7.1.2 Dispersal Origin of New Zealand Forest Plants	195
7.1.2.1 Mechanisms of Long-Distance Dispersal.....	197
7.2 Overseas Relationships of the New Zealand Flora	198
7.2.1 The Trans-Tasman Connection	199
7.2.3 The Pacific Connection.....	199
7.2.4 Have Plants Dispersed From New Zealand?	200
7.3 What Events May Have Had An Influence on the Evolution and Biogeography of the New Zealand Forest flora?	201
7.3.1 The Cretaceous	201
7.3.2 The 'Oligocene Drowning'.....	202
7.3.3 The Pliocene Mountain Building.....	202
7.3.4 The Pleistocene Ice-Ages	203
7.4 Conclusions.....	204
7.5 Directions for Future Work	205
Bibliography	206
Appendix I: Geological Time Scale	221
Appendix II: Herbarium Voucher Numbers.....	222
Appendix III: Herbarium Voucher Numbers for <i>Nothofagus menziesii</i> Population Samples	226
Appendix IV: DNA sequence of AFLP Derived Markers	231
Appendix V: Nexus Files	232

LIST OF FIGURES

Figure 1.1: The changing outline of the New Zealand Archipelago during the Cenozoic.....	4
Figure 1.2: Model of the New Zealand South Island including the Alpine Fault sector.....	5
Figure 1.3: Distribution of glaciers and vegetation in New Zealand during the Otiran glaciation...	7
 Figure 2.1: Overview of different steps performed under the protocol for Amplified Fragment Length Polymorphism (AFLP).....	38
Figure 2.2: Interrelationship among five models for estimating the number of nucleotide substitutions among a pair of sequences.	46
Figure 2.3: Path length comparison for the relative rates test of Steel <i>et al.</i>	55
 Figure. 3.1: Splitsgraph for Araucariaceae.	64
Figure 3.2: Rooted phylogeny for Araucariaceae.	66
Figure 3.3: Scheme of divergence for Araucariaceae.	69
Figure 3.4: Splitsgraph for Podocarpaceae.....	74
Figure 3.5: Rooted phylogeny for Podocarpaceae.....	75
Figure 3.6: Splitsgraph for New Zealand and overseas species of <i>Metrosideros</i>	80
Figure 3.7: Splitsgraph for New Zealand <i>Metrosideros</i>	82
Figure 3.8: Unrooted quartet puzzling tree from <i>Metrosideros</i> nuclear rDNA.	83
Figure 3.9: Splitsgraph from nuclear ITS and 5.8S rDNA sequence data for Myrsinaceae.	89
Figure 3.10: Splitsgraph from nuclear ITS and 5.8S rDNA sequence data for Myrsinaceae without <i>M. salicina</i>	90
Figure 3.11: Rooted phylogeny for Myrsinaceae based on ITS 1, ITS 2 and 5.8S.....	91
Figure 3.12: Splitsgraph for <i>Nothofagus</i> from nuclear rDNA sequence	99
Figure 3.13: Rooted phylogeny for <i>Nothofagus</i> based on nuclear rDNA.	100
Figure 3.14: Splitsgraph for <i>Nothofagus</i> based on plastid sequence data.....	101
Figure 3.15: Rooted phylogeny for <i>Nothofagus</i> based on <i>tmL</i> intron and <i>tmL-tmF</i>	102
Figure 3.16: Scheme for the divergence between Australian and New Zealand <i>Lohpozon</i> ia and <i>Fusca</i> spora beeches.....	105
Figure 3.17: Splitsgraph for Proteaceae based on plastid coded <i>atpB</i> sequence data.	109
Figure 3.18: Rooted phylogeny for Proteaceae based on plastid coded <i>atpB</i> sequence data...	111
Figure 3.19: Splitsgraph for Winteraceae based on nuclear rDNA sequence data.....	115
Figure 3.20: Unrooted quartet puzzling tree for Winteraceae based on nuclear rDNA.	116

Figure 4.1: AFLP profile from <i>M. australis</i> and <i>M. divaricata</i>	128
Figure 4.2: AFLP profile from <i>M. divaricata</i>	129
Figure 4.3: Ma-af1 amplification products for some accessions of <i>Myrsine</i> sp.....	131
Figure 4.4: Repetitive DNA sequence motif of 14 bp length from <i>M.australis</i>	131
Figure 4.5: Correlation between molecular markers.....	134
Figure 4.6: Splitsgraphs for New Zealand species of <i>Myrsine</i> and <i>Elingamita</i>	135
Figure 4.7: Distance Hadamard spectra for New Zealand Myrsinaceae.....	136
Figure 4.8: AFLP profile for <i>Nothofagus menziesii</i>	139
Figure 4.9: Nm-af2 allele combinations found in <i>N. menziesii</i>	141
Figure 4.10: Repetitive sequence motifs from <i>N. menziesii</i>	142
Figure 4.11: Correlation between molecular markers Nm-af2 and nuclear rDNA.....	143
Figure 4.12: Splitsgraphs for <i>Nothofagus</i> subgenera <i>Lophozonia</i> and <i>Fuscaspora</i>	144
Figure 4.13: Distance Hadamard spectra for <i>Nothofagus</i>	145
 Figure 5.1: <i>Myrsine divaricata</i>	151
Figure 5.2: <i>ndhF</i> sequence types displayed by <i>Myrsine</i> sp. and <i>Rapanea</i> sp.....	154
Figure 5.3: Splitsgraph for <i>Myrsine</i> sp. and <i>Elingamita johnsonii</i> based on nuclear rDNA	156
Figure 5.4: Splitsgraph for <i>Myrsine</i> sp. and <i>Elingamita johnsonii</i> based on sequence of the AFLP derived marker Md-af1	157
Figure 5.5: Splitsgraph for <i>Myrsine</i> sp. and <i>Elingamita johnsonii</i> based on sequence of the AFLP derived marker Md-af9.	158
Figure 5.6: Geographical distribution of <i>ndhF</i> sequence types CCA and CAA in <i>Myrsine</i> <i>divaricata</i> and <i>M. argentea</i>	160
Figure 5.7: Splitsgraph for samples of <i>Myrsine divaricata</i> and <i>M. argentea</i>	161
Figure 5.8: AFLP Profiles for <i>Myrsine divaricata</i> and <i>M. argentea</i>	163
Figure 5.9: Splitsgraph for <i>Myrsine</i> sp. based on nuclear rDNA.....	166
Figure 5.10: Splitsgraph for <i>Myrsine australis</i> and <i>M. divaricata</i> based on sequence data of the AFLP derived marker Ma-af1	166
 Figure 6.1: Distribution of <i>Nothofagus menziesii</i> in New Zealand	174
Figure 6.2: Distribution of <i>Nothofagus moorei</i> in Australia.....	176
Figure 6.3: Splitsgraph for <i>N. menziesii</i> based on nuclear rDNA sequence.	178
Figure 6.4: Splitsgraph for <i>N. menziesii</i> population samples based on Nm-af2 sequence.	178

Figure 6.5: Splitsgraph for <i>N. menziesii</i> based on plastid sequence data.....	180
Figure 6.6: Distribution of <i>N. menziesii</i> plastid sequence types.....	180
Figure 6.7: Nm-af2 allele combinations found in <i>N. menziesii</i>	181
Figure 6.8. Nm-af2 allele frequencies for four <i>Nothofagus menziesii</i> provenances.....	184
Figure 6.9: Splitsgraph based on nuclear rDNA data of <i>N. moorei</i>	186
Figure 6.10: Splitsgraphs based on nuclear Nm-af2 data of <i>N. moorei</i>	187

LIST OF TABLES

Table 1.1: Overview over the geological history of New Zealand	6
Table 1.2: Examples from the pollen record of New Zealand plant families.....	14
Table 1.3: Suspected dispersal events for New Zealand plant groups.....	16
Table 2.1: Composition of two CTAB-based buffers used for DNA extraction.	26
Table 2.2: Thermocycling protocols for amplification of 6 gene regions from genomic DNA	33
Table 2.3: Thermocycling protocols for amplification of 3 gene regions from PCR products	34
Table 3.1: Details and results of substitution rate estimate for the <i>rbcL</i> gene region of Araucariaceae	68
Table 3.2: Hamming distances between species of <i>Metrosideros</i> subgenus <i>Metrosideros</i>	85
Table 3.3: Hamming distances between species of <i>Metrosideros</i> subgenus <i>Mearnsia</i>	85
Table 3.4: Details and results of substitution rate estimate for the nuclear ITS regions of New Zealand species of <i>Myrsine</i>	93
Table 3.5: Details and results of substitution rate estimate for the nuclear ITS regions of <i>Nothofagus</i>	103
Table 3.6: Details and results of substitution rate estimate for the <i>trnL</i> intron and <i>trnL-trnF</i> intergenic spacer regions of <i>Nothofagus</i>	104
Table 3.7: Divergence time estimates between Australian and New Zealand <i>Lophozonia</i> and <i>Fuscaspora</i> beeches derived from two molecular markers.	104
Table 3.8: Mean hamming distance between New Zealand and overseas species of <i>Nothofagus</i>	106
Table 3.9: Details and results of substitution rate estimate for the nuclear ITS regions of Winteraceae.	117
Table 3.10: Substitution rate for plastid regions for Araucariaceae and <i>Nothofagus</i>	120
Table 3.11: Substitution rates for nuclear ITS regions of three New Zealand plant groups.	121
Table 4.1: Relative success to isolate four markers for <i>Myrsine</i> from AFLP profiles	128
Table 4.2: Relative success to isolate seventeen markers for <i>Myrsine</i> from AFLP profiles.	129
Table 4.3: Potential new molecular markers for <i>Myrsine</i>	130
Table 4.4: Comparison of pairwise hamming distance for New Zealand species of <i>Myrsine</i>	133
Table 4.5: AFLP fragments extracted from <i>Nothofagus</i> AFLP profiles.....	139
Table 4.6: Potential new molecular markers for <i>Nothofagus</i>	140
Table 4.7: Sizes of three Nm.af2 alleles of <i>N. menziesii</i>	141

Table 4.8: Pairwise hamming distances for <i>Nothofagus</i>	143
Table 4.9: Substitution rate estimates for the new molecular marker Nm-af2 for <i>Nothofagus</i>	146
Table 4.10: Overview over total efforts for obtaining molecular markers for <i>Myrsine</i> and <i>Nothofagus</i>	148
Table 4.11: Possible uses for AFLP derived molecular markers.....	150
Table 5.1: Morphological characters to distinguish <i>M. argentea</i> and <i>M. divaricata</i>	152
Table 5.2: Species of Myrsinaceae used in Section 5.2.	153
Table 5.3: Species of Myrsinaceae used only in Section 5.2.1.	153
Table 5.4: Accession of <i>Myrsine</i> used in Section 5.3	164
Table 5.5: <i>ndhF</i> sequence types of samples taken from locations where two <i>Myrsine</i> species occur sympatrically.	165
Table 6.1: Variable sites from aligned rDNA sequence of <i>N. menziesii</i>	177
Table 6.2: Variable sites from aligned Nm-af2 sequence of <i>N. menziesii</i>	178
Table 6.3: Variable sites from <i>N. menziesii</i> <i>trnL</i> intron and <i>trnL-trnF</i> intergenic spacer..	179
Table 6.4: Nm-af2 alleles of <i>N. menziesii</i> samples in the central North Island.....	181
Table 6.5: Nm-af2 alleles of <i>N. menziesii</i> samples in the lower North Island.....	182
Table 6.6: Nm-af2 alleles of <i>N. menziesii</i> samples in the northern South Island	182
Table 6.7: Nm-af2 alleles of <i>N. menziesii</i> samples in the southern South Island.....	183
Table 6.8: Fixation index (F_{st}) and migration rate (Nm)	185
Table 6.9: Variable sites from aligned rDNA sequence of <i>N. moorei</i> population samples.....	186
Table 6.10: Variable sites from aligned Nm-af2 sequence of <i>N. moorei</i>	186