ALLOZYME AND OTHER ASPECTS OF VARIATION IN THE GENUS *BULBINELLA* IN NEW ZEALAND

By

Lesley Dawn Milicich

A thesis

submitted to the Victoria University of Wellington

in fulfilment of the

thesis requirements for the degree of

Doctor of Philosophy

VUW 1993

ABSTRACT

This thesis examines some aspects of morphological, cytogenetic and allozyme variation in the six species of the genus *Bulbinella* in New Zealand. Because evidence was found suggesting that fragmentation and reduction of the habitat of some species of the study genus had occurred, aspects of the conservation status of *Bulbinella* were also investigated.

Some of the morphological characters described and used by Moore (1964) to separate the species were employed in this study as well as other characters recorded by the author in actively growing plants. Generally, the seven taxa could be successfully distinguished using selected morphological characters, although in some species or populations a range of morphological forms was observed.

Increased human land use (mainly mining, farming and associated activities) has reduced some populations of *Bulbinella* to low numbers by destroying large areas of habitat. In some cases once vast areas of *Bulbinella* have been reduced to fragments or probably exterminated.

The karyotypes of five of the seven taxa were determined and these were all consistent with published data. G-banding was achieved in only one slide from one plant. A total of four bands (restricted to two pairs of chromosomes) was observed in the entire chromosome complement of 14. Each band was located on a separate chromosome.

Inflorescence material from 61 natural populations of *Bulbinella* in New Zealand was examined for enzyme activity using starch gel electrophoresis. Activity was detected for eight of a total of 43 enzyme stains. Three monomorphic and 11 polymorphic loci were resolved. While no completely fixed differences between all

the taxa could be demonstrated, four almost fixed differences were found. In some instances where populations belonging to different species were not geographically separated by great distances (<50km) shared alleles between species were demonstrated, indicating that introgression had occurred and may still be taking place.

Overall, the genetic distance (Nei 1978) within taxa was less than that between taxa. The dendrogram resulting from cluster analysis of Nei's unbiased genetic distances divided the genus into four groups, three of which corresponded to three currently recognised taxa. The other group contained the remaining four taxa. Although the component taxa of this cluster could be readily separated using morphological characters, they could not be distinguished using allozyme data.

The endemic distribution of *B. rossii* (Campbell Island and Auckland Island Group) and fixed morphological differences justify its remaining a separate taxon. The formal raising of *B. gibbsii* var. *gibbsii* to a separate specific status is subject to the analysis of further samples of this taxon. *B. angustifolia, B, talbotii,* and *B. gibbsii* var. *balanifera* also remain separate taxa, with *B. gibbsii* var. *balanifera* being raised to a separate specific status. *B. modesta*, which is genetically closely related to *B. hookeri*, becomes a sub-species of this taxon.

ACKNOWLEDGEMENTS

I sincerely thank both my supervisors for constructive criticism of the manuscript; Dr C.H. Daugherty for encouragement and assistance with the analysis and Dr G.K. Rickards for suggesting the study topic, collecting study material and refrigerating plants while I was away on field work.

I am grateful to the Department of Conservation (DoC) for issuing permits to collect study material in protected areas including national parks. I also thank the DoC and information centre staff with whom I made contact for their assistance and advice on *Bulbinella* distribution in their local areas.

I acknowledge the financial assistance given by the Internal Research Committee (IRC), Victoria University of Wellington (VUW), in providing research grants for the extensive field work.

I thank my husband Mr G. Milicich who helped to locate *Bulbinella* populations in the field and took some of the photographs.

The following people are thanked for their assistance in collecting study material: Mr D.M. Cunningham, Mr B.D. Lloyd and Dr P.J. Moore (DoC, Wellington); Dr D.R. McQueen, Dr B.V. Sneddon (also for information on population localities and other aspects of the study) and Mrs C. Thorn (VUW); Mr W. Sykes (DoC, Christchurch) and Ms J. Maxwell and Mr I. Flux (DoC, Te Anau).

I acknowledge the valuable assistance in attempting to locate populations of plants provided by the following members of the public, a number of whom are farmers and also gave me access to their land; Mrs L. Buick (Blue Mountain Station), Mr D. Cameron (Puhi Peaks Station), Mrs N. Christie (Atarau), Mr B. Dunnett (Kaikoura), Mr A. Erickson (Akaroa), Mrs K. Gorringe (Mangaweka), Mr P.

Green and staff (Mangaohane Station), Mr R. Guise (Lilburn valley), Mr P. Hocking (Kaikoura), Mr J. James (Stonehurst Station), Mr R. Johnson (Ashley Gorge), Mr C. and Mrs L. MacKay (Le Bons Bay Road), Mr A. Orbell (Clayton Station), Mr G. Patterson (Mount Nimrod Station), Mrs M. Parsons (Kaikoura district), Mr A. Templeton (Tuatapere), and Mrs E. Wilkie (Gladrook Station).

My thanks are expressed to all the following, and my apologies to anyone I have overlooked. Mr A.P. Druce (Wellington) advised on the localities of some *Bulbinella* populations. Dr G.C. Lindsay (MAFTechnology Horticultural Research Centre, Levin) helped me by suggesting laboratory techniques and drawing my attention to relevant references. Mrs B. Matthews (Waikanae) advised on the cultivation of *Bulbinella* and seed germination. Mr R. Mole (Otari) supplied seed, advised on cultivation techniques and suggested localities to search. Dr B. Molloy (DoC, Christchurch) sent me young seedlings and foliage samples from *Bulbinella* plants growing in his garden and advised on localities of populations. I sent many boxes of plants to Dr G. Ridley (formerly VUW) who placed them in the refrigerator along with other material which arrived while I was away doing field work. Dr G. Rogers (FRI, Rotorua) suggested localities to search for *Bulbinella* in the central North Island and suitable contacts. Mr W. Shaw (FRI, Rotorua) advised on exact locations of *Bulbinella* in the Urewera district. Mr R. Smith (formerly VUW) mentioned the population at Umukarikari Range.

At the beginning of the study I wrote to a large number of people for help and advice on various aspects of the topic. All these people are also thanked for their assistance.

I thank staff of the following herbaria [acronyms follow Wright (1984)] for their help and for allowing me to study *Bulbinella* specimens; Auckland Institute and

Museum Herbarium (AK), Forest Research Institute Herbarium (FRI), National Museum Herbarium (WELT) and Botany Division, D.S.I.R. Herbarium [now Manaaki-Whenua, Landcare Research New Zealand Ltd.] (CHR).

I am grateful to members of the technical staff, VUW, who have helped with various aspects of this study. These people are sincerely thanked. I especially thank Mrs C. Thorn who taught me the technique of starch gel electrophoresis and Ms A. Conwell who oversees the electrophoresis laboratory.

Last but not least I thank the many colleagues, not named individually, who during the course of this study have assisted me in some way with their helpful discussions and suggestions on a variety of topics connected with this thesis.

TABLE OF CONTENTS page

ABSTRACT ii
ACKNOWLEDGEMENTS iv
Table of contents vii
List of tables xii
List of figures xv

CHAPTER 1: INTRODUCTION 1
1.1 Goal of the study 3
1.2 Structure of the thesis 4

CHAPTER 2: BIOLOGY and NATURAL HISTORY of *BULBINELLA*
2.1 Introduction 5
2.2 Materials and methods
2.2.1 Distribution and habitat of *Bulbinella* in New Zealand 5
2.2.2 Other relevant information 7
2.2.3 Comparison with *Bulbinella* in South Africa 8
2.3 Results and discussion
2.3.1 Features of the genus *Bulbinella* in New Zealand
2.3.1.1 Distribution and habitat 9
2.3.1.2 Morphology 9
2.3.1.3 Pollination and seed dispersal 10
2.3.2 Important features of the individual species
2.3.2.1 *B. hookeri* 11
2.3.2.2 *B. gibbsii* 11
2.3.2.3 *B. angustifolia* 12
2.3.2.4 *B. modesta* 12
2.3.2.5 *B. talbotii* 13
2.3.2.6 *B. rossii* 14
2.3.3 Features of the South African genus *Bulbinella* 14

CHAPTER 3: ASPECTS OF CONSERVATION
3.1 Introduction 17
3.2 Materials and methods 19
3.3 Results and discussion
3.3.1 Location of study populations 21
3.3.2 The effects of habitat reduction on *Bulbinella* populations 22
3.3.2.1 *B. hookeri* 22
3.3.2.2 *B. gibbsii* var. *balanifera* 23
3.3.2.3 *B. modesta* 24
3.3.2.4 *B. talbotii* 26
3.3.2.5 *B. angustifolia* 26
3.3.3 General discussion and conclusions 28

CHAPTER 4: GROWTH AND MORPHOLOGY
4.1 Introduction 30
4.2 Materials and methods
4.2.1 Collection and storage of material 31
4.2.2 Plant cultivation 31

4.2.3 Seed germination 33
4.2.3.1 Germination regime 1986 A 34
4.2.3.2 Germination regime 1986 B 35
4.2.3.3 Germination regime 1987 36
4.2.3.4 Germination regime 1988 36
4.2.4 Recording of morphological characters 37
4.3 Results and discussion
4.3.1 Adult plants
4.3.1.1 Survival in cultivation 37
4.3.1.2 Recorded character states 38
4.3.2 Seedlings
4.3.2.1 Results of germination regime 1986 A 40
4.3.2.2 Results of germination regime 1986 B 41
4.3.2.3 Results of germination regimes 1987 and 1988 41
4.3.2.4 Conclusions 42
4.3.2.5 Morphological Characters of the Seedlings 42

CHAPTER 5: CYTOGENETICS
5.1 Introduction 44
5.2 Materials and methods
5.2.1 Materials 45
5.2.2 Preparation of material to study mitosis 45
5.2.2.1 Propionic orcein staining 45
5.2.2.2 Feulgen staining technique 46
5.2.2.3 Mounting 46
5.2.3 Chromosome banding techniques 47
5.2.3.1 Giemsa banding technique (Schweizer 1973) 47
5.2.3.2 Giemsa banding technique (Filion 1974) 48
5.2.4 Photography 49
5.3 Results and discussion
5.3.1 Dividing cells 49
5.3.2 Chromosome banding 51
5.3.3 Summary 52

CHAPTER 6: ALLOZYME TECHNIQUES
6.1 Introduction 53
6.1.1 Principle of electrophoresis 53
6.1.2 Limitations 54
6.2 Materials and methods
6.2.1 Materials for the pilot study using *Bulbinella* seed and tuber and tissues (leaf, root and stem) from related genera 55
6.2.1.1 Collection and treatment of *Bulbinella* tubers 56
6.2.1.2 Collection and treatment of *Bulbinella* seeds 56
6.2.1.3 Collection and treatment of other tissues from related genera 56
6.2.2 Materials for the secondary study using the full range of *Bulbinella* tissues 57
6.2.2.1 Collection and treatment of tissues from *Bulbinella* 57

6.2.3 The genetic basis of polymorphism 58
6.2.4 Nomenclature 59
6.2.5 Allozyme analysis
6.2.5.1 The preparation and assembly of gels 60
6.2.5.2 The staining of gel slices 61
6.2.5.3 Precautions taken during the laboratory procedures 62
6.2.5.4 Recording 63
6.2.5.5 Storage of gels 63
6.3 Results and discussion
6.3.1 Results of the pilot study using *Bulbinella* seed and tuber and tissues (leaf, root and stem) from related genera 64
6.3.1.1 Tubers 64
6.3.1.2 Seeds 64
6.3.1.3 Related Genera 65
6.3.2 Results of the secondary study using *Bulbinella* tissues 65
6.3.3 Conclusions pertaining to future analysis of *Bulbinella* tissue 66

CHAPTER 7: GEOGRAPHIC VARIATION in ALLOZYMES of *BULBINELLA* in NEW ZEALAND
7.1 Introduction 68
7.2 Materials and methods
7.2.1 Samples 69
7.2.1.1 Collection and treatment 70
7.2.1.2 Preparation for analysis 71
7.2.2 Allozyme analysis
7.2.2.1 Basic work pattern 71
7.2.2.2 Modifications made to technique 72
7.2.2.3 Recording of results 72
7.2.2.4 Quality control 73
7.2.3 Analysis: statistical methods 74
7.3 Results and discussion
7.3.1 Variation at individual loci 76
7.3.1.1 Adh-1 77
7.3.1.2 Gdh-1 77
7.3.1.3 G6pdh-1 77
7.3.1.4 Gpi-1 77
7.3.1.5 Gpi-2 77
7.3.1.6 Icd-1 78
7.3.1.7 Icd-2 78
7.3.1.8 Mdh-1, Mdh-2 and Mdh-3 78
7.3.1.9 6Pgd-1 79
7.3.1.10 6Pgd-2 79
7.3.1.11 Pgm-1 79
7.3.1.12 Pgm-2 79
7.3.2 Genetic variability 79
7.3.3 Conformity of data to Hardy-Weinberg proportions 81
7.3.4 Allelic frequencies 83
7.3.4.1 Adh-1 83

page

7.3.4.2 Gdh-1 83
7.3.4.3 G6pdh-1 84
7.3.4.4 Gpi-1 84
7.3.4.5 Icd-1 84
7.3.4.6 Icd-2 84
7.3.4.7 Mdh-1 85
7.3.4.8 Mdh-2 85
7.3.4.9 Mdh-3 85
7.3.4.10 6Pgd-1 86
7.3.4.11 6Pgd-2 86
7.3.4.12 Pgm-1 86
7.3.5 Patterns of geographic differentiation 87
7.3.6 Correlation of taxonomic level with genetic distance 90
7.3.6.1 Genetic distance distribution relating to taxonomic levels 92
7.3.6.2 Summary 93
7.3.7 Patterns of relationships 93

CHAPTER 8: APPLICATION TO TAXONOMY: CONCLUSIONS
8.1 Taxonomy
8.1.1 Introduction 96
8.1.2 Evaluation 99
8.1.2.1 Justification for taxonomic decisions 102
8.2 Phylogenetic analyses
8.2.1 Introduction 105
8.2.2 Materials and methods 106
8.2.3 Results and discussion 107
8.3 Implications for conservation
8.3.1 Introduction 108
8.3.2 Appropriate conservation measures 109
8.4 Conclusions 111

CHAPTER 9: SUMMARY 112

APPENDIX I **Extent of field work** 115

APPENDIX II **Plant deaths in cultivation** 121

APPENDIX III **Solutions for cytogenetic stains** 122

APPENDIX IV **Grinding buffers** 123

APPENDIX V **Recipes for the electrophoretic buffer systems** . . 124

APPENDIX VI **Detailed gel preparation technique** 125

APPENDIX VII **Stain recipes** 126

page

APPENDIX VIII **Photographs of starch gels** 129

REFERENCES 131

LIST OF TABLES

following page

Table 1.1 New Zealand *Bulbinella* taxa (Moore 1964) 1

Table 3.1 The 61 populations of *Bulbinella* found 21

Table 3.2 The relative difficulty involved in locating *Bulbinella* populations 22

Table 3.3 *B. hookeri* populations sought 22

Table 3.4 *B. gibbsii* var. *balanifera* populations sought 23

Table 3.5 *B. modesta* populations sought 24

Table 3.6 *B. talbotii* populations sought 24

Table 3.7 *B. angustifolia* populations sought 26

Table 4.1 Experimental germination regime 1986 B (1) 36

Table 4.2 Experimental germination regime 1986 B (2) 36

Table 4.3 Seeds planted outdoors and kept moist in 1987 36

Table 4.4 The 18 morphological characters and character states recorded in the study populations of *Bulbinella* 37

Table 4.5 The morphological characters of the species 38

Table 4.6 Results of the 1986 germination experiments A (1-6) 40

Table 4.7 Results of the 1987 seed germination experiments 41

Table 4.8 Results of the 1988 seed germination experiments 41

Table 6.1 Plant tissues examined for allozyme activity in the pilot study using seed and tuber of *Bulbinella* and tissues (leaf, root and stem) of related genera 56

Table 6.2 Plant tissues from *Bulbinella* examined for allozyme activity 57

Table 6.3 The electrophoretic buffer systems used in both the pilot and the secondary studies 60

Table 6.4 Enzyme Commission names and numbers of enzymes examined in the pilot and secondary studies 61

following page

Table 6.5 Results of the pilot study of enzyme activity in Liliaceae 64

Table 6.6 The allozyme activity and loci resolved in New Zealand *Bulbinella* 65

Table 6.7 Standard laboratory protocol for routine analysis of *Bulbinella* flower tissue 67

Table 7.1 Genetic variability at 14 loci in 61 natural populations of *Bulbinella* in New Zealand 69

Table 7.2 The number of alleles per locus following starch gel electrophoresis of *Bulbinella* flower extracts 76

Table 7.3 Mean genetic variability in *Bulbinella* in New Zealand 79

Table 7.4 The 14 populations tested for Hardy-Weinberg proportions 81

Table 7.5 Allelic frequencies in the 11 variable loci detected in 61 natural populations of *Bulbinella* in New Zealand 83

Table 7.6 The alleles for each variable locus which were present in each taxon 88

Table 7.7 The F-statistics (F_{IS}) within Moore's (1964) taxa and taxa identified by allozymes 89

Table 7.8 Pairwise F-statistics (F_{ST}) between *Bulbinella* taxa (Moore 1964) in New Zealand 89

Table 7.9 Pairwise F-statistics (F_{ST}) between taxonomic groupings inferred from allozyme data 89

Table 7.10 Mean unbiased genetic distances (Nei 1978) between and within *Bulbinella* taxa in New Zealand 90

Table 7.11 The distribution of D values within individual species 92

Table 7.12 The distrubution of D values between pairs of species 93

Table 7.13 The distribution of D values over all the taxonomic levels studied 93

Table 8.1 The data sets used to construct phylogenies using PAUP 106

following page

Table 8.2 Summary of the phylogeny results 106

Table 8.3 General phylogeographic patterns discussed by Avise et al. (1987) and applied to *Bulbinella* 109

LIST OF FIGURES

		following page
Figure 2.1	N.Z. map showing distribution of taxa	9
Figure 2.2	*Bulbinella* root system	10
Figure 2.3	*B. hookeri* (Cobb Valley)	11
Figure 2.4	*B. hookeri* (Waipakihi River)	11
Figure 2.5	*B. gibbsii* var. *balanifera* (Lake Marian)	11
Figure 2.6	*B. gibbsii* var. *balanifera* (Lake Marian)	11
Figure 2.7	*B. angustifolia* (Macraes Flat)	12
Figure 2.8	*B. angustifolia* (Clayton Wetlands)	12
Figure 2.9	*B. modesta* (Okarito Swamp)	12
Figure 2.10	*B. modesta* (Lake Ellery Track)	12
Figure 2.11	*B. talbotii* (Gouland Downs) in flower	13
Figure 2.12	*B. talbotii* (Gouland Downs) in fruit	13
Figure 2.13	*B. rossii* (Plants from Campbell Island growing in cultivation)	14
Figure 2.14	as figure 2.13	14
Figure 3.1	The locations of the 61 populations of *Bulbinella* found	21
Figure 3.2	The locations of the 19 previously recorded populations of *Bulbinella* sought but not found	22
Figure 4.1	The south garden (from east gate)	32
Figure 4.2	The south garden (from west gate)	32
Figure 4.3	The north garden	32
Figure 5.1	*B. hookeri*, Jacks Pass (2n = 14)	49
Figure 5.2	*B. gibbsii* var. *balanifera*, Murchison mountains (2n = 14)	49
Figure 5.3	*B. rossii*, female plant from Campbell Island (2n = 14 + 1'B')	49

following page

Figure 5.4 *B. modesta*, Atarau (2n = 14) 49

Figure 5.5 *B. angustifolia*, Blackmount (2n = 14) 49

Figure 5.6 *B. angustifolia*, Porters Pass (mitotic metaphase) 51

Figure 5.7 *B. angustifolia*, Porters Pass (interphase cell) 51

Figure 6.1 Loaded gel on ice 61

Figure 6.2 Loaded gel ready to begin run 61

Figure 6.3 Gel running 61

Figure 7.1 Sample preparation on ice 71

Figure 7.2 Zymogram interpretation of ADH 77

Figure 7.3 Zymogram interpretation of GDH 77

Figure 7.4 Zymogram interpretation of G6PDH 77

Figure 7.5 Zymogram interpretation of GPI 77

Figure 7.6 Zymogram interpretation of ICD 77

Figure 7.7 Zymogram interpretation of MDH 78

Figure 7.8 Zymogram interpretation of 6PGD 78

Figure 7.9 Zymogram interpretation of PGM 78

Figure 7.10 The relationship between variability and sample size 81

Figure 7.11 The frequencies of the alleles in the locus Adh-1 83

Figure 7.12 The frequencies of the alleles in the locus Gdh-1 83

Figure 7.13 The frequencies of the alleles in the locus Gpi-1 84

Figure 7.14 The frequencies of the alleles in the locus Icd-1 84

Figure 7.15 The frequencies of the alleles in the locus Icd-2 84

Figure 7.16 The frequencies of the alleles in the locus Mdh-1 85

following page

Figure 7.17 The frequencies of the alleles in the locus Mdh-2 85

Figure 7.18 The frequencies of the alleles in the locus Mdh-3 85

Figure 7.19 The frequencies of the alleles in the locus 6Pgd-1 86

Figure 7.20 The frequencies of the alleles in the locus 6Pgd-2 86

Figure 7.21 The frequencies of the alleles in the locus Pgm-1 86

Figure 7.22 Nei's D means and ranges within individual species 90

Figure 7.23 Nei's D means and ranges between species, individual pairings 91

Figure 7.24 Nei's D: means and ranges within species and between congeneric species 91

Figure 7.25 Distribution of Nei's D values within individual species 92

Figure 7.26 Distribution of Nei's D values between pairs of species 93

Figure 7.27 Distribution of Nei's D values relating to taxonomic levels 93

Figure 7.28 Dendrogram resulting from cluster analysis using Nei's unbiased D 93

Figure 8.1 One of the seven phylogenetic trees constructed using allozyme data alone 107

Figure 8.2 The phylogenetic tree constructed using morphological data alone 107

Figure 8.3 The phylogenetic tree constructed using both allozyme and morphological data 107

Photographs in appendix VIII

VIII (a) ADH on RW gel 129

VIII (b) GDH on RW gel 129

VIII (c) GPI on RW gel 129

VIII (d) ICD on AC gel 129

VIII (e) MDH on TC_2 gel 129

following page

VIII (f) MDH on TC_3 gel 129

VIII (g) 6PGD on TC_2 gel 129

VIII (h) PGM on AC gel 129

CHAPTER 1: INTRODUCTION

Bulbinella, a member of the family Liliaceae, is a genus of perennial herbs producing leaf rosettes and flowers during summer, and remaining dormant below the ground surface over winter. In New Zealand the genus has been divided into six species [Moore (1964), table 1.1], all of which have yellow flowers and swollen fleshy roots.

The genus *Bulbinella* has been recorded only in New Zealand and South Africa. In New Zealand the genus is virtually confined to sub-alpine boggy areas, where it exists in isolated populations.

The generic name "*Bulbinella*" was established by Kunth (1843 p. 569, cited by Moore 1964) when he placed six species from the Cape of Good Hope in this new genus. Cheeseman (1906) first described the two (then recognised) New Zealand species under the generic name "*Bulbinella*". Prior to this publication these two plant species had been variously referred to under the generic names *Chrysobactron* (Hooker 1845, cited by Moore 1964) or *Anthericum* (Buchanan 1868). *Bulbinella, Chrysobactron* and *Anthericum* were considered closely related by workers involved in reviewing their taxonomy (for example Baker [1876], and Cockayne [1909] both cited by Moore [1964]; and Cockayne and Laing [1910]). The three genera were separated by morphological characters such as the possession of a deciduous versus a persistent perianth and the number of ovules or bearded filaments present in the flower. In the latest taxonomic review of the New Zealand genus (Moore 1964) six species were described, one of which contained two varieties. Morphological features were used to separate these seven taxa.

Table 1.1. New Zealand *Bulbinella* taxa (Moore 1964).

Species	Variety
hookeri	-
gibbsii	*gibbsii*
gibbsii	*balanifera*
rossii	-
modesta	-
talbotii	-
angustifolia	-

Webb et al. (1990) adopted the systematic treatment of *Bulbinella* described by Dahlgren et al. (1985) who placed the genus *Bulbinella* in the family Asphodelaceae; subfamily Asphodeloideae.

Common names for the genus *Bulbinella* in New Zealand include "Maori onion", "bog lily" and "golden star lily" (Moore and Edgar 1970), with Maori onion being used most often. Goulding (1971) presented evidence that *Bulbinella* leaves were made into plaited baskets and floor mats by the Maori. Both Fisher et al. (1970) and Skinner (1981) stated that *Bulbinella* roots were edible but neither publication referred to their being eaten by either the Maori or the early Europeans. Fuller (1978) stated that the Maori onion (*B. rossii*) was eaten by the Maori but gave no references. However it was considered unlikely that *Bulbinella* was used as a food source by the Maori because it tasted neither sweet nor mealy (pers. comm. Dr H. Leach, Department of Anthropology, University of Otago).

Although I found no references which suggested that *Bulbinella* was rare or endangered, my field observations indicated that land use has reduced some populations to low levels and has probably exterminated others. Because the status of some species has changed since the study by Moore (1964), a conservation aspect for the study was decided upon. Such extensive habitat destruction would have reduced not only *Bulbinella* numbers but those of other species as well.

The use of new methods such as allozyme analysis for examining taxonomic relationships has increased markedly over the past 20 years. This technique, which was initially widely applied to animal systematics (Avise 1974), is now also commonly used to help resolve problems in plant taxonomy. For example allozyme analysis was used by Brunsfeld et al. (1991) to help understand the patterns of evolution and the nature of the taxonomic difficulty associated with *Salix* section

Longifoliae in North America. Allozyme analysis has been used to demonstrate the effect of founder events (bottlenecks) on the genetic variability of plant populations (Schwaegerle and Schaal 1979). Morikawa and Leggett (1990) used allozyme analysis to quantify the range of available genetic diversity within and between wild populations of *Avena canariensis* (oat). The results provided a better basis for the sampling and use of wild germplasm for possible transference of advantageous characters to cultivated crops. However, biochemical studies using allozyme analysis are rare on New Zealand plants. A recent example, a study by Hawkins and Sweet (1989), examined genetic variation in Rimu (*Dacrydium cupressinum).* They attributed the low genetic variability found within Rimu populations to an evolutionary bottleneck. The present study employs allozyme analysis to test the existing taxonomy of *Bulbinella.*

Unless otherwise stated the species definition used in this thesis is that given by Conquist (1978), cited by Grant (1981).

'Species are the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means.'

'Consistently distinct' means that the vast majority of the individuals under consideration clearly belong to one group or another, not somewhere in between. 'By ordinary means' indicates a traditional approach, for example the use of a compound microscope to distinguish species of fungi, and a dissecting microscope or hand lens to distinguish species of higher plants.

1.1 Goal of the study

The overall goal of this study was a systematic review, to which end the aims were:

1. To assess the habitat stability of the genus by documenting reduction, fragmentation and probable extinction of previously recorded populations.

2. To determine whether the reported morphological characters of the species were present in all populations studied and to find whether these features were stable in a different habitat by performing transplant experiments.

3. To confirm the reported karyotypes of the seven taxa.

4. To compile a set of allozyme data from as many populations as possible and including at least one population of each taxon.

Aims 2 to 4 are aspects of testing existing taxonomy. Aim 4 uses a modern technique.

1.2 Structure of the thesis

Natural history and biological aspects of the genus essential for the fulfilment of the aims are outlined in Chapter 2. Aspects of conservation are discussed in detail in Chapter 3. Morphological characters typical of each species, from both literature and personal observation, are recorded in Chapter 4. The cytogenetic aspect is presented in Chapter 5. The allozyme analyses are discussed in Chapters 6 (techniques) and 7 (geographic variation). Overall conclusions concerning taxonomy and conservation, including a phylogeny are given in Chapter 8. The results obtained in this study are summarized in Chapter 9.

CHAPTER 2: BIOLOGY and NATURAL HISTORY of *BULBINELLA*

2.1 Introduction

Before attempting to fulfil the aims outlined in Chapter 1 it was necessary to become familiar with relevant aspects of *Bulbinella* biology and natural history. Distribution of the species in New Zealand, especially past records of populations, habitats, reproduction, pollination mechanisms and general appearance of plants including obvious morphological differences between species were considered important. A comparison between the genus *Bulbinella* in New Zealand and representatives of the genus in South Africa was also considered appropriate.

2.2 Materials and methods

2.2.1 Distribution and habitat of *Bulbinella* in New Zealand

I obtained the distributions of the individual species initially from Moore (1964). The maps included in her paper were particularly useful in demonstrating the overall distribution pattern, especially the disjunct distributions shown by *B. hookeri* and *B. gibbsii* var. *balanifera*. I studied several hundred herbarium specimens including some from each of the following herbaria: Auckland Institute and Museum Herbarium (AK), Forest Research Institute Herbarium (FRI), the H.D. Gordon Herbarium (WELTU), National Museum Herbarium (WELT) and Botany Division, D.S.I.R. Herbarium [now Manaaki-Whenua, Landcare Research New Zealand Ltd.] (CHR). As well as plant habitat and general appearance, herbarium specimens provided information such as exact locations of plants, abundance at those

locations, accompanying vegetation, dates observed and reproductive status of the population, that is flowering and fruiting times. Personal communications were also valuable in locating possible study populations. A written record was kept of all herbarium specimens examined, and information obtained through personal communications was also recorded in writing. Additional literature was consulted for sitings of the lesser known species such as *B. modesta* (Robins 1968).

The information on habitat, relative abundance and accompanying vegetation recorded from the herbarium specimens was valuable in identifying areas where *Bulbinella* populations might be found. Abundance indicated the probable ease of finding plants at previously recorded sites. In the **initial field work** attempts were made

(1) to study some material from each of the seven documented taxa and

(2) to cover as much of New Zealand as possible geographically.

Thus optimum use was made of the collecting time and funding available. This approach meant not searching localities which were several days away on foot, unless this was considered absolutely essential. An exception was the Gouland Downs, the only recently recorded location of *B. talbotii*. Localities were considered suitable to search on the initial field work if the following criteria were satisfied.

(a) The abundance of *Bulbinella* plants was recorded as abundant or frequent, as opposed to rare or occasional.

(b) More than one herbarium specimen had been collected from the same locality including at least one specimen obtained after 1965. Localities represented only by older collections or by a single specimen were not searched initially.

(c) Recent personal sitings of populations by colleagues were considered very useful, particularly if their information was supported by herbarium records.

On subsequent field work the resulting geographic gaps were noted and appropriate locatities were searched. This was accomplished by

(i) looking in localities not searched during the initial field work,

(ii) writing to contacts for detailed information on the location of *Bulbinella* populations where difficulty had previously been encountered in finding plants,

(iii) asking people the exact whereabouts of *Bulbinella* in their local area and

(iv) sometimes searching previously unrecorded sites where typical accompanying vegetation and suitable habitat were present.

Where personal observations of plants in their natural habitat could not be made by the author, live material consisting of entire plants collected by colleagues was studied [Tin Range, Stewart Island, *B. gibbsii* var. *gibbsii* (Mr B.D. Lloyd) and Campbell Island, *B. rossii* (Mr D.M. Cunningham)]. These plants were grown in cultivation in Wellington.

2.2.2 Other relevant information

Moore (1964) gave a detailed morphological description of the genus as a whole and of each individual species. This information was supplemented by the study of photographs (Philipson and Hearn 1962, Salmon 1985 and Salmon 1986), drawings (Moore and Irwin 1978, Mark and Adams 1979 and Wilson 1982) and herbarium specimens to establish the general appearance of the plants. Obvious differences between species were noted, especially features which were likely to cause difficulty finding populations in the field. Species having a prostrate growth habit or small, inconspicuous inflorescences were noted. A detailed account of morphological differences between species is presented later (Chapter 4).

Information on pollination and reproduction of *Bulbinella* obtained from Moore (1964) was supplemented by personal observations of living plants in the field.

The mechanism of seed dispersal was determined from a study of seed morphology, and field observations of plants having ripe capsules. The general conclusions made were confirmed by Webb et al. (1990).

2.2.3 Comparison with *Bulbinella* in South Africa

A letter to the director of the National Botanic Gardens in Pretoria, South Africa, resulted in personal contact being established with Ms P.L. Perry who was reviewing the taxonomy of the genus in South Africa. A number of dormant plants and packets of seed were sent to New Zealand by Ms Perry. As some South African *Bulbinella* species can be grown successfully in New Zealand conditions (Harrison 1977, Matthews and Matthews 1968 and pers. comm. Mrs M. MacKenzie, Lower Hutt), I decided that it was worth attempting to grow these plants. Three other South African plants were purchased in September 1986 from a local gardening centre. All adult plants were placed in individual pots in a peat/sand mixture (Chapter 4) and some seeds from each packet sent by Ms Perry were sown in small pots of seed soil mixture (Chapter 4). The exchange of letters and a personal visit to New Zealand by Ms Perry in November 1988 were most useful in learning important features of the South African plants.

2.3 Results and discussion

2.3.1 Features of the genus *Bulbinella* in New Zealand

2.3.1.1 Distribution and habitat

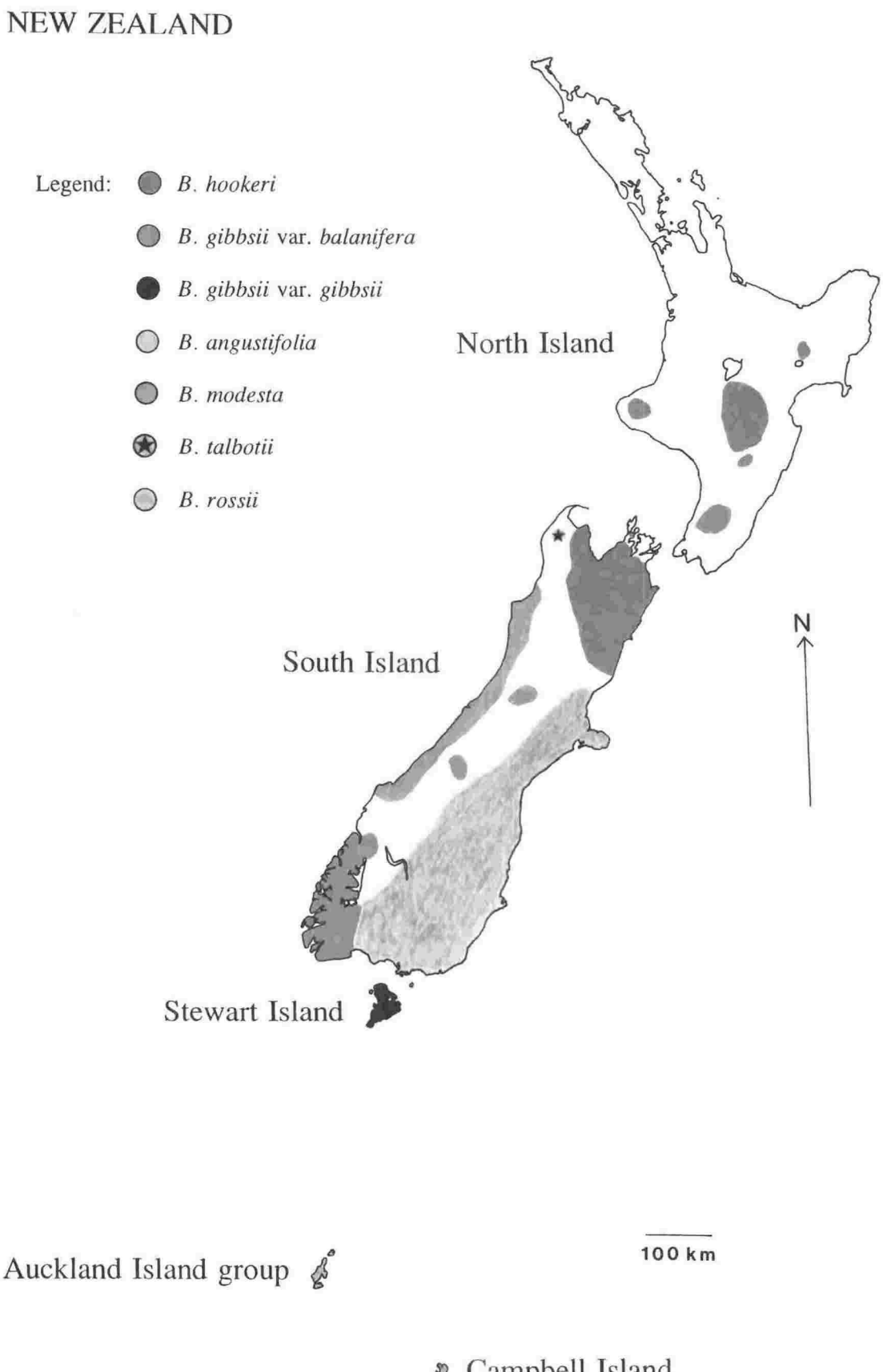
The six species of *Bulbinella* are entirely allopatric in New Zealand (fig. 2.1). Detailed ranges for each species are presented under their specific names.

A common feature of the habitats of all six species was their high water content. Examples of suitable habitats included permanent bogs, the banks of streams or rivers and seepage sites in wet grassland usually on shaded slopes.

2.3.1.2 Morphology

Plants of all six species have the following basic appearance: The crown of each plant produces a rosette of up to 12 strap-shaped leaves, which show prominent longitudinal ribbing in some species. The stem, which is erect with leaf insertions crowded over a short length, continues as the fleshy, leafless peduncle, varying in height. The peduncle bears the flowers, which are yellow in all New Zealand species. Flowers have a star-like appearance with two whorls each of three periath segments (tepals) and two whorls each of three anthers. Flowers are borne on flexible pedicels, subtended by small, leaf-like bracts (Moore 1964). The number of individual flowers contained in one inflorescence varies from 10 or less to more than 100 depending on the species. While in some species the peduncle elongates considerably as the flowers open, raising the inflorescence well above the rosette of leaves, in other species it remains short making the inflorescence barely visible unless looking vertically down on the plant. Ovaries are green in flowers and young ripening capsules, changing through amber to brown and drying prior to dehiscence.

Figure 2.1. The geographic areas where the six taxa of *Bulbinella* have been reported in New Zealand.

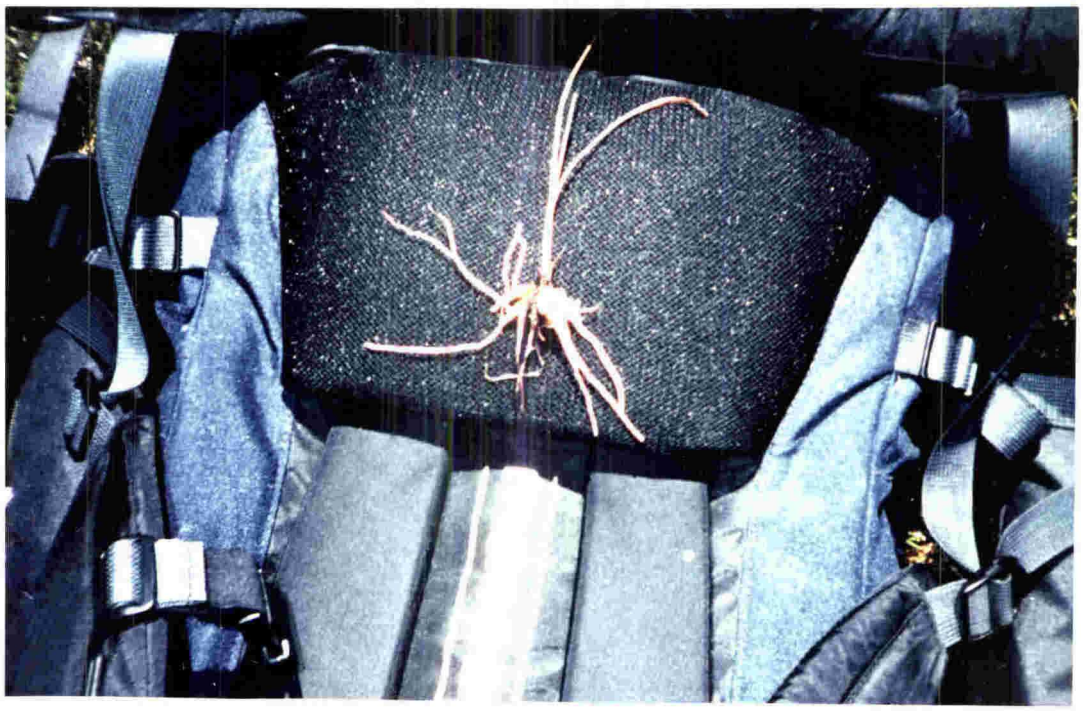


One capsule may contain up to six seeds, which are triangular in cross section. The new roots which are produced each year function as storage organs and swell to a long fusiform shape (fig. 2.2). These tubers are tough, similar to those of commercial ginger, and appear to resist rotting or fungal attack. Shrunken roots of previous seasons may be retained by their remaining attached to the plant.

2.3.1.3 Pollination and seed dispersal

The flowers of all *Bulbinella* species are brightly-coloured (yellow) and produce a faint scent. None have the feathery anthers typical of wind-pollinated species. Representatives of several different insect families were observed on flowers of some populations of *B. hookeri, B. gibbsii, B. angustifolia* and *B. modesta* in the wild. The insects observed included honey bees, flies and bugs, suggesting that insects are likely to be involved in *Bulbinella* pollen transport. It was therefore considered likely that in nature wind-pollination occurred only if pollination by insects (the primary method) did not take place.

Although Moore (1964) described the seeds of some species as "winged", these seeds were still too heavy to be carried far by wind. Personal observations indicated that seed dispersal was achieved by wind shaking seeds from their capsules on to the ground in the vicinity of the parent plant. Webb et al. (1990) suggested that seed dispersal was accomplished by wind. Because seeds were usually dispersed into the immediate vicinity of the parent, it was considered likely that pollen was often transferred between related individuals.



2.3.2 Important features of the individual species

2.3.2.1 *B. hookeri* (figures 2.3 and 2.4)

This species is found in the North Island: Urewera Country, Mount Egmont, parts of the Volcanic Plateau and the Ruahine Range; and the South Island: north of Waiau, North Canterbury, Marlborough and Nelson, in wet tussock grassland down to 150m. *B. hookeri* is hermaphroditic. Generally flowering occurs between November and January (Moore and Edgar 1970). Field observations indicated that most populations flower during December and January. Racemes of flowers which are easily visible above the erect leaves, usually contain more than 50 flowers. Some populations, notably those of Mount Stokes (Marlborough Sounds) and the Cobb Valley (North-West Nelson), have a blue-green colouration (glaucous sheen) to their leaves and peduncles.

2.3.2.2 *B. gibbsii* (figures 2.5 and 2.6)

B. gibbsii var. *balanifera* shows a widely disjunct distribution pattern. This plant occurs in the North Island: Tararua Range and parts of the Ruahine Range; and the South Island: in the mountains of the Southern Alps from Arthur's Pass south to Fiordland, in wet tussock grassland. Most racemes contained more than 50 flowers but ones with fewer flowers were seen. Inflorescences were prominently cone-shaped when the lowermost flowers were just open. Some populations experienced "non-flowering" years, when only two or three plants produced flowers and seed out of a population of several hundred. The paucity of flower heads made the plants less obvious when searching for populations. Plants which flowered without setting seed were also encountered. Material showing this phenomenon was observed in

Figure 2.5. *B. gibbsii* var. *balanifera* (Lake Marian) Note large racemes of flowers.

Figure 2.6. *B. gibbsii* var. *balanifera* (Lake Marian, cool, damp habitat)

herbarium collections and field studies. Such plants were not as easy to find as those which set seed normally.

B. gibbsii var. *gibbsii* is restricted to Stewart Island. Generally plants of this variety are smaller than those on the mainland and produced fewer flowers per raceme (40 or less).

Both varieties of *B. gibbsii* are gynodioecious [populations contain both hermaphroditic and functional female plants (Moore 1964)], and flower during January and February (Moore and Edgar 1970). Study of herbarium material indicated that flowering sometimes began in December. This was confirmed by field observations of *B. gibbsii* var. *balanifera*.

2.3.2.3 *B. angustifolia* (figures 2.7 and 2.8)

B. angustifolia is locally common south of Waiau (South Island) in the eastern hills of Canterbury, Otago and Southland, in damp tussock grassland, but is absent from the western mountains. This species is hermaphroditic. Flowering occurs during November and December (Moore and Edgar 1970). Generally plants of this species are smaller overall than those of *B. hookeri* but not in all cases. Most plants produce racemes having 50 flowers or less, but ones with more flowers do occur.

2.3.2.4 *B. modesta* (figures 2.9 and 2.10)

B. modesta is confined to the West Coast of the South Island from Buller District as far south as Jackson Bay in damp lowland areas especially pakihi land. [The soil of pakihi land is extremely leached. In low-lying areas when drainage is impeded by both the iron pan and the nature of the relief, the ground is exceedingly wet, often approaching semi-bog conditions (Rigg 1962)]. *B. modesta* is

Figure 2.9. *B. modesta* (Okarito Swamp) growing with *Sphagnum* and under manuka (*Leptospermum scoparium*) forest. Inflorescence to left of white arrow.

Figure 2.10. *B. modesta* (Lake Ellery Track) in wet habitat under beech (*Nothofagus*) forest.

hermaphroditic. Flowering occurs during December and January (Moore and Edgar 1970). However, all of the four study populations began flowering in November and had virtually finished by the end of December by which time they were setting seed. The study of herbarium specimens and living plants in natural populations demonstrated that flowering often began in November. The leaves are similar in length to those of *B. hookeri*, but considerably thinner and tend to grow flat along the ground (prostrate) rather than erect like those of the three previously mentioned species. The prostrate habit demonstrated by live plants was not obvious from the study of herbarium specimens belonging to this species. Peduncles were spindly and delicate. The racemes of most populations contained 10-20 flowers, although ones with more flowers were observed.

2.3.2.5 *B. talbotii* (figures 2.11 and 2.12)

Although at least one herbarium specimen exists which was collected from "Taupo", *B. talbotii* is now known for certain only from the Gouland Downs, North-West Nelson, in open, boggy areas. This species is also hermaphroditic. Flowering occurs during December and January (Moore and Edgar 1970). When straggling up through the abundant *Gleichenia* of the bog, the leaves of this species are long and thin. However, leaves are short, thin and prostrate when plants are growing in an open habitat. In both situations, the peduncles bearing inflorescences are so short that they almost remain hidden among the leaf bases, even at fruiting. Most racemes contain only about 10 flowers.

Figure 2.11. *B. talbotii* (Gouland Downs) in flower.

Figure 2.12. *B. talbotii* (Gouland Downs) in fruit.

2.3.2.6 *B. rossii* (figures 2.13 and 2.14)

B. rossii is endemic to the herbfields of the Auckland Island Group and Campbell Island. This species is dioecious (Moore 1964). Flowering was most common during December, but could occur as early as October or as late as January (Moore and Edgar 1970). Obvious differences were observed between plants of different sexes which were transplanted from Campbell Island. While flowers of male plants possessed prominent anthers but no ovary, style or stigma; flowers of female plants completely lacked anthers but had a prominent gynoecium. The inflorescences of *B. rossii* were cylindrical in shape, and generally contained more than 50 flowers with short (1cm) pedicels. The flowers were so closely crowded that the peduncle was almost invisible.

2.3.3 Features of South African genus *Bulbinella*

In South Africa *Bulbinella* is clearly separated from related genera such as *Bulbine, Trachandra* and *Kniphofia* by its simple compact raceme of stellate flowers, smooth filaments and ovarian shape (Perry 1987). Ms Perry used morphological characters to divide *Bulbinella* into 16 species, two of which she further sub-divided each into two varieties.

Plants of the South African genus *Bulbinella* are much more variable than those of the New Zealand genus. Among the South African species, there is great variation in size and appearance of plants as documented below.

Plant height (not including inflorescence) varies from 0.25m to 1m.

Tubers are less uniform in appearance than those of the New Zealand genus. Swellings can be found adjacent to the root base, or some distance from it.

Figure 2.13. *B. rossii* plants originally obtained from Campbell Island, growing in the south garden.

Figure 2.14. as figure 2.13.

Alternatively tubers may be absent and instead the root is swollen to an elongated sausage shape over the entire length.

The leaves are erect in all South African species, but their cross sectional appearance varies greatly, from thick and fleshy to thin and deeply channelled. Leaf width ranges from very thin and grass-like to 65mm. Fibrous, sheathing leaf bases, which vary in height, are present in all the South African species, even the smallest ones. Of the six New Zealand species, only *B. rossii* possesses such fibrous leaf bases and is therefore considered to bear the closest physical resemblance to plants of the South African genus (pers. comm. Ms P. Perry).

The colour of the perianth segments varies both among and within some species in South Africa. Tepals may be white, some with a pink central stripe, through ivory, cream and yellow to bright orange. Flowering times vary for each species, coinciding with their respective wet seasons, and are from 1-5 months duration.

Whereas plants of the South African genus examined by the author experienced a period of total dormancy during the dry season, when they showed no external sign of life, plants of the New Zealand genus had already formed visible young leaf buds for the following season before the current season's leaves had completely died away. A number of the African species favoured habitats similar to those preferred by the New Zealand genus, that is damp shaded slopes and peaty seepages. In South Africa, wet sites almost completely disappear during the dry season (pers. comm. Ms P. Perry) which is different from the situation in New Zealand.

Of the South African material sent by Ms Perry, the adult plants survived at least one growing season. Some flowered during their first season in New Zealand but all had died by September 1990. Although some of the seeds germinated, all

except one seedling died after transplantation into individual pots. This young plant remained alive at November 1991. Of the three African plants purchased in New Zealand, all have survived and two have flowered for the last two seasons, since being transplanted from individual pots into a very dry garden plot. It is likely that the New Zealand summer was not dry enough to induce dormancy or to prevent rotting in the plants sent from South Africa. Invasion by fungi absent from their natural habitat may also have been a contributing factor to their deaths.

During her visit to New Zealand, Ms Perry examined living plants (some of which were in flower) and infructescences of all except one (*B. gibbsii* var. *gibbsii*) of the seven taxa of *Bulbinella* in New Zealand. She also viewed a selection of habitat slides of these same taxa. She compared these observations to those of the South African genus she had studied. Despite the wide range in form and appearance of plants of the different species of the genus *Bulbinella* in South Africa compared to plants of the New Zealand genus, Ms Perry (pers. comm.) considered that these two widely separated groups of plants should remain in the same genus, *Bulbinella.*

CHAPTER 3: ASPECTS OF CONSERVATION

3.1 Introduction

I have adopted the categories used in documenting New Zealand rare plants. These categories are based on those established by the Threatened Plants Committee of the Survival Service Commission of Nature and Natural Resources (IUCN). These categories are:

(1) Extinct: The plant is believed to no longer occur naturally in the wild, although it may be growing in cultivation.

(2) Endangered: Survival in the wild is unlikely if factors causing endangerment continue to operate and the plant is in danger of becoming extinct.

(3) Vulnerable: The plant is considered likely to move into the endangered category in the near future if the factors causing depletion continue to operate. Usually plants in this category are obviously diminishing in abundance or geographic range.

(4) Rare: Only small populations are known or the plant is found only in restricted areas where it may be locally common. Usually the numbers of plants and localities where it is found are reasonably stable.

(5) Local: The plant is not particularly at risk but has a local distribution which suggests that in time the species could move into one of the above categories. Local is not an IUCN category.

Bulbinella has not to my knowledge been officially listed as rare or endangered except for *B. talbotii*, the miniature species from Gouland Downs, which has been classed as "local" (Given 1981). While the species was not seen as particularly at

risk by Given, the local distribution suggested that in time it could move into another category such as vulnerable, endangered or rare.

Upritchard (1986) listed the Maori onion, *Bulbinella* species, under "pasture and wasteland weeds" in a field guide to the identification of common weeds. Evans (1987) stated that *Bulbinella* was one of the few native plants which had spread as a result of pastoralism because of its unpalatability to stock and its tuberous roots which enabled plants to resist burning. *Bulbinella* is often considered a weed by farmers because in their view the plants take up land on which pasture could be grown (pers. comm. Mr R. Johnson). Where the terrain allows the use of a tractor, land is usually ploughed, effectively eliminating *Bulbinella* plants. Swampy farmland may be drained and sometimes filled exterminating any *Bulbinella* present. Thus farming practices have reduced some *Bulbinella* habitats from vast meadows to small areas of verge by the roadside (pers. comm. Mr C. MacKay) and probably exterminated others.

One aim of the present study was to assess the habitat stability of the genus by comparing previously recorded distributions to present ones, and by making serial observations of as many populations as possible to determine whether the status was currently changing. This was achieved by:

(1) obtaining past records of the distribution of *Bulbinella* populations,

(2) seeking previously recorded populations and

(3) making serial observations of those populations located.

3.2 Materials and methods

Moore (1964) and the herbarium and personal communication records compiled (Chapter 2) were used initially to seek populations for study. For reasons such as difficult access and absence of recent collections not all recorded populations were sought. Plants of all the species were more obvious when in flower than when vegetative or in fruit. Therefore flowering times in particular were noted or estimated from herbarium records for the populations sought.

When a population could not be found at a recorded locality after an initial search, help was sought. Contact was first made with personnel of the local Department of Conservation (DoC) office or information office in smaller centres. Usually names and addresses of local people having knowledge of plants in the area would be obtained. In back country areas with no settlement nearby, enquiries were made from local farmers about the whereabouts of *Bulbinella* populations.

Photographs were taken of representative plants, habitat and any outstanding features of all populations located. The features documented below were recorded for each population.

(a) Numbers of plants present were estimated by inspection of the population using the criteria small (<200), moderate (200-1000) and many (>1000). The natural loss of genes from a population due to drift occurs at the rate 1/2N, where N = number of individuals. A population comprising a minimum of 50 individuals was considered adequate for short term (decades) survival and of 500 individuals for long term (centuries) survival (Frankel and Soulé 1981). The numbers 50 and 500 have been criticised for a number of reasons (Simberloff 1988) and hence have not been

used in this study. No magic numbers or specific rules are valid when applied to minimum viable population size because:

(i) Not all individuals contribute gametes equally to the next generation therefore the effective population size is almost invariably less than the census size.

(ii) In most natural populations, age structure is non-stationary, especially in many populations of conservation concern where age structure may be changing rapidly because of habitat alteration.

(iii) In nature, generations usually overlap.

(b) The spatial arrangement of plants was noted. The density of plants was recorded (scattered or densely crowded) as well as the distribution of plants within the population (in pockets or continuous).

(c) The habitat stability of each population was determined by examination and assessment of the habitat (was the habitat stable, threatened or diminishing?) by seeking answers to the following questions:

(i) Was there evidence of past disturbance or alteration to the habitat?

(ii) Did the habitat change during the course of the study?

(iii) Were the plants confined to small areas outside fences (suggesting that land inside fences had been altered)?

(d) Habitat including accompanying vegetation, slope and altitude was recorded.

(e) The reproductive status was noted.

(f) Any other relevant information was recorded including landmarks or features which could help in relocating the population. Most of those populations found were studied on more than one occasion (appendix I).

3.3 Results and discussion

3.3.1 Location of study populations

Variation in flowering time occurred both between successive seasons and between different populations of a single species. However, populations which flowered either earlier or later than the majority of their species did so consistently. Some populations were not found initially because they were not flowering at the time of the first search, but were located on a subsequent visit to the area. This emphasised the importance of accurate information on flowering times.

Help was not required to find some recorded populations of *Bulbinella* which were clearly visible from a public road. Others, however, were more difficult to locate. Local people suggested by DoC or information centre staff were contacted. Often they were able to supply detailed information on the locations of populations in the district. Occasionally they telephoned friends and associates in the neighbourhood who were better able to help in suggesting suitable sites to search for *Bulbinella*. Sometimes up to five people were consulted in order to find one population (Le Bons Bay). In some areas, although previous records of plants existed, local people had no knowledge of them and did not recognise the plants from photographs (Southland). Searching such localities was abandoned.

Weather often posed a problem and hazard when searching sub-alpine areas. In such places, thick fog and mist often greatly reduced visibility and making travel along lightly marked routes hazardous (Mount Fox and Mount Tuhua).

Fifty-seven of the 61 populations located were examined by the author (table 3.1 and fig. 3.1).

Table 3.1. The 61 populations of *Bulbinella* found. All populations except four [Granity Pass (12), Murchison Mountains (27), Tin Range (29) and Campbell Island (30)] were examined by the author. Legend: Where populations were found by others; [BVS] = Dr B.V. Sneddon, [PJM] = Dr P.J. Moore, [BDL] = Mr B.D. Lloyd and [DMC] = Mr D.M. Cunningham. Localities of the populations are shown in figure 3.1. The taxa are identified as: [h] = *B. hookeri*, [b] = *B. gibbsii* var. *balanifera*, [g] = *B. gibbsii* var. *gibbsii*, [r] = *B. rossii*, [m] = *B. modesta*, [t] = *B. talbotii* and [a] = *B. angustifolia*. Altitudes (where applicable) are recorded in meters (m). Plant numbers are recorded as: + = small (<200), ++ = moderate (200 - 1000) and +++ = many (>1000). Habitat stability is recorded as: s = stable, t = threatened (although no evidence was found of past habitat reduction, the population may face this threat in the future) and d = diminuished (evidence of previous habitat reduction was found and further destruction is likely). "Local" means the population is concentrated into pockets of suitable habitat rather than being continuous. "Fragmented" means that there is evidence for the present population being fragments of a once larger population. "Scattered" means that individual plants occur at intervals throughout the habitat rather than being in pockets or clumps.

Locality, Taxon, Number	**Habitat**	**Accompanying Vegetation**	**Plant Numbers, Habitat Stability**
Desert Road[h] (1)	roadside bog, rolling, steep 1000m	tussock, sedges	+++ / s
Umukarikari Range[h] (2)	rolling, steep, boggy 1270m	*Dracophyllum*, tussock	+++ / s
Waipakihi River[h] (3)	grassland by river 1000m	pasture grasses, toitoi, tussock	+ / d, fragmented
Poled Track[h] (4)	rolling, wet 1350m	tussock	++ / s, scattered
Mangaio Stream[h] (5)	streamsides, rolling 1080m	tussock, toitoi, shrubs	++ / t, local, on army land
Mangaohane[h] (6)	swampy, steep-rolling 1170m	tussock	+++ / s
Pouakai Plateau[h] (7)	plateau with tarns, steep 1213m	flax, *Sphagnum*, *Dracophyllum*	+++ / s
Ahukawakawa Swamp[h] (8)	rolling bog 910m	tussock, flax, *Sphagnum*	+++ / s
Purity Hut[h] (9)	boggy, steep 1120m	tussock, *Aciphylla*	+++ / s
Cobb Valley[h] (10)	flat-rolling grassland 819m	tussock	+++ / s
Red Hills[h] (11)	bog near hut	tussock	++ / s, local
Granity Pass[h] (12)			+++ / s [BVS]
Puhi Peaks[h] (13)	steep slopes 607m	tussock	+++ / s-t, on farmland, stock present
Molesworth[h] (14)	seepages, steep	tussock	+++ / s, local
Acheron Valley[h] (15)	seepages, steep	tussock	+++ / s, local
Jacks Pass[h] (16)	rolling-steep 1000m	tussock, *Celmesia*	+++ / s

Table 3.1 continued

Locality, Taxon, Number	Habitat	Accompanying Vegetation	Plant Numbers, Habitat Stability
Mt. Isobel[h] (16)	grassland, scree, steep 1300m	grasses, exotic forest	+++ / s-t, forestry
Whanahuia Range[b] (17)	damp, steep slopes 1500m	tussock, shrubs	+++ / s, local
Holdsworth[b] (18)	damp slopes, steep gullies 1400m	tussock, shrubs	+++ / s, local
Bridge Peak[b] (19)	damp slopes, steep gullies 1400m	tussock	+++ / s, local
Carroll Hut[b] (20)	seepage sites, rolling 1200m	tussock, *Ranunculus, Celmesia*	++ / s, local
Mt. Tuhua[b] (21)	damp, steep slopes 1100m	tussock, shrubs	++ / s, local
Mt. Fox[b] (22)	steep gullies near summit 1213m	tussock, small shrubs	+ / s, local
Red Tarns[b] (23)	damp, steep slope 1213m	tussock, shrubs	++ / s, local
Hooker Valley[b] (23)	raised boggy plateau, bounded by streams 1153m	tussock, shrubs	++ / s, local
Lake Marian[b] (24)	slopes at lake head 700m	grasses, shrubs	+++ / t, prone to avalanches and flooding
Lake Harris[b] (25)	steep slopes, water courses 14700m	tussock, shrubs	+++ / s, some scattered, some local
Homer[b] (26)	steep slopes 800m	grasses, shrubs	+++ / t, prone to avalanches
Monkey Creek[b] (26)	stream banks, rolling 800m	tussock, shrubs	+++ / s
Murchison Mts[b] (27)	wet area beside stream 1031m	tussock	+++ / s [PJM]
Wilmot Pass[b] (28)	damp, steep slopes 700m	tussock, shrubs	+ or ++ / s, local
Tin Range, Stewart Island[g] (29)	damp soil		+++ / s [BDL]
Campbell Island[r] (30)			+++ / s [DMC]

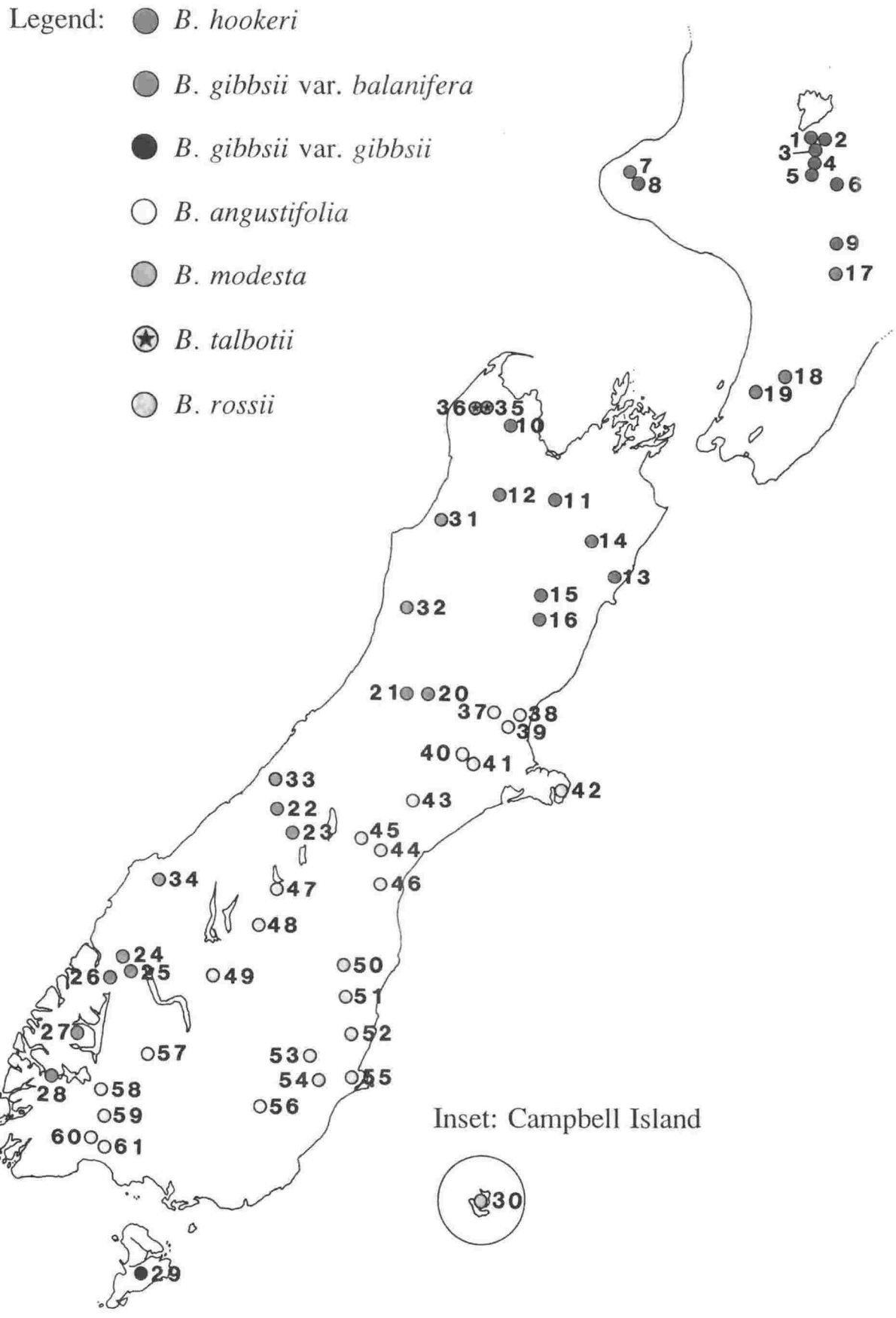
Table 3.1 continued

Locality, Taxon, Number	Habitat	Accompanying Vegetation	Plant Numbers, Habitat Stability
Charming Creek[m] (31)	gentle slope, soggy 100m	manuka, *Sphagnum*	+++ / s
Atarau[m] (32)	ditch by road <100m	pasture grasses, open	+ / d, fragmented
Okarito Swamp[m] (33)	rolling, boggy almost sea level	manuka, *Sphagnum*	+++ / s
Lake Ellery Track[m] (34)	wet river bank <100m	beech forest	+ / t, local
Gouland Downs[t] (35)	soggy soil around streams 576m	*Gleichenia*, small shrubs	+++ / s, local
Saxon Hut[t] (36)	wet ground on stream banks 667m	*Gleichenia*, small shrubs	+++ / s, local
Virginia Road[a] (37)	damp roadside, shaded slopes, rolling	flax, tussock, grasses	+ / d, scattered fragments
Okuku Pass[a] (38)	rolling farmland, steep slopes	tussock, grasses, sedges	++ / t, local, grazing, scattered
Ashley Gorge[a] (39)	farmland, steep, damp slopes	tussock, grasses, leafless broom	++ / t, grazing
Lake Lyndon[a] (40)	steep, shaded slopes	tussock, reeds, mosses, thistles	++ / t, local, grazing
Porters Pass[a] (41)	steep shaded slopes and gullies	tussock, *Aciphylla*, matagouri, *Celmesia*	+++ / s
Le Bons Bay[a] (42)	rolling slope above road	grasses, shrubs and trees	+ / d, fragmented
Lakes Emma and Clearwater[a] (43)	rolling farmland, lakeshore	grasses, rushes, matagouri	+++ / t, some grazing
Road between Fairlie and Geraldine[a] (44)	steep, shaded slope	grasses	+ / d, fragmented
Clayton Wetlands[a] (45)	rolling, permanent bog	tussock, matagouri, grasses, leafless broom	++ / t, local, mostly fenced from stock
Backline Road[a] (46)	roadside, rolling-steep	grasses, buttercups	+ / d, scattered, fragmented
Lake Ohau[a] (47)	steep-rolling, boggy	grasses, sedges, tussock, ferns manuka	+++ / s, with stock

Table 3.1 continued

Locality, Taxon, Number	Habitat	Accompanying Vegetation	Plant Numbers, Habitat Stability
Lindis Pass[a] (48)	steep-rolling, seepages	grasses, tussock	+++ / s
Crown Range[a] (49)	steep, shaded slopes and gullies	tussock, matagouri, wild roses	+++ / s
Danseys Pass[a] (50)	steep, damp slopes and gullies	tussock, matagouri, *Aciplylla*	+++ / s, grazing
Road between Palmerston and Ranfurly[a] (51)	steep, damp slope near stream	tussock, *Aciphylla*	++ / s, local
Macraes Flat[a] (52)	damp slopes, roadside, rolling	grasses, tussock	+++ / d, fragmented
Rock and Pillar Range[a] (53)	steep, damp, shaded slopes	tussock, grasses, thistles	+++ / s, with stock
Road between Dunedin and Middlemarch[a] (54)	by main road	tussock, grasses, gorse	+ / d, fragment
Flagstaff Hill[a] (55)	roadside, rolling	tussock, flax, exotic pine forest	++ / t, scattered, forestry area
Raes Junction[a] (56)	roadside and in paddock, rolling	grasses	+ / t, farming
Road between Te Anau and Mossburn[a] (57)	roadside, damp 500m	grasses, tussock	++ / t, fragmented
Road between Manapouri and Blackmount[a] (58)	roadside ditch 200m	tussock, grasses	+ / t, fragmented
Blackmount[a] (59)	boggy plateau, rolling, in foothills 400m	tussock, exotic pine plantation	++ / t, grazing
Lilburn Valley[a] (60)	damp slopes, rolling 200m	tussock, pasture grasses	+++ / t, on farmland
Alton Burn[a] (61)	roadside bank and ditch	tussock, grasses	+ / d, fragmented

Figure 3.1. The locations of the 61 populations of *Bulbinella* found. The population numbers correspond to those in table 3.1.



3.3.2 The effects of habitat reduction on *Bulbinella* populations

Most prospective study sites were sought or visited several times during the course of the field work (appendix I). The relative difficulty involved in locating populations of five of the seven taxa has been summarised (table 3.2). As *B. gibbsii* var. *gibbsii* and *B. rossii* were not observed personally by the author in their natural habitat, they have not been assessed in this manner. There is, however, no reason to suspect that either of these taxa is threatened. *B. gibbsii* var. *gibbsii* is locally common on Stewart Island (Moore 1964), as it has been since first being described by Cockayne (1909) cited by Moore (1964). *B. rossii* was one of the few if not the only herb to thrive because of its unpalatability when sheep were released on Campbell Island (Moore 1964 and Peat 1990). However, in the Auckland Islands, pigs were reported to dig up and eat the roots of *B. rossii,* while cattle and goats avoided the plants (Johnson and Campbell 1975). Hence on Enderby Island where cattle but not pigs are present, *Bulbinella* is dominant in the meadow. On the main island, where pigs are present *Bulbinella* is abundant only on sites inaccessible to pigs (Johnson and Campbell 1975).

3.3.2.1 *B. hookeri* (table 3.3)

While I was unsuccessful in finding the population at Mount Manuoha (fig. 3.2), I was assured that plants were still present but localised on large landslide scars and small bluffs several hundred feet below the summit (pers. comm. Mr W. Shaw).

Several attempts were made to find the recorded population near Tukino Ski Road (fig. 3.2), including a search at the peak of flowering. These were unsuccessful, despite using precise locality data obtained from Tongariro National

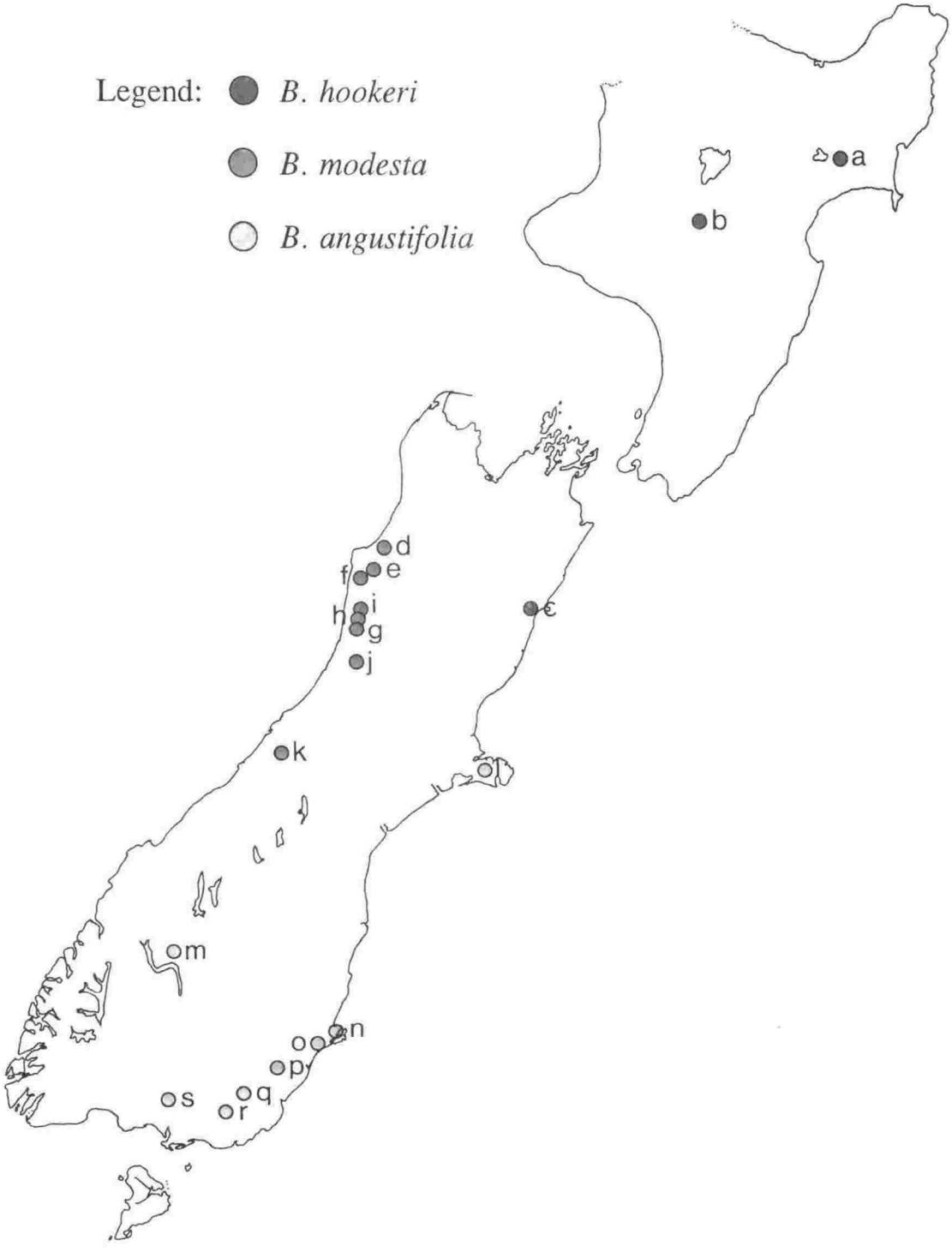
Table 3.2. The relative difficulty involved in locating *Bulbinella* populations. Legend: *B. h.* = *B. hookeri; B. gvb.* = *B. gibbsii* var. *balanifera; B. m.* = *B. modesta; B. t.* = *B. talbottii; B. a.* = *B. angustifolia.* T = total number of populations sought; F = number of populations found easily; D = number of populations found with difficulty (at least one person was consulted or extensive searching was required); N = number of populations not found (possibly extinct); C = number of populations found by chance or by searching suitable but previously unrecorded localities.

	B. h.	***B. gvb.***	***B. m.***	***B. t.***	***B. a.***
T	18	12	12	1	33
F	13 (72%)	9 (75%)	1 (8%)	1 (100%)	17 (52%)
D	3 (17%)	3 (25%)	3 (25%)	-	8 (24%)
N	2 (11%)	-	8 (67%)	-	8 (24%)
C	-	-	-	1	-

Table 3.3. *B. hookeri* populations sought. Legend: Status: F = found easily; D = found with difficulty (at least one person was consulted or extensive searching was required); N = not found and C = found by chance or searching suitable but previously unrecorded habitats. Comments: BVS = Dr B.V. Sneddon, VUW.

Locality	Status	Comments
Mt. Manuoha, Ureweras	N	local distribution, pers. comm. Mr W. Shaw
Desert Road	F	by roadside, obvious
Umukarikari Range	F	Pers. comm. Mr R. Smith
Waipakihi River	F	scattered population, 1 area containing 10 large plants washed away during study period
Tukino Ski Road	N	specific location obtained, possibly extinct
Poled Track (Kaimanawas)	F	scattered population
Mangaio Stream	F	by Desert road, obvious
Mangaohane	D	very plentiful on high farmland, steep shaded slopes and streamsides
Pouakai Plateau	F	locally common
Ahukawakawa Swamp	F	locally common
Purity Hut	D	pers. comm. Mrs K. Gorringe
Cobb Valley	F	very abundant in seepage sites
Red Hills	F	occurs locally in bog
Granity Pass	F	plentiful, pers. comm. BVS
Puhi Peaks	D	on high farmland, pers. comm. Mr B. Dunnett and Mr D. Cameron
near Oaro, south of Kaikoura	N	specific locality was obtained pers. comm. Mrs M. Parsons, possibly extinct
Molesworth	F	common around seepage sites
Acheron Valley	F	common around seepages
Hanmer - Jacks Pass and Mt. Isobel	F	common on shaded slopes and around seepages

Figure 3.2. The locations of the 19 previously recorded populations of *Bulbinella* sought but not found. Key to populations: a = Mount Manuoha, Ureweras, b = Tukino Ski Road, c = Oaro, d = Stockton, e = Caroline Terrace, south of Westport, f = south of Charleston, g = Blackball-Roa road, h = Blackball rubbish dump, i = Croesus track, j = Kumara-Stafford area, near Dillmanstown, k = Poerua State Forest, South Wanganui River, l = Mount Sinclair, m = Coronet Peak, n = Signal Hill, Dunedin, o = Lake Waipori, p = Waitahuna, south of Milton, q = Tapanui, west of Balclutha, r = Clinton-Mataura road and s = Otapiri gorge, Southland.



Park Headquarters. I therefore suspect that the small population which once existed in the area may be extinct.

The population near Oaro (fig. 3.2) which was on farmland may be either extinct or very localised. Although specific directions were given (pers. comm. Mrs M. Parsons) these plants were not found.

Difficulty arose in finding some populations of this species only because they grew on high farmland. Hence information centre staff were consulted and landowners were contacted to obtain access and information on exact locations of plants (Mr P. Green, Mangaohane Station and Mr D. Cameron, Puhi Peaks Station).

The only population of those studied which was considered endangered was the one at Waipakihi river which consisted of scattered plants or groups of plants. During the course of this study, a bank on which ten large, established plants (part of the population), had been growing was no longer present on searching in December 1990. The entire bank plus two large marker posts had disappeared, probably swept away by a flood. It is likely that a once extensive population of *Bulbinella* has been reduced to fragments. The effects of naturally occurring events (floods and landslides) may be enhanced as a result of human activity in this area.

3.3.2.2 *B. gibbsii* var. *balanifera* (table 3.4)

The difficulty in finding some documented populations occurred only because of their local distribution. None of the populations studied was threatened. A number were very localised along watercourses and in swampy areas (Mount Cook, Mount Tuhua, Carroll Hut and Mount Fox), where they had probably always been. All the populations studied of this variety were too high or on land too steep to be suitable for farming or other human activities apart from recreation.

Table 3.4. *B. gibbsii* var. *balanifera* populations sought. Legend: Status: F = found easily; D = found with difficulty (at least one person was consulted or extensive searching was required); N = not found and C = found by chance or searching suitable but previously unrecorded habitats. PJM = Dr P.J. Moore, DoC (formerly Wildlife Service), Wellington.

Locality	Status	Comments
Whanahuia Range	F	
Mt. Holdsworth	F	
Bridge Peak, Tararua Range	F	
Carroll Hut	F	local; only 2 pockets of plants found
Mt. Tuhua	F	local; on shaded slopes
Mt. Fox	D	local; in shaded gullies at top of Fox range
Mt. Cook	D	local; in bogs and seepages
Lake Marian	F	local; at head of lake on shaded slopes and in gullies
Lake Harris	F	visible from Routeburn track, along seepages
Homer	F	obvious from main road to Milford Sound, near Homer tunnel
Murchison Mts	F	plentiful, collected by PJM
Wilmot Pass	D	local; on shaded slopes, most of population probably inaccessible

3.3.2.3 *B. modesta* (table 3.5)

Populations of this species were difficult to find because of their prostrate growth habit and flowering relatively early in the season. This may be a partial explanation for only 33% of the populations sought actually being found. The modest size of the plants may also have resulted in their being overlooked in some cases even by local people. Plants of this species were rooted less deeply in the soil than any of the others, making them more prone to being washed away by flooding.

Populations were often localised within a general area. Altered or increased land use had visibly fragmented some populations and probably eliminated others. The deleterious effects of coal mining on the landscape of parts of the West Coast of the South Island, and Stockton (fig. 3.2) in particular, were mentioned by Given (1981), as well as being observed by the author.

The most common habitat of the four populations found was under manuka (*Leptospermum scoparium*) forest with *Sphagnum*. The two populations growing in this habitat (Charming Creek and Okarito swamp) were stable and considered neither vulnerable nor endangered.

A number of existing records referred to the population at Atarau under different local names such as the names of local creeks and landmarks in the area. Local people had plants growing naturally on their land in the 1960's (Moore 1964 and pers. comm. Mrs N. Christie) and at least one of these populations was still present in 1987 (pers. comm. Mrs N. Christie). The only plants seen at Atarau (excluding those on Mrs N. Christie's land) were confined to two pockets on opposite sides of the main road separated by 500m. These plants grew in ditches and along fencelines at the road edge. None were seen in nearby paddocks which looked like suitable habitat but which had probably been ploughed or drained. The presence

Table 3.5. *B. modesta* populations sought. Legend: Status: F = found easily; D = found with difficulty (at least one person was consulted or extensive searching was required); N = not found and C = found by chance or searching suitable but previously unrecorded habitats.

Locality	Status	Comments
Charming Creek	D	stable population, locality pers. comm. Mr A.P. Druce
Stockton - Millerton	N	locality pers. comm. Mr A.P. Druce, habitat altered by coal mining, population possibly reduced or fragmented
Caroline Terrace, south of Westport	N	possibly extinct
south of Charleston (pakihi)	N	habitat looked suitable, but no plants found
Atarau	D	exact location obtained, pers. comm. Mrs Christie, plants restricted to ditches by main road
Blackball - Roa Road	N	possibly eliminated when road widened and sealed
Blackball rubbish dump	N	type specimen collected in 1960's, dump probably filled since then
Croesus Track	N	habitat appeared suitable
Kumara - Stafford area near Dillmanstown	N	habitat possibly altered by farming
Okarito Swamp	F	stable population
Poerua State Forest South Wanganui River	N	habitat may have been altered by planting of exotic forest, population may be extinct
Lake Ellery Track	C	habitat wet but otherwise atypical, under tall beech/broadleaf forest

Table 3.6. *B. talbottii* populations sought. Legend as for table 3.5.

Locality	Status	Comments
Gouland Downs, Heaphy Track	F	local distribution in bogs and along stream banks
near Saxon Hut, Heaphy Track	C	not previously recorded near this hut, but found to be locally abundant in boggy sites along stream banks

of several now localised populations suggests that the plants once comprised a single large population which has been fragmented by farming practices and other human activities such as road making. The remaining plants, however, may have adapted to their habitat of **open** grass verge because they now consistently produce more flowers per inflorescence than most plants in the other populations of *B. modesta* studied. That *B. modesta* plants growing under manuka forest at Okarito Swamp produce only 10-20 flowers per inflorescence while those in **open** swamp habitat in the same locality consistently produce many more (>40) supports this interpretation. The population at Atarau was considered vulnerable, since the remaining plants could easily be covered by further widening of the road or swept away during a flood. Reproduction by seed could be reduced by the use of a mower to cut long roadside grass.

A record was found of a specimen of *B. modesta* collected from Jackson Bay, Southland. The only population found in this area, on Lake Ellery track, which may or may not be the one documented, occupied a small area under beech forest with some *Bulbinella* plants actually growing on the track. I suspected that other small local populations existed under this forest, but could find none. Although the small number of plants present were thriving under the constantly wet forest, the population was considered vulnerable because it was confined to a small area and could be affected by a relatively minor catastrophe. A natural event such as the falling of a large tree in the immediate area could remove some *Bulbinella* plants in the associated root plate. If the nearby river flooded or altered course plants could easily be swept away. The population is in no danger from human activities except for those plants growing directly on the track which are prone to being trampled.

3.3.2.4 *B. talbotii* (table 3.6, following p. 24)

In the Gouland Downs area *B. talbotii* was locally abundant. Plants were difficult to find only because they were much smaller even than *B. modesta.* The peduncles were very short making inflorescences barely visible between the leaf bases. Plants were localised around seepages and along stream banks, occurring in patches over a large area. They were observed from Shiner Brook (near Gouland Downs hut) to Saxon hut, over a distance of 5km. Plants were seen in the middle of a track to the river and on a popular camping site near Saxon hut. *B. talbotii* is listed as local by Given (1981) because the Gouland Downs is the only recent site where plants have been recorded. No special protection is given to *B. talbotii* other than that afforded to all flora and fauna in the vicinity of a public track. The plants are more likely to be affected by natural disasters than by human activities in the area since most trampers would not see *Bulbinella* plants even if they were in flower. The flowerheads are neither large nor showy enough to be picked by curious walkers. Most of the habitat of *B. talbotii* is away from the track in swampy ground where walkers are unlikely to go.

Although herbarium specimens were studied which had been collected last century from Taupo, no recent collections and no actual plants of *B. talbotii* from this locality were seen by the author. Thus the Taupo population is probably extinct.

3.3.2.5 *B. angustifolia* (table 3.7)

Populations of this species are generally not difficult to find, especially when in flower, although as a rule the overall size of plants is smaller than that of *B. hookeri.* It was therefore likely that recorded populations which were not found on searching

Table 3.7. ***B. angustifolia*** **populations sought.** Legend: Status: F = found easily; D = found with difficulty (at least one person was consulted or extensive searching was required); N = not found and C = found by chance or searching suitable but previously unrecorded habitats.

Locality	Status	Comments
Virginia Road	F	confined to road verges
Okuku Pass	F	
Ashley Gorge	D	on farmland, pers. comm. Mr R. Johnson, probably part of a once large population, including Okuku Pass and Virginia road, now fragmented
Lake Lyndon	F	local; in seepage sites
Porters Pass	F	obvious from Arthur's Pass highway, plants followed seepages
Mt. Sinclair, Banks Peninsula	N	possibly extinct
Le Bons Bay, Banks Peninsula	D	after consulting 5 people, I was personally taken to the remnant population by Mr C. MacKay
Lakes Emma and Clearwater	F	scattered along roadside, concentrated around small lakes
Road between Fairlie and Geraldine	F	some under exotic forest, original habitat fragmented
Clayton Wetlands	D	on high farmland, pers. comm. Mr A. Orbell
Backline Road	D	small roadside fragments of a once extensive population were found with assistance, pers. comm. Mr G. Patterson
Lake Ohau	F	extensive and stable population although on farmland
Lindis Pass	F	visible from Lindis Pass highway, plants followed seepages
Crown Range	F	very large population, visible from Wanaka - Arrowtown road
Coronet Peak	N	habitat probably altered by ski resort and associated activities
Danseys Pass	F	extensive population, easily visible from Dansey's Pass road
Road between Palmerston and Ranfurly	F	local, in seepages

Table 3.7 continued

Locality	Status	Comments
Macraes Flat	F	still large population but fragmented now, mainly restricted to road verges and fencelines, effects of herbicide use were observed
Rock and Pillar Range	D	on farmland, pers. comm. Mrs E. Wilkie and Mr J. James
Road between Dunedin and Middlemarch	F	remnant population, effects of burning were observed
Flagstaff Hill, Dunedin	F	under exotic forest, and in tussock grassland
Signal Hill, Dunedin	N	no access, altered habitat, suspect extinct
Lake Waipori	N	habitat altered by farming practices
Waitahuna, west of Milton	N	
Tapanui, west of Balclutha	N	
Clinton - Mataura road	N	local farmer did not recognise the plant when shown photograph
Otapiri Gorge, Southland	N	
Raes Junction	F	roadside, and in paddock
Road between Te Anau and Mossburn	F	roadside
Road between Manapouri and Blackmount	F	roadside
Blackmount	D	on tussock-covered farmland, and under exotic forest, habitat atypical
Lilburn Valley	D	on private farmland, pers. comm. Mr R. Guise
Alton Burn	D	small remnant population, pers. comm. Mr A. Templeton

were either extinct or their range had been reduced to such an extent that they were no longer visible from the site of the original record.

Land alteration mainly by farming practices has reduced or fragmented some populations. The majority of presumed extinctions occurred in Southland where land was not too steep to allow ploughing (table 3.7 and fig. 3.2). The small number of *Bulbinella* plants growing in a verge above Le Bons Bay were confirmed as being a remnant of a once much larger population by Mr C. MacKay. He said that *Bulbinella* plants which had been present in the paddocks beyond the fence near the present population had disappeared following ploughing. It is likely that other small populations where plants were seen only in verges and along fencelines but not in paddocks (for example Virginia Road, Backline Road and Alton Burn) had their habitat reduced in a similar manner to those above Le Bons Bay.

Other observed human activities which may be affecting this species include gold mining at Golden Point (part of Macraes Flat). Tailings from the mine have disrupted the landscape where plants had previously (1989) been found. None were seen during a search in November 1990.

Human activities from which *Bulbinella* appeared to suffer no long-term effects included herbicide application along grass verges (Macraes Flat). Here *Bulbinella* in the sprayed area showed twisted and distorted leaves and stems. Although production of viable seed for that season may have been reduced or prevented by chemical application, it was likely that the underground tubers were unaffected, enabling the plants to survive. The habitat of one population (road between Dunedin and Middlemarch) had been burnt either accidently or deliberately. Tussocks in the area showed charred bases, while *Bulbinella* plants in the vicinity were unaffected.

The land was probably fired while the *Bulbinella* plants were dormant below the ground surface and their underground tubers had enabled them to survive burning.

3.3.3 General discussion and conclusions

Although *Bulbinella* tissues are reported to be distasteful to stock (Moore and Irwin 1978, Salmon 1985 and Webb et al. 1990, all referring mainly to *B. angustifolia*), some species (*B. hookeri* and *B. angustifolia*) were browsed, perhaps inadvertently, by animals. It is unlikely that browsing was a threat to the long-term survival of those populations where it occurred. At most seed production was reduced when some flower heads were removed as well as leaves.

Major threats to the future survival of some *Bulbinella* populations include farming procedures such as the ploughing and draining of land. Plants can survive burning and possibly the application of some herbicides. When *Bulbinella* plants are present on farmland where stock graze, they do so without affecting the animals. No references suggest that *Bulbinella* is poisonous or likely to upset animals eating it, in contrast to tutu (*Coriara arborea*). The only detrimental effect of *Bulbinella* plants on farmland is their taking up land on which pasture or crops could be grown.

Mining activity in some areas has affected the survival of local populations by causing habitat destruction. Given (1981) pointed out that the effects of associated roads, buildings and spoil heaps must also be considered when looking at the disturbance caused by quarries and mines. Away from actual mining sites there are associated activities such as burning vegetation, exploratory roading and the dumping of overburden. Given (1981) illustrated this point with photographs showing the effects of coal mining above Stockton and gold mining in Otago. These effects were

seen by the author in the form of coal mining at Stockton (*B. modesta*) and gold mining at Golden Point, Macraes Flat (*B. angustifolia*).

Although the only **species** of *Bulbinella* presently at risk is *B. talbotii* because of its local distribution, some individual populations of other species (*B. hookeri, B. angustifolia*, and *B. modesta*) are considered vulnerable. A marked increase in human activity causing habitat destruction is the reason for their being assigned this status.

CHAPTER 4: GROWTH AND MORPHOLOGY

4.1 Introduction

The following features were used by Moore (1964) to separate pairs of taxa: tepal position after flowering, ovary and capsule shape, seed shape and colour, width of the broadest leaves, the relative lengths of peduncle and raceme, raceme shape (cylindrical or conical), flower arrangement in the raceme (lax or closely crowded) and the relative lengths of bracts and pedicels.

The aims of this section of the study were:

(1) To record the character states of a set of morphological features of plants both in their natural habitat and following transplant from natural populations, to identify and compare the taxa.

(2) To determine whether the character states recorded in the adult plants were stable in progeny grown in a different environment.

(3) To assess the validity of existing taxonomy and species identification.

(4) To investigate relationships and evolutionary history.

These aims were fulfilled by recording the character states of selected morphological features of:

(a) adult plants from each of the populations examined and

(b) seedlings germinated from seed collected from wild populations and grown in cultivation.

4.2 Materials and methods

4.2.1 Collection and storage of material

After being carefully dug out in the field, intact plants were placed in labelled plastic bags. A large ball of wet soil was left around the roots to increase the chance of the material surviving transportation. When a large amount of material was collected, plants were sent by air freight to a colleague at Victoria University. They were refrigerated on arrival. Most plants were collected in late summer, when they had begun to die back in preparation for winter dormancy, to give material the best chance of surviving several months refrigeration.

Seed should be harvested when ripe before the capsules split open (pers. comm. Mrs B. Matthews). In accordance with this advice, seedheads with ripe (but not split) capsules were collected from different plants in the field and placed in separate labelled paper bags. When the capsules did split, seeds were shed into the bags where they were stored until required.

4.2.2 Plant cultivation

A cool damp situation was recommended by Cartman (1985) for the cultivation of New Zealand *Bulbinella* plants. He also mentioned that they resent being dug up and divided and therefore suggested that seedlings should be planted out in their permanent positions as soon as possible and not disturbed thereafter.

A peaty, acid, sandy soil was suggested as best suiting South African *Bulbinella* plants (Harrison 1977). No references were found recommending suitable soils for the growing of New Zealand species. A mixture of equal parts of coarse river sand and peat moss was suggested for sowing seeds of the New Zealand species

(pers. comm. Mrs B. Matthews). This recommended mixture, when enriched and the proportions of ingredients altered, was found to be suitable for growing adult plants. The following "adult soil mixture" was used.

Ingredient	**Proportion (parts)**
peat (Smith's)	10
sand*	3
compost (Lang's organic garden mushroom compost)	1
potting mix (Smith's or Yates)	1
bark (Lang's medium granulated)	cover

*Coarse sand was collected at a Wellington beach, and soaked in several changes of tap water before use.

Mature plants were removed from the refrigerator and planted out in the above soil mixture in appropriately sized pots and covered with bark chips to deter weed growth and to help retain moisture. At the planting stage, the young leaf shoots for the next season could usually be seen between the remains of the current season's rosette of leaves. These young shoots were easily broken, so care was required.

Once potted, the plants were placed in a south-facing, enclosed garden/grass area, to be known hereafter as the south garden (figs 4.1 and 4.2), which received strong, direct sun only during summer, that is for about four to five months of the year. During the winter it was completely in shade. This situation was the best available to mimic the natural habitat of most plants. Plants from areas which experienced the wettest and coldest conditions; those from Fiordland, Stewart Island and the sub-antarctic islands, were placed against a wall facing south, which was the dampest and coolest part of the garden. Temperature readings taken in a pot against

Figure 4.1. The south garden (from east gate).

Figure 4.2. The south garden (from west gate).

Figure 4.3. The north garden.

this wall varied from 4°C or less in winter to 20°C in summer. As more plants were collected and further space was required, plants of *B. angustifolia*, the most drought resistant species, were placed in a north-facing gravelled area, fenced on three sides, to be known hereafter as the north garden (fig. 4.3, following p. 32). Special care was taken to prevent these plants becoming too dry as they were exposed to the prevailing northerly wind and in full sun from approximately September to April. Although the north and south gardens did not provide a completely uniform environment, an effort was made to place plants in a situation which best resembled their natural habitat regarding temperature and moisture levels, to give them the best chance of survival.

4.2.3 Seed germination

As seeds were best planted while fresh in autumn (pers. comm. Mrs B. Matthews) and they ripened the following spring (Matthews 1979), seed collections were planted out in autumn of the year in which they were obtained whenever possible. As mentioned previously, Mrs Matthews (pers. comm.) recommended a mixture of equal parts of coarse river sand and peat moss for sowing seeds. Coarse beach sand was collected and treated as described for adult plant cultivation. The following seed soil mixture was prepared for the sowing of seeds and the raising of seedlings.

Ingredient	**Proportion (parts)**
peat moss	2
sand	1
Smith's seed-raising mixture	1

Both the germination regimes attempted and the seed collections used were different each year and are therefore described separately. The year in which the experiments were conducted indicates the year in which the seeds were sown, not necessarily the year in which germination occurred or when the experiment was completed. All seeds used for the germination experiments were collected from wild populations unless otherwise stated. Where petri dishes were used as seed containers, about 20 seeds were placed in each dish. Sterile distilled water was used to keep the seeds damp in all experiments except those performed outdoors in pots or plastic bags, in which case tap water was used.

4.2.3.1 Germination regime 1986 A

These experiments were conducted from March to May 1986. The experiments were not continued after October 1986 unless otherwise stated. Seeds from Cobb Valley (*B. hookeri*) were used for regimes 1-3, and seeds from both Cobb Valley and Junction Knob, Tararuas (*B. gibbsii* var. *balanifera*) were used for treatments 4 - 6. The presence of both small and large seeds in the collections was noted. This phenomenon is common in the family Liliaceae (pers. comm. Dr G.K. Rickards). Experiments (1) and (2) were attempted to show if both types of seed were viable. These two experiments were also designed to demonstrate whether splitting the seed coat reduced the time taken for germination. Seeds which germinated were carefully transplanted into small pots of seed soil mixture and placed in the south garden.

(1) The following seeds were placed on moist filter paper in sterile petri dishes, which were wrapped in aluminium foil to maintain darkness and placed at room temperature (15 - 20°C). They were checked weekly for 16 weeks. Some seeds of each size were "cracked" by squeezing gently with forceps until the seed coat split.

Type of seed	Number of seeds
small	40
large	35
cracked	40

(2) The following seeds were treated as for (1) except that they were placed at 4°C for 14 weeks and then continued at room temperature until germination occurred.

Type of seed	Number of seeds
small	81
large	59
cracked	93

(3) Fifty seeds were placed in a dry paper bag in a refrigerator for 16 weeks and then planted outdoors in moist seed soil mixture.

(4) Forty seeds from each of the two populations above were planted outside in moist seed soil mixture.

(5) Eighty seeds from each population were placed in moist seed soil mixture in plastic bags (to retain moisture) in the south garden for 14 weeks, then planted in trays. The experiment was continued until November 1987.

(6) Two hundred seeds from each population were placed in moist seed soil mixture at 4°C (refrigerator) for 14 weeks and then placed at room temperature until germination occurred. The experiment was continued until November 1989.

4.2.3.2 Germination regime 1986 B

These experiments were conducted from September 1986 onwards when more seed collections were available for testing. Seeds from Wardle River (Inland Kaikoura Mountains) were collected in February 1986 from plants growing at Otari

Native Plant Museum which had been transplanted from the wild. Only a few seeds remained in the seed capsules of the Reporoa Bog plants in May 1986 when they were collected. The Campbell Island seeds had been collected in 1984. Regime B(1) [table 4.1] attempted to duplicate A(6) and treatment B(2) [table 4.2] was an expanded version of A(2), designed to determine more precisely the period of moist chilling required to induce germination.

(1) Seeds from four populations (table 4.1) were placed in moist seed soil mixture in plastic bags in the refrigerator for 16 weeks. They were then placed at room temperature and inspected every week for germination until December 1987 when the experiment was terminated.

(2) Twenty seeds from five populations (table 4.2) were placed on moist filter paper in sterile petri dishes, wrapped in aluminium foil and placed in the refrigerator. The dishes were removed to room temperature at intervals as indicated (table 4.2).

4.2.3.3 Germination regime 1987

Seeds were placed outdoors in pots of seed soil mixture and kept moist (table 4.3). The experiment was continued for three years.

4.2.3.4 Germination regime 1988

Thirty-five seeds from Mt. Patriarch (*B. hookeri*) and 100 seeds from Lake Marian (*B. gibbsii* var. *balanifera*) were planted outside in pots of seed soil mixture and kept moist. The experiment was continued for three years.

Table 4.1. Experimental germination regime 1986 B (1). The seeds were placed in moist seed soil mixture in plastic bags in the refrigerator for 16 weeks, then at room temperature.

Population	Number of seeds
Cobb Valley (*B. hookeri*)	50
Wardle River, Inland Kaikouras (*B. hookeri*)	25
Junction Knob, Tararuas (*B. gibbsii* var. *balanifera*)	50
Campbell Island (*B. rossii*)	50

Table 4.2. Experimental germination regime 1986 B(2). Twenty seeds were placed on moist filter paper in petri dishes at 4°C, then placed at room temperature after the time indicated by *. The populations are: 1 (Cobb Valley), 2 (Wardle River), 3 (Reporoa Bog), 4 (Junction Knob, Tararuas) and 5 (Campbell Island). Populations 1-3 are *B. hookeri*, 4 is *B. gibbsii* var. *balanifera* and 5 is *B. rossii*.

	Population				
Weeks at 4°C before placing at room temperature	**1**	**2**	**3**	**4**	**5**
2	*				
4	*			*	
6	*				
8	*			*	*
10					
12	*	*	*	*	*
14	*		*	*	*
16		*			*

Table 4.3. Seeds planted outdoors in seed soil mixture and kept moist in 1987.
Legend: * denotes populations from Campbell Island.

Population	Number of seeds	Species
Jacks Pass	20	*B. hookeri*
Mt. Isobel	20	"
Cobb Valley	20	"
Mt. Owen	11	"
Red Hills	20	"
Lake Lyndon	20	*B. angustifolia*
Porters Pass	20	"
Lake Emma	20	"
Lindis Pass	20	"
Crown Range	20	"
Flagstaff Hill	20	"
Red Tarns	20	*B. gibbsii*
Lake Harris	20	"
Murchison Mts.	20	"
Atarau	10	*B. modesta*
Gouland Downs	10	*B. talbotii*
Enderby Island	20	*B. rossii*
Ramp Point*	20	"
Bull Rock*	20	"
Beeman Cove*	20	"
Mt. Yvon Villerceau*	20	"

4.2.4 Recording of morphological characters

A list of suitable morphological characters was compiled using some of those employed by Moore (1964) and characters observed by the author which appeared to be consistent within a population or species, and which were therefore likely to be useful. The states of the selected characters (table 4.4) were recorded during flowering for several adult individuals in each natural population studied. These same character states were recorded for adult plants which had been transplanted from natural populations. It was essential to record most of the features, (especially the **colour** of leaves, peduncles, pedicels and bracts) early in the growing season. These features changed as the season advanced, as did other characters such as leaf form and surface appearance. The ideal time to assess most characters was when the first flowers were opening. Characters such as tepal position after flowering were assessed later in the season when the plants were beginning to set fruit.

All the characters of the germinated seedlings could not be recorded until the plants had attained adult size. A period of about three years from seed to flowering was considered appropriate for New Zealand *Bulbinella* plants (pers. comm. Mrs B. Matthews).

4.3 Results and discussion

4.3.1 Adult plants

4.3.1.1 Survival in cultivation

The majority (310/364 = 85.2%) of adult plants removed from the wild survived transportation, storage and replanting in a different environment (appendix II). Plants belonging to *B. gibbsii* and *B. rossii* showed the highest proportion of

Table 4.4. The 18 morphological characters and character states recorded in the study populations of *Bulbinella*.

Character	Character States
leaves (length)	short (s) / long (l)
leaves (thickness)	fleshy (f) / thin (t)
leaves (surface appearance)	prominantly ribbed (r) / not ribbed (n)
leaves (form)	erect (e) / arched (a) / prostrate (p)
leaves (width of broadest where leaf base separates from rosette)	wide (w) / medium (m) / narrow (n) > 3 cm / 1.5-3 cm / < 1.5 cm
leaves (colour)	green (gr) / brown (br)
peduncle (height)	short (s) / long (l)
peduncle (thickness)	stout (s) / medium (m) / thin (t)
peduncle (colour)	green (gr) / brown (br)
pedicels (length)	short (s) / long (l)
pedicels (colour)	green (gr) / brown (br)
bracts (length)	length compared to pedicel: short (s) 1/2 pedicel length / long (l) same length as pedicel or longer (where pedicel >1cm long)
bracts (colour)	green (gr) / brown (br)
inflorescence (appearance)	lax (l) / compact (c)
inflorescence (shape)	cylindrical (cy) / conical (co)
number of flowers per inflorescence	many (+++) / moderate (++) / few (+) >50 / <50 / 10 - 20
tepal position after flowering	twisted and pendant below fruit (p) / appressed to fruit (a)
reproduction	hermaphroditic (h) / gynodioecious (g) / dioecious (d)

deaths [14/78 (18%) and 8/16 (50%) respectively]. This was possibly because their natural habitats (high altitude North Island and South Island, Fiordland, Stewart Island, Auckland Island group and Campbell Island) were so different from Wellington in factors such as temperature and rainfall. Because of the size of these plants and the terrain of their respective habitats, they were the most difficult to remove intact. This may have been a contributing factor to the relatively high death rate.

Some difficulties were encountered with the growth and maintenance of plants in the north and south gardens. Small pots were easily blown over by wind. In both gardens cats were held responsible for knocking over pots, breaking inflorescences, uncovering plants and breaking new leaf shoots. Dogs physically moved pots in the north garden, spilling soil and disturbing plants.

Attack by aphids was very common, especially during the flowering season, and infestation was much worse in the north garden. Regular applications of detergent diluted with water were the best method of control. In the south garden, however, fungal infections were more prevalent. They rotted the leaves and young inflorescences on some plants to the extent that affected parts disintegrated. Affected plants did not die as a result, but remained dormant for the rest of that season. The only successful treatment was "Yates Rose and Floral Dust" applied at the first signs of infection.

4.3.1.2 Recorded character states

The states of all 18 characters recorded in wild populations (table 4.5) were maintained in plants in cultivation. Populations which differed in any respect from the remainder of their species, and individual plants which differed from the

Table 4.5. Morphological characters of the species. Legend: B.h. = *B. hookeri*, B.gvb. = *B. gibbsii* var. *balanifera*, B.gvg. = *B. gibbsii* var. *gibbsii*, B.r. = *B. rossii*, B.m. = *B. modesta*, B.t. = *B. talbotii* and B.a. = *B. angustifolia*. Abbreviations used for the character states have been explained previously (table 4.4). The state recorded for each character for each taxon is that of the majority of plants/populations. Exceptional observations are noted in parentheses.

Feature	B.h.	B.gvb.	B.gvg.	B.r.	B.m.	B.t.	B.a.
leaves: **s**hort/ **l**ong	l	l	s	l	l	s	l
leaves: **f**leshy/ **t**hin	f	t	t	t	t	t	f (t)
leaves: **r**ibbed/ **n**ot ribbed	n	r	r	r	n	n	n
leaves: **e**rect/ **a**rched/ **p**rostrate	e	a	a	a	p	p	e (p)
leaves: width of broadest: **w**ide/ **m**edium/ **n**arrow	m	m	m	w	n	n	m (w) (t)
leaves: colour	fresh green, brown-pink edges	bright green	bright green	bright green	olive green to brown	long: green, pink base; short: olive green	green: fresh to olive to dull
peduncle: **s**hort/ **l**ong	l	l	s	l	l	s	l

Table 4.5 continued

Feature	B.h.	B.gvb.	B.gvg.	B.r.	B.m.	B.t.	B.a.
peduncle: **s**tout/ **m**edium/ **t**hin	m (s)	m	m	s	t	t	m
peduncle: colour	green	green	green	green	brown	brown	brown
pedicels: **s**hort/ **l**ong	l	l	l	s (1cm)	l	s (very)	l
pedicels: colour	green	green	green	green	olive green to brown	brown	green
bracts: **s**hort/ **l**ong	s	s	l	s	s	s	s
bracts: colour	green centre, clear edges and tips	green, with clear edges	green, with clear edges	green, with clear edges	green, with brown edges and tips	green, with brown edges and tips	green, with brown edges
inflorescence: **l**ax/ **c**ompact	c	c	c	c	l	c	c (l)
inflorescence: **co**nical/ **cy**lind-rical	co	co	co	cy	co	co	co
number of flowers per inflorescence	+++	+++	++	+++	+ (++)	+	++ (+++)

remainder of their population maintained their differences when transplanted and they are mentioned specifically below.

The leaves and peduncles of plants from the Cobb Valley had a glaucous sheen (blue-green colouration), in contrast to all the other populations of *B. hookeri* studied.

Three populations of *B. angustifolia* from the south-west of the South Island (Blackmount, Lilburn Valley and Alton Burn), possessed inflorescences having a lax flower arrangement as opposed to the compact inflorescence of plants in other localities studied. Plants from the three localities mentioned above also had thin leaves (<1.5cm wide) as opposed to leaves of medium width (1.5 - 3cm).

In some populations of *B. angustifolia* (Lake Lyndon, Clayton Wetlands and Raes Junction), some plants produced inflorescences which had very long leaf-like bracts subtending the lower one or two flowers. The remainder of the bracts were of normal appearance. Where one such inflorescence was seen in a clump (single plant), all other flower heads present in the same clump showed this feature as well.

In some populations of *B. angustifolia* (Lake Clearwater, Danseys Pass, Rock and Pillar Range, Blackmount, Lilburn Valley and Alton Burn) most plants had bright green erect leaves but occasional ones had brown prostrate leaves. This variation may be explained by the different forms observed representing plants at different growth stages or small habitat differences within the populations concerned.

In one population of *B. modesta* (Charming Creek) most plants had long spindly peduncles (typical of the species) but a few had the short peduncles bearing small compact inflorescences more typical of *B. talbotii*.

Of the 18 characters studied, nine (leaf form, peduncle length and colour, pedicel length and colour, bract length, inflorescence shape, number of flowers per inflorescence and tepal position after flowering) were the most useful for species

identification. Assessment of tepal position after flowering divided the genus into two distinct (non-overlapping) groups. *B. gibbsii* and *B. rossii* (appressed tepals) were thus separated from *B. hookeri, B. angustifolia, B. modesta* and *B. talbotii* (pendant tepals). *B. rossii* can be easily distinguished from *B. gibbsii* by the cylindrical inflorescence (as opposed to conical) and very short pedicels (1cm) producing a closely crowded arrangement of flowers. *B. gibbsii* var. *balanifera* is separated from *B. gibbsii* var. *gibbsii* mainly by possession of bracts half as long as the pedicels (var. *balanifera*) as opposed to bracts as long or longer than the pedicels (var. *gibbsii*).

B. hookeri can be distinguished from the remainder of the group with pendant tepals by possession of a green (as opposed to brown) peduncle. Green pedicels, an inflorescence containing >40 flowers and erect leaves distinguish *B. angustifolia* from *B. modesta* and *B. talbotii*, which both have brown pedicels and prostrate leaves. A very short peduncle which is barely visible above the leaf bases, bearing a few (10) closely crowded flowers, distinguishes *B. talbotii* from *B. modesta* which has a long slender peduncle carrying a visible inflorescence containing 10-20 flowers on long pedicels.

4.3.2 Seedlings

The results of the germination experiments are described separately under the year in which they were conducted.

4.3.2.1 Results of germination regime 1986 A

Moist chilling by either overwintering outdoors or placing in a refrigerator was successful in germinating a maximum of 10% of seeds in the first season (table 4.6).

Table 4.6. Results of the 1986 germination experiments A (1-6). Legend: The treatments were; (1) kept moist in petri dishes at room temperature, (2) kept moist in petri dishes at 4°C for 14 weeks then at room temperature, (3) dry chilled for 16 weeks then planted outdoors, (4) planted outdoors soon after collection, (5) moist chilled outdoors then planted and (6) moist chilled in soil in a refrigerator for 14 weeks then placed at room temperature.

Treatment / Population	**Germination**	**Success**
	1986	**1987**
(1) Cobb Valley		not
small	-	continued
large	-	
cracked	-	
(2) Cobb Valley		
small	5/81 (6%)	not
large	5/59 (10%)	continued
cracked	9/93 (10%)	
(3) Cobb Valley	2/50 (4%)	not continued
(4) Cobb Valley	4/40 (10%)	not continued
Junction Knob	3/40 (8%)	
(5) Cobb Valley	8/80 (10%)	20/80 (25%)
Junction Knob	7/80 (9%)	18/80 (23%)
(6) Cobb Valley	20/200 (10%)	39/200 (20%)
Junction Knob	16/200 (8%)	29/200 (15%)

More seeds germinated (maximum 25%) the second season after sowing in those experiments continued for more than one year.

4.3.2.2 Results of germination regime 1986 B

No germination was detected in either of the treatments (1) using petri dishes or (2) using soil, both of which involved moist chilling for several weeks before warming to room temperature. As germination had been observed in experiments 1986 A (2) and A (6), and in other treatments using moist chilling then warming, it was surprising to find that none of the second lot of seeds to which such a protocol was applied germinated.

The information provided by Mrs B. Matthews (pers. comm.), that the seeds were best sown fresh, was supported by the results of experiments 1986 B procedures (1) and (2). It is possible that the seed collections had lost their viability. This applied particularly to the seeds from Campbell Island which were collected in 1984, two years before sowing. The other populations tested in 1986 B were collected earlier the same year and thus were expected to be still viable at the time of sowing.

4.3.2.3 Results of germination regimes 1987 and 1988

Although the results of the experiments conducted in 1987 were variable (table 4.7), results of the 1988 experiments (table 4.8) supported the findings of 1986 A procedures (5) and (6) in that more seeds germinated after a second season of moist chilling than after only one season.

Table 4.7. Results of the 1987 seed germination experiments. The seeds were planted outdoors in seed soil mixture soon after collection and kept moist. Legend: * denotes populations from Campbell Island.

Population	Species	Germination	Success
		1987	1988
Jacks Pass	*hookeri*	-	2/20 (10%)
Mt. Isobel	"	1/20 (5%)	-
Cobb Valley	"	-	4/20 (20%)
Mt. Owen	"	-	-
Red Hills	"	2/20 (10%)	1/20 (5%)
Lake Lyndon	*angustifolia*	1/20 (5%)	1/20 (5%)
Porters Pass	"	-	-
Lake Emma	"	2/20 (10%)	-
Lindis Pass	"	-	-
Crown Range	"	1/20 (5%)	-
Flagstaff Hill	"	-	2/20 (10%)
Red Tarns	*gibbsii*	-	-
Lake Harris	"	-	-
Murchison Mts.	"	-	-
Atarau	*modesta*	1/10 (10%)	-
Gouland Downs	*talbotii*	-	-
Enderby Island	*rossii*	-	-
*Ramp Point	"	-	-
*Bull Rock	"	-	-
*Beeman Cove	"	-	-
*Mt. Yvon Villerceau	"	-	-

Table 4.8. Results of the 1988 seed germination experiments. The seeds were planted outdoors in seed soil mixture as soon as possible after collection and kept moist.

Population	Species	Germination	Success
		1988	1989
Mount Patriarch	*hookeri*	1/35 (3%)	2/35 (6%)
Lake Marian	*gibbsii*	6/100 (6%)	19/100 (19%)

4.3.2.4 Conclusions

Overall results indicated that an extended period of moist chilling was required before optimum seed germination occurred. This could be achieved by refrigeration or by placing the seeds outdoors in a cold situation.

The seeds of all New Zealand *Bulbinella* species exuded a brown, water-soluble pigment when soaked in water for several days (Chapter 6). Since seeds of *B. gibbsii* and *B. rossii* exuded the largest amount of this brown substance, required the longest time to germinate and showed the lowest germination success, it is possible that this pigment acted as a germination inhibitor in a similar manner to that described in *Melicytus ramiflorus* seeds by Partridge and Wilson (1990). In *Bulbinella* also it is likely that such an inhibition mechanism allowed the seed to germinate only when conditions were most likely to be optimum for its survival. According to Berrie (1984) naturally-occurring germination inhibitors are often found in seeds, especially their coverings, and may be specific (a hormone), or non-specific (phenolic acids). As phenolic acids are water-soluble, they are gradually lost by leaching, releasing the seed from inhibition after sufficient rainfall in nature, simulated by soaking in the laboratory. Since gentle crushing of *Bulbinella* seeds did not reduce the time required for germination, a chemical inhibitor, as opposed to a mechanical one, present in the seed coat was considered the most likely cause of dormancy.

4.3.2.5 Morphological characters of the seedlings

Most of the seedlings alive in 1991 (November) were not large enough to score for all of the 18 characters (table 4.4). However, two of the seedlings which germinated in 1986 [ex. Cobb Valley, (*B. hookeri*)] each produced one inflorescence

in summer 1990-91. These two plants demonstrated the character states typical of *B. hookeri* populations and the glaucous sheen to the leaves and peduncle typical of plants from this particular locality. It was noted that even very young seedlings (<1 year old) from this population possessed leaves with a glaucous sheen. No seedlings of any of the other species have produced flowers as of 1991 (November). A two year-old seedling of *B. gibbsii* var. *balanifera* (ex. Junction Knob, Tararuas) showed the prominently ribbed leaves typical of this species. A four year-old seedling from Lake Emma showed the typical vegetative characters of *B. angustifolia* populations. This plant had produced no flowers as of 1991, November.

CHAPTER 5: CYTOGENETICS

5.1 Introduction

A somatic (2n = 2x) chromosome number of 14 has been assigned to all the New Zealand species of *Bulbinella* (Hair and Beuzenberg 1966). These workers found that all six species had one metacentric or submetacentric, three metacentric and three acrocentric chromosome pairs. Karyotype differences between species existed only in the relative sizes of corresponding pairs of chromosomes.

Limited information was available on the cytogenetics of the South African genus *Bulbinella.* Darlington and Wylie (1955) reported a somatic chromosome number of 14 for *B. robusta.* Nordenstam (1982) assigned a somatic number of 14 to *B. setosa* and 12 to *B. cauda-felis.* Both these publications preceeded the recent taxonomic review of the genus (Perry 1987). No supernumary or 'B' chromosomes were reported in any of the New Zealand or South African species.

The aims of the chromosome work were:

(1) to obtain chromosome numbers and karyotypes of plants from the New Zealand populations studied and

(2) to use chromosome banding to detect inversions or other chromosomal rearrangements that might distinguish different populations and/or species.

5.2 Materials and methods

5.2.1 Materials

Adult plants originally obtained from wild populations and planted in pots as described previously (Chapter 4) were used for the collection of root tips for chromosome study. Seedlings were not used initially since they took too long to germinate and to attain a suitable size (Chapter 4). It was likely that a further year would be required for rootlets to develop if the tip of the main root was removed.

5.2.2 Preparation of material to study mitosis

1. Young, fresh-looking root tips were collected into 0.1% aqueous colchicine at various times during the day from 8am to 2pm, and throughout the year.

2. Following three hours of colchicine treatment, the root tips were washed in water, drained and placed in freshly-made glacial acetic acid/ethanol (1:3) fixative for at least one hour, or overnight at 4°C if neccessary. They were then transferred to 70% ethanol for storage at 4°C, or processed immediately.

3. The root tips were washed in three changes of distilled water over a period of five minutes, then drained.

They were next stained using either the propionic orcein or the Feulgen technique.

5.2.2.1 Propionic orcein staining

4. Two or three root tips were heated with a liberal quantity of propionic orcein (appendix III) plus two drops of 1N hydrochloric acid on a watchglass for ½ minute.

5. When the mixture had cooled, one root tip was placed on a slide and the meristem removed. The meristem was macerated in a small drop of propionic orcein and squashed using thumb pressure.

5.2.2.2 Feulgen staining technique

4. Two or three drained root tips were placed in a small stoppered vial of 1N hydrochloric acid preheated at 60°C and left for 10 minutes.

5. The material was cooled rapidly, washed in distilled water and drained.

6. Root tips were placed in Feulgen stain (appendix III) in the dark for one hour at room temperature or overnight at 4°C.

7. The material was washed in distilled water and drained.

8. One root tip was placed on a slide with a small drop of 45% acetic acid (HAc) and macerated. A coverslip was applied and the preparation squashed as for the propionic orcein technique.

5.2.2.3 Mounting

Preparations were viewed under a light microscope. Good slides (having chromosome spreads where most individual chromosomes were separated) were made permanent using liquid nitrogen (or by placing in a -80°C freezer if one was readily available) to remove the coverslip. Slides were then put through the following series of solutions; two changes of 95% ethanol, two changes of 100% ethanol, 50/50 eucalyptus/ethanol and eucalyptus oil, for one minute each. Slides were drained, mounted in euparal and left flat at 20-25°C for the mountant to harden for two to three days.

5.2.3 Chromosome banding techniques

Banding techniques obtained from the following references were applied to unstained root tip preparations which had been viewed under a phase-contrast microscope to confirm the presence of suitable chromosome spreads; Schweizer (1973), Filion (1974), Marks (1975), Marks (1983) and Turner (1985). Minor variations of these methods (for example, the timing of steps) were investigated. The different buffers, solution concentrations and pH conditions used by these authors were also tried. Examples of two of these banding methods are given below.

5.2.3.1 Giemsa banding technique (Schweizer 1973)

1. Root tips were placed in 0.05% colchicine for four hours in the dark.

2. They were then placed in ethanol/glacial acetic acid (3:1) for three to five hours or overnight.

3. They were placed in 90% ethanol for one to four days.

The root tips were further processed using either series 4-6 or 4A-6A.

4. 45% HAc on slide and heated gently.	4A. 45% HAc for two to three hours @ room temperature.
5. Chopped, applied coverslip and tapped.	5A. Dissected apical meristem.
6. Heated gently and squashed.	6A. Made squash preparation using gentle heat.

7. Coverslip was removed.

8. Slides were placed in two changes of 90% ethanol, two changes of 100% ethanol and air dried.

9. Either step 9 or 9A was applied.

9. Slides were placed in two changes of Saline Sodium Citrate (SSC) over two hours.	9A. Slides were placed in two changes of McIlvaine's buffer over three hours.

10. Either method 10 or 10A was applied.

10. Three changes of 70% ethanol
Two changes of 90% ethanol
One treatment with 100% ethanol

10A. Three changes of distilled water

11. Slides were air dried.

12. Slides were placed in 2% Giemsa in M/15 Sörensons phosphate buffer (appendix III) for about two hours at room temperature.

13. The slides were then rinsed in several changes of distilled water, air dried and mounted in euparal.

5.2.3.2 Giemsa banding technique (Filion 1974)

1. Root tips were placed in 0.05% colchicine for four hours in the dark.

2. They were fixed in fresh ethanol/glacial acetic acid (3:1) for 48 hours.

3. They were stored in 70% ethanol at 4°C.

4. The material was hydrolysed in 1N HCl for eight minutes at room temperature.

5. The meristems were squashed in 45% acetic acid.

6. The coverslips were removed, the slides dipped in absolute ethanol and air dried.

7. Slides were stored in a vacuum desiccator for at least five days.

8. The slides were immersed in aqueous 0.064M barium hydroxide solution for 50 minutes at room temperature.

9. They were washed in two changes of distilled water over five minutes.

10. Slides were incubated in 2 x SSC at 60°C for 40 minutes.

11. They were rinsed in two changes of distilled water and air dried.

12. Slides (maximum two per staining tube) were placed in 2% Giemsa in M/15 Sörensons phosphate buffer (pH 6.8) for 20 minutes at room temperature. They were monitored during staining.

13. Slides were rinsed in distilled water, dried and mounted.

5.2.4 Photography

Photomicrographs were taken with a Zeiss Photomicroscope III using Ilford Pan F film at either 50 or 25 A.S.A. with maximum illumination (12V).

5.3 Results and discussion

5.3.1 Dividing cells

In most root tip material studied from New Zealand species, a great paucity of suitable cells was obtained. Varying the time and concentration of colchicine treatment produced no improvement. Neither the time of day nor the season of the year when the material was collected had a detectable effect on the number of mitoses observed. As few cells were undergoing mitosis, very few suitable chromosome spreads were obtained. However, chromosomes of at least one plant from the following populations were examined: Ahukawakawa swamp, Waipakihi River, Mangaohane, Cobb Valley, Puhi Peaks, Jacks Pass, (*B. hookeri,* fig. 5.1); Murchison Mountains, (*B. gibbsii* var. *balanifera*, fig. 5.2); Campbell Island (*B. rossii*, fig. 5.3); Atarau, (*B. modesta*, fig. 5.4) and Lake Lyndon, Lake Emma, Crown Range, Blackmount, (*B. angustifolia*, fig. 5.5). No suitable preparations were obtained from root tip material of either *B. talbotii* or *B. gibbsii* var. *gibbsii.* This was unfortunate because it would have been useful to confirm the presence of the

The preparations illustrated in figures 5.1 to 5.5 are chromosome spreads (x 1250) which were stained using the Feulgen technique. In each figure, the drawing (a) is the interpretive sketch of the photograph (b). The arrows indicate the acrocentric chromosomes.

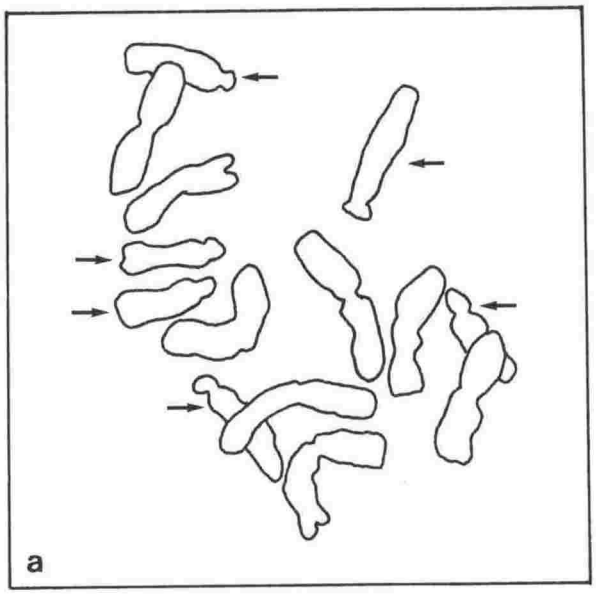


Figure 5.1. *B. hookeri*, Jacks Pass (2n = 14).

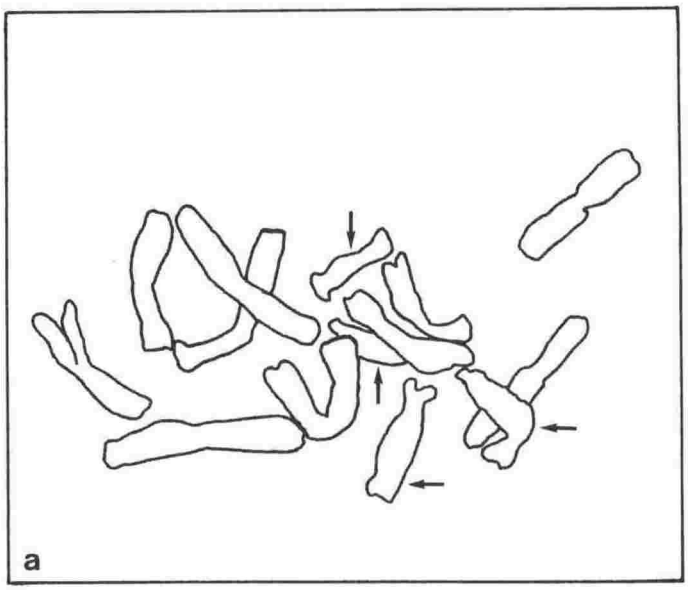
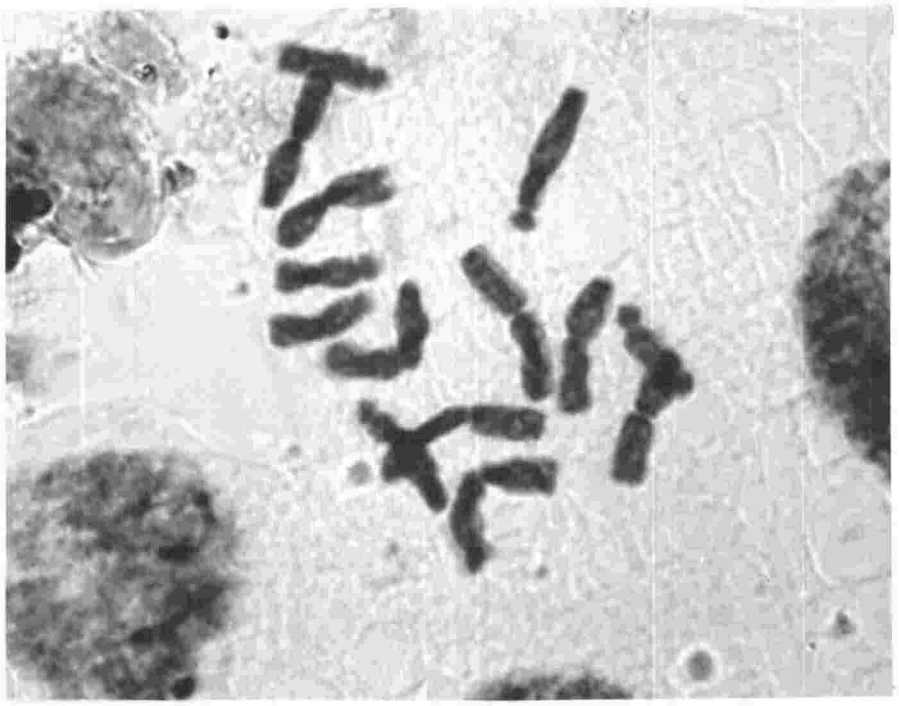
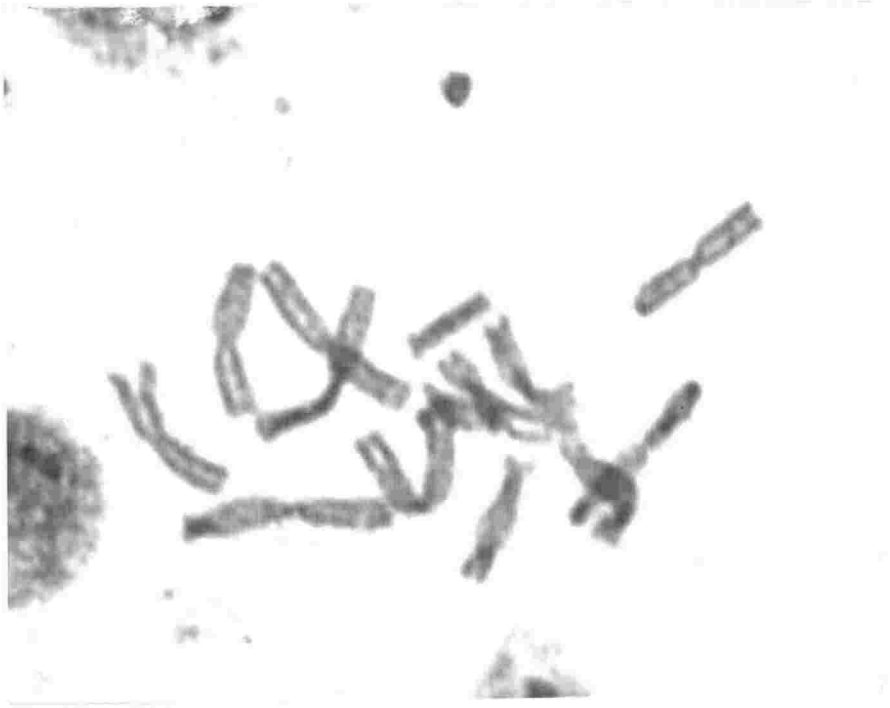


Figure 5.2. *B. gibbsii* var. *balanifera*, Murchison mountains (2n = 14).



b



b

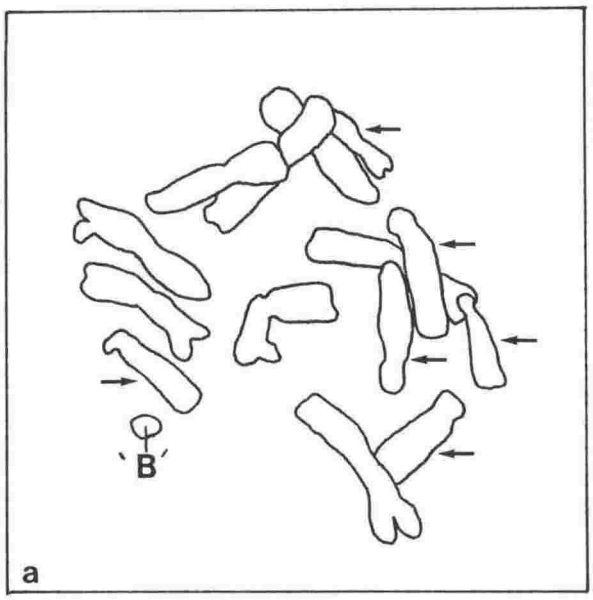


Figure 5.3. * *B. rossii*, female plant from Campbell Island, (2n = 14 + 1'B').

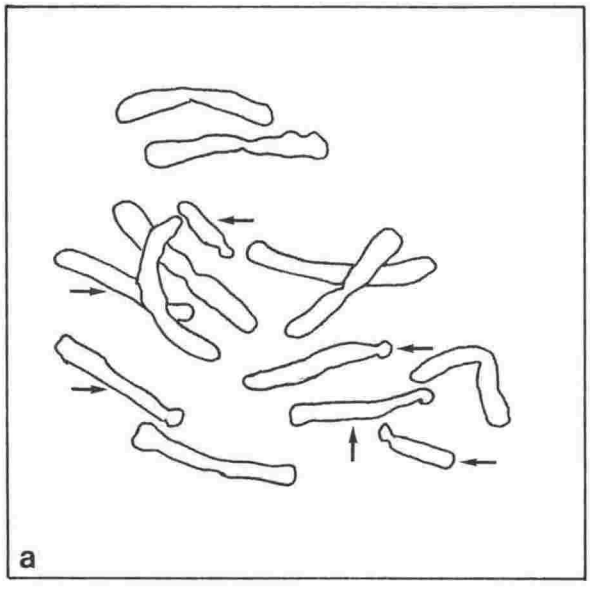
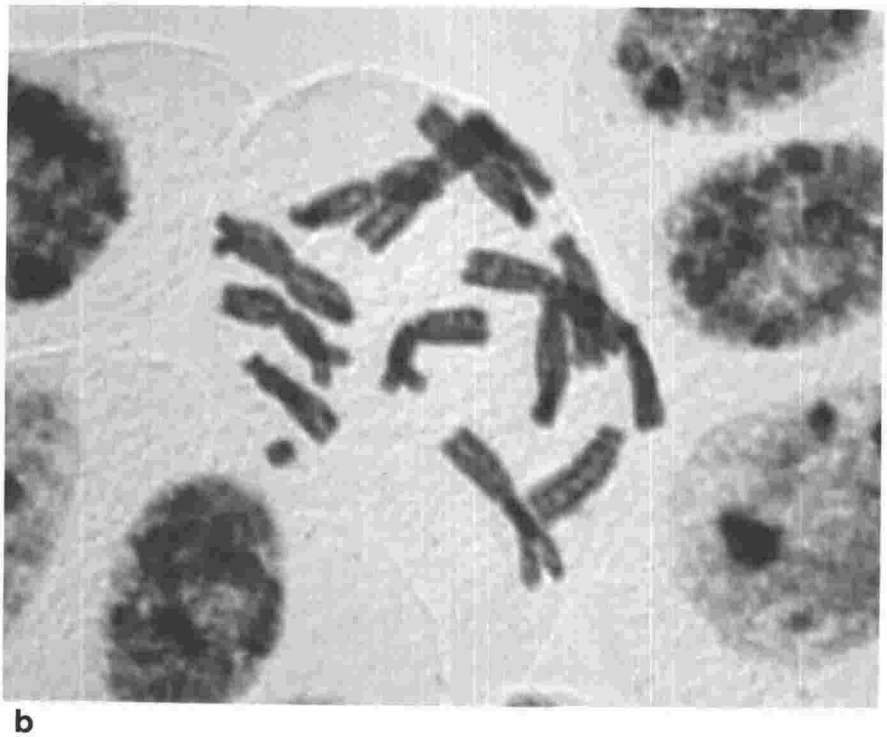


Figure 5.4. *B. modesta*, Atarau (2n = 14).

* One 'B' chromosome was clearly seen in only two of the four cells examined.



b

b

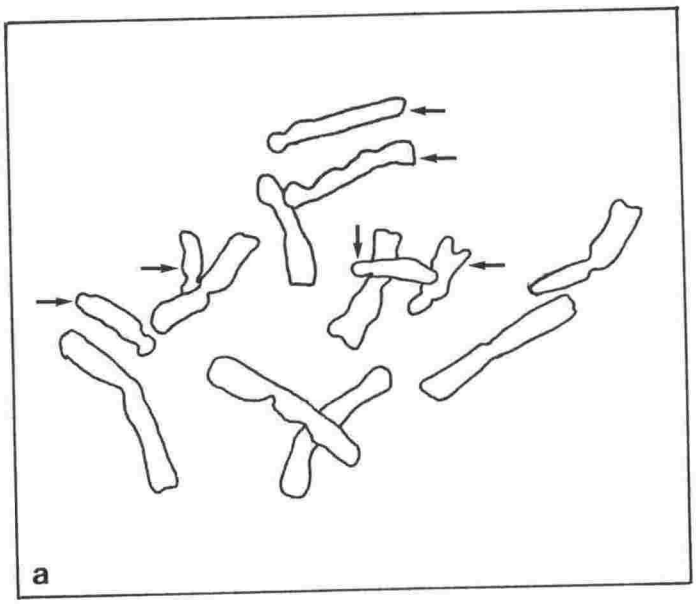
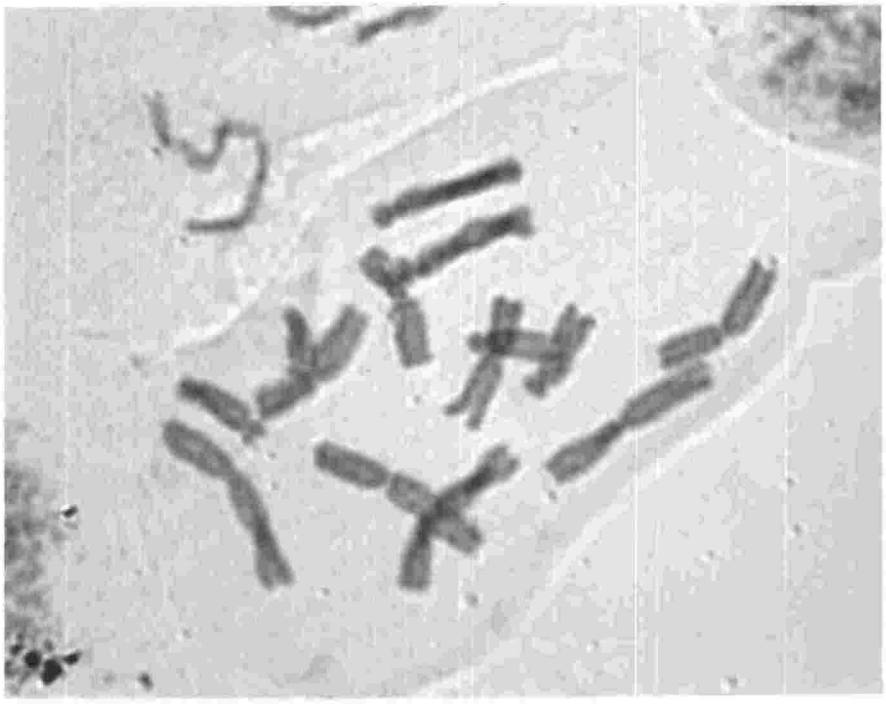


Figure 5.5. *B. angustifolia*, Blackmount (2n = 14).



b

pair of heteromorphic acrocentric chromosomes found in one plant of *B. gibbsii* var. *gibbsii* by Hair and Beuzenberg (1966).

Three acrocentric pairs could be identified in all individuals except the plant examined from Murchison Mountains, where only two could be seen clearly, almost certainly because of the orientation of the chromosomes on the slide. It was considered very unlikely that the karyotype of this plant differed from that published for *B. gibbsii* var. *balanifera* (Hair and Beuzenberg 1966). All the chromosome spreads examined revealed a total of 14 chromosomes, except where material had obviously been lost, or where the presence of an additional 'B' chromosome was suspected (Campbell Island, fig. 5.3). Of four spreads examined in the same preparation, one 'B' chromosome could be seen clearly in only two. The orientation of the large chromosomes in the other two spreads may have concealed the 'B'.

The three South African *Bulbinella* plants purchased in New Zealand, however, produced more suitable-looking root tips than those of the New Zealand genus. Although mitosis was not detected in many root tip cells from these plants either, the chromosome complement was assessed as far as possible. The three plants examined all had a different karyotype: plant A, 17 chromosomes (8 pairs 1 'B') [5 cells]; plant B, either 23 chromosomes (11 pairs + 1 'B') or 24 chromosomes (12 pairs) [1 cell], and plant C, 28 chromosomes (14 pairs) and no 'B's [2 cells].

No suitable root tips were obtained from the South African plants which were sent to New Zealand by Ms Perry in early 1987.

5.3.2 Chromosome banding

Of the many techniques and variations attempted, the methods described by Schweizer (1973) and Filion (1974) produced the closest results to 'banding' observed in any of the preparations.

Using the method of Schweizer (1973), no difference was observed between the two alternative slide preparation techniques (steps 4-6 or 4A-6A). The use of McIlvaine's buffer was preferable to SSC for the incubation stage in some preparations. Although the original paper suggested 2x SSC for 1-2 days for *Scilla* (also in the family Liliaceae), this treatment was not advantageous for *Bulbinella* and an incubation time of two hours was routinely used. Either step 10 or 10A could be applied prior to staining. Using this technique, chromosomes stained a uniform pinkish colour. 'Banding' as such appeared as swellings along the length of the chromosome rather than an actual difference in colour between segments. The resulting G-banded preparations were neither consistent nor distinct enough to be useful even in matching members of a pair of chromosomes.

The use of Filion's (1974) technique produced purple bands on mauve-staining chromosomes in only one slide examined (fig. 5.6). Bands were detected in only two pairs of chromosomes of the total complement of 14 (7 pairs). One pair possessed one small band seen at one end of each chromosome and the other pair had one large band located in the centre of each chromosome. The remainder of the chromosomes stained a uniform mauve colour. These bands were useful in matching individual members of the two pairs of chromosomes on which they occurred, but were not considered useful for detecting rearrangements. The same two small and two large bands could be distinguished in many interphase cell nuclei in this same preparation (fig. 5.7).

The preparation illustrated in figures 5.6 and 5.7 was G-banded using the method of Filion (1974). In both figures, the drawing (a) is the interpretive sketch of the photograph (b). Legend: L denotes large bands, S denotes small bands and N is the nuclear membrane.

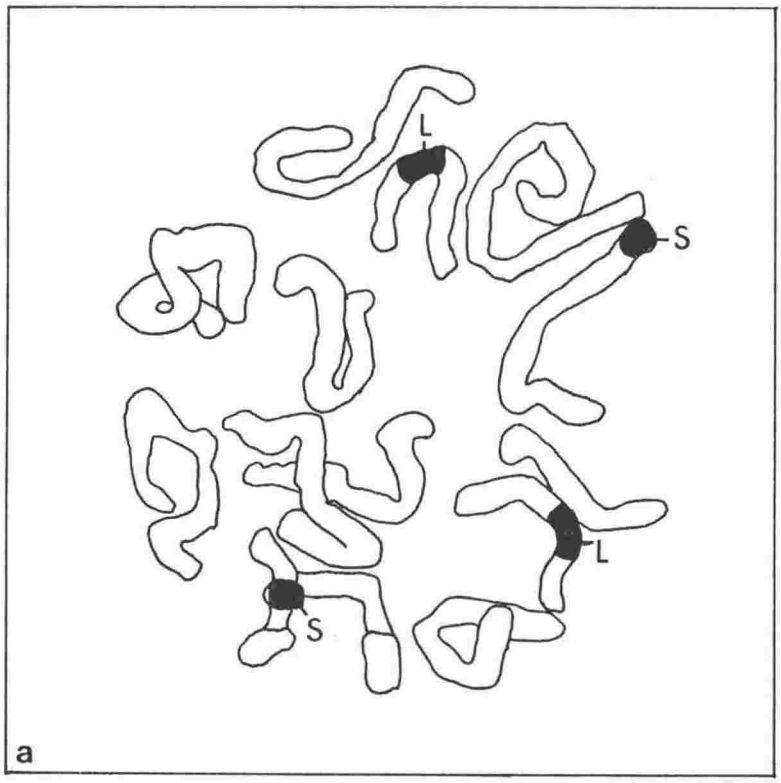


Figure 5.6. *B. angustifolia*, Porters Pass (mitotic metaphase).

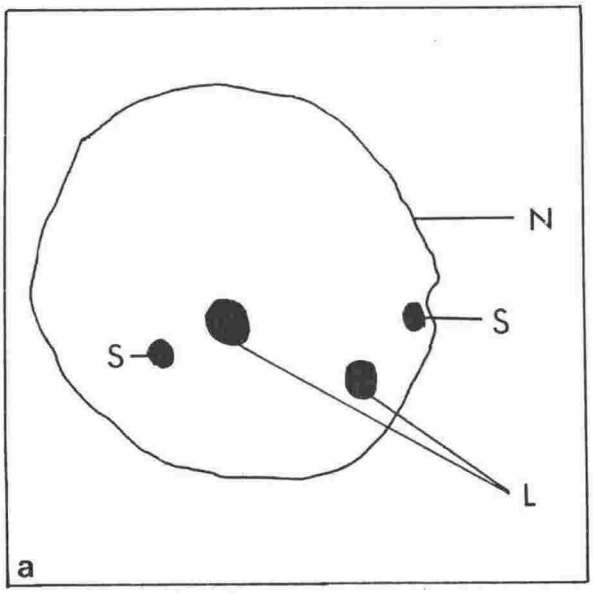
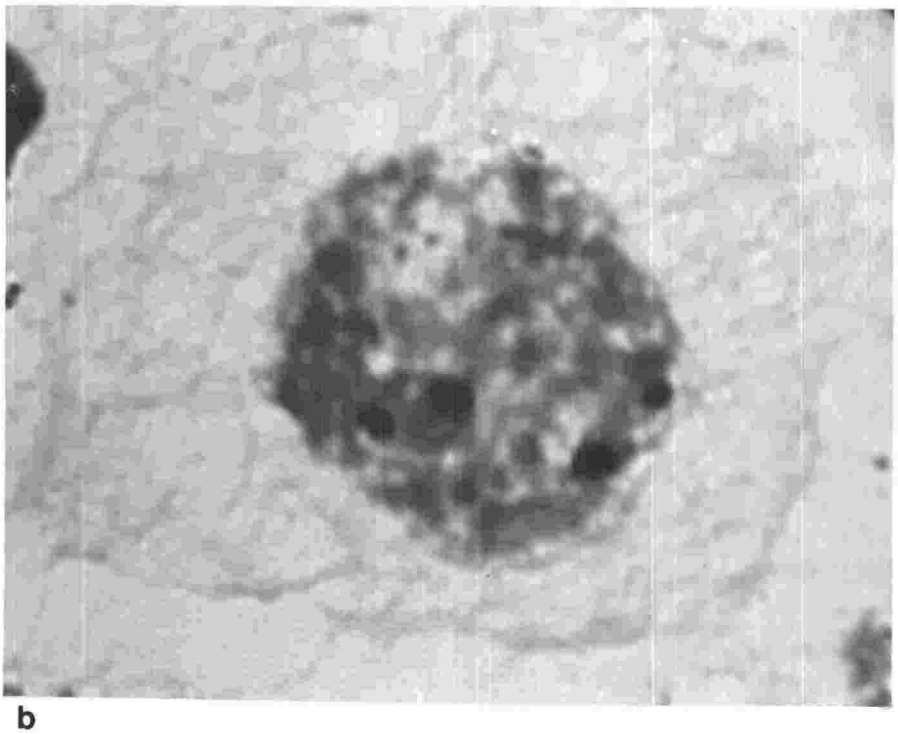


Figure 5.7. *B. angustifolia*, Porters Pass (interphase cell).

b



b

With both these banding techniques great variation occurred between different preparations which had been treated in exactly the same manner. On a single slide, great variation in staining intensity and clarity was observed between adjacent areas, which is usually the case with G-banding (pers. comm. Dr G.K. Rickards).

5.3.3 Summary

(1) For plants from 12 populations the diploid chromosome number was 14, confirming the results of previous studies. In one further population from which one plant was examined (Campbell Island, *B. rossii*) the presence of an additional 'B' chromosome was suspected.

(2) Karyotyping was achieved for at least one plant from each of 13 populations.

(3) Giemsa banding of chromosomes was successful in one slide of material from one plant.

CHAPTER 6: ALLOZYME TECHNIQUES

6.1 Introduction

The aim of this part of the study was to determine whether the use of allozyme analysis to study *Bulbinella* was feasible. Three questions required answers.

(1) Could enzyme activity be detected in any *Bulbinella* tissues?

(2) Which tissue(s) of *Bulbinella* yielded the greatest number of loci with allozymes which were clear and easy to interpret?

(3) Could differences between taxa be detected using allozyme analysis?

These three questions were answered by

(a) analysing seeds and tubers (the only tissues available initially) of *Bulbinella* and leaves, root tips and stems from related genera and

(b) analysing other *Bulbinella* tissues (leaves, root tips, stems, pedicels, bracts, flowers and pollen) when available.

Starch gel electrophoresis was the technique chosen because of its relatively low cost, ease and speed for screening a large number of individuals when compared to cellulose acetate electrophoresis, polyacrylamide gel electrophoresis or molecular techniques.

6.1.1 Principle of electrophoresis

The pattern (zymogram) of enzyme bands on a gel after electrophoresis (number, spacing, relative intensity) depends on the particular enzyme assayed, its mode of inheritance and the genotype and ploidy of the individual examined (Gottlieb 1981). Different forms of an enzyme which catalyse the same reaction are

called **isozymes** if they are coded by different gene loci and **allozymes** if they are coded by the same locus. In plants, most commonly examined enzymes have several isozyme forms, often with specific subcellular locations, and the majority of isozymes have different allozymic forms (Gottlieb 1981). Loci and alleles separate according to the size, charge and shape of the protein molecules and the pH of the buffer system used.

The data generated from enzyme electrophoresis differ fundamentally from other information routinely employed by plant systematists because the banding patterns in gels produced by staining for specific enzymes may be interpreted in genetic terms (Crawford 1989). Because allozymes are inherited as codominants in a simple Mendelian fashion, allelic frequencies for a population can be calculated. These data can be used to quantify the similarities and differences between populations, groups of populations, species and groups of species in a genus.

6.1.2 Limitations

Standard protein electrophoresis detects variation only in the small proportion of enzymes for which staining methods have been developed. Therefore estimates of genetic variation and distances obtained from electrophoretic data among taxa are based on only a subset of the total number of loci in the genome. They represent relative as opposed to total levels of variation and divergence. However such estimates are still valuable for the purposes of comparison.

Amino acid substitutions are only revealed if they produce a difference in the size, charge or shape of the protein which leads to a different migration rate on a gel. The use of several different electrophoretic (gel and electrode) buffer systems and pH conditions, rather than just one system can overcome this problem to a large extent.

Thus the maximum possible number of loci and alleles can be detected. According to Lewontin (1974) a maximum of 30% of variation is detectable. However, Hurka (1980) estimated that as little as one quarter of all substitutions are detected. Because some types of enzymes are more variable than others, [the esterases, phosphatases and peroxidases frequently analysed in plant studies are highly variable (Gottleib 1981)] it is important to include as many loci as possible in the data. The most fundamental assumption to be made is that the variation detected represents alleles at a Mendelian locus. Banding patterns can also be affected by environmental conditions. Multiple bands can be produced by protein interaction with buffer components, or by instability of molecules caused by the undesirable treatment of samples such as several freeze/thaw cycles.

The tissues of some plants, especially woody plants, contain phenolics which interfere with protein extraction. A variety of buffer additives available have been reported to improve extract quality by retarding or overcoming this interference (Wendel and Parks 1982). Such techniques have considerably extended the number of plant genera which can be studied using allozyme analysis.

6.2 Materials and methods

6.2.1 Materials for the pilot study using *Bulbinella* seed and tuber and tissues (leaf, root and stem) from related genera

The only tissues available initially for the study of *Bulbinella* were seeds and tubers because the plants had died down for the winter dormancy period when the pilot study was undertaken. Useful allozymes for genetic studies have been found in both seeds (Crawford and Julian 1976, Cherry et al. 1972 and Panda et al. 1986) and

storage tissues such as tubers (Martinez-Zapater and Oliver 1984) and corms (Schaal 1975). Other tissues available from related genera (*Arthropodium, Agapanthus* and *Crocosmia)* were also analysed (table 6.1).

6.2.1.1 Collection and treatment of *Bulbinella* tubers

Small pieces of tuber were cut from the main clump and placed in labelled plastic bags for transport to the laboratory. Tubers were cut into small cubes and ground with an equal volume of either distilled water, TRIS/DTT buffer (pH 7.4) [pers. comm. Dr G. Lindsay, appendix IV] or phosphate buffer (pH 7.5) {Conkle et al. 1982, appendix IV} using a pestle and mortar. The preparation was then centrifuged in a Sorval refrigerated centrifuge at 15,000 revs per minute for 20 minutes. The supernatant was ultra-frozen at -80°C for later analysis.

6.2.1.2 Collection and treatment of *Bulbinella* seeds

Seeds collected from wild plants (table 6.1) were placed in individual, labelled vials, soaked in distilled water for two days, then ground in fresh distilled water, or one of the two buffers mentioned above. One seed was prepared with one drop of grinding solution. Soaking the seeds softened them, aiding grinding and may have allowed undesirable compounds present in the seed coat to leach out before sample preparation. Non-viable and therefore useless seeds rotted when soaked and could thus be easily identified and discarded before grinding.

6.2.1.3 Collection and treatment of other tissues from related genera

In addition to seeds and storage tissues (tubers), useful enzyme activity has been found in other plant genera in vegetative buds (Gottlieb 1973 and Martinez-

Table 6.1. Plant tissues examined for allozyme activity in the pilot study using seed and tuber of *Bulbinella* and tissues (leaf, root amd stem) of related genera. Legend: Locality/source is the place from which entire plants were obtained. The required tissues were separated in the laboratory after plant collection.

Tissue	Taxon	Locality / Source
seed	*B. hookeri*	Cobb Valley
	B. gibbsii	Junction Knob
tuber	*B. hookeri*	Reporoa bog
		Mt Egmont
	Arthropodium	VUW gardens
	Crocosmia	town belt
young leaves	*Arthropodium*	
	Crocosmia	
	Agapanthus	own garden
	Scilla	own garden
root tips	*Arthropodium*	
	Crocosmia	
	Agapanthus	
	Scilla	
stems	*Agapanthus*	
	Crocosmia	

Zapater and Oliver 1984), leaves (Baker et al. 1975, Babbel and Selander 1974, Crawford and Wilson 1979 and Torres et al. 1982), root tips, flowers and buds (Heywood and Levin 1984 and Bayer and Crawford 1986), stem and pollen (Weeden and Gottlieb 1979) and newly germinated seedlings (Crawford and Smith 1984 and Lowrey and Crawford 1985). The availability of material was often a prime consideration. For this reason extracts were usually obtained from vegetative tissues such as leaves, stems, terminal portions of roots, or parts of or entire germinating seedlings (Wendel and Weeden 1989). The importance of the material being in a state of active growth was emphasised. Because the only *Bulbinella* tissues available for study initially were seeds and tubers, other tissues (leaves, root tips and stems) from related genera were analysed (table 6.1). These tissues were ground with an equal volume of distilled water or buffer.

After preparation (grinding with water or buffer) each sample was stored ultra-frozen in a plastic Auto-Analyzer (AA) tube having a 1ml capacity and a tightly-fitting lid.

6.2.2 Materials for the secondary study using the full range of *Bulbinella* tissues

The following *Bulbinella* tissues were prepared and analysed when available to determine which gave the most useful results: mature leaves, young leaf buds, root tips, peduncles (stems), pedicels, bracts, open flowers, flower buds, pollen, leaves of germinated seedlings and roots of germinated seedlings.

6.2.2.1 Collection and treatment of tissues from *Bulbinella*

The tissues were collected from mature plants growing in cultivation, that were originally removed from wild populations as adults (table 6.2). Each tissue was

Table 6.2. Plant tissues from *Bulbinella* examined for allozyme activity. The *Bulbinella* species used were: *B. hookeri* [Molesworth (M), Jacks Pass (JP), Ahukawakawa Swamp (AS) and Cobb Valley (CV)]; *B. gibbsii* var. *balanifera* [Bridge Peak (BP), Lake Harris (LH) and Junction Knob (JK)]; *B. angustifolia* [Flagstaff Hill (FH) and Porters Pass (PP)] and *B. modesta* [Atarau (A)].

Tissue	Locality
mature leaves	M, FH, BP, A.
young leaf buds	JP, PP, LH.
root tips	M, FH, A.
peduncles (stems)	AS.
pedicels	M, FH, A.
bracts	M, FH, A.
open flowers	M, FH, A.
large (old) buds	M, FH, A.
small (young) buds	M, FH, A.
pollen	FH
germinated seedling leaves	CV, JK.
germinated seedling roots	CV, JK.

collected at an early (young) stage of growth. For example the tips of mature leaves were used, while they were still green, before they turned red. Young leaf buds were harvested while still underground, that is, before they emerged in spring. Plants were removed from their pots and new, fresh-looking root tips were pinched off with forceps. Small sections of peduncle were collected while still elongating. Pedicels, bracts, flowers and buds were collected at the same time. Whole inflorescences were sometimes taken or the required parts were plucked off inflorescences with forceps.

After collection the tissues were placed into labelled AA tubes, stored in a refrigerator for one hour and then placed in a vaccuum flask of crushed ice before being taken to the laboratory.

Each sample was prepared in distilled water and each of the two buffers mentioned earlier (TRIS/DTT and phosphate), giving three subsamples of each tissue. Samples were ground, using a specially designed polystyrene instrument, in AA tubes with an equal volume of grinding solution. The prepared samples were not centrifuged since any solids present settled out and the additional heating effect produced by centrifugation was considered undesirable. Pollen was prepared using the technique of Weeden and Gottlieb (1979).

The collection and preparation of tissue samples was done well in advance, at least the day before the laboratory work was scheduled. After preparation the tissue was stored ultra-frozen until required.

6.2.3 The genetic basis of polymorphism

In previous studies concerning annual plants or those with a short life history, selected crosses were generally performed between plants of known genotype. The resulting progeny were analysed to demonstrate Mendelian inheritance. The results

of these experiments showed that the loci detected were genetically controlled as opposed to being determined by environmental or other external influences. Since *Bulbinella* seed germination was more likely after two seasons, and a period of four years from germination to flowering was found (Chapter 4), experimental crosses were not attempted in this study.

Pollen analysis was attempted (section 6.2.2) for comparison of haploid to diploid tissues from the same plant.

Plants in cultivation were sampled in successive years to confirm that they were assigned the same genotype on each occasion. Plant genotypes obtained from the analysis of samples collected from wild populations were compared to those obtained using samples from plants presently grown in cultivation, but originally from the same wild populations.

6.2.4 Nomenclature

The labelling of enzymes, genetic loci and alleles followed Murphy and Crabtree (1985). Enzymes were designated by upper case letters (for example PGM for phosphoglucomutase). Corresponding loci or isozymes were designated by an initial upper case letter, followed by lower case, then a hyphen and numeral were used to indicate the specific allozyme locus (Pgm-1). When an isozyme was represented by more than one locus, the loci were sequentially numbered from cathode (lowest) to anode (highest). Alternative alleles at a locus were labelled, in order of discovery, using a lower case letter in parentheses [Pgm-1(a)].

6.2.5 Allozyme analysis

6.2.5.1 The preparation and assembly of gels

Seven gel and electrode buffer systems were used for the pilot study and the secondary *Bulbinella* tissue study (table 6.3). Recipes for these buffers are given later (appendix V).

The gels were prepared the day before the laboratory work was scheduled (appendix VI), using the technique of Allendorf et al. (1977).

1. The following day, each gel was unwrapped and a scalpel was used to free the plastic sides of the mould. These and the rubber bands were carefully removed leaving the gel on the glass plate.

2. An "origin" was cut about 4cm from one long edge using a straight edge and scalpel. The two pieces were pulled apart to leave a gap of about 1cm to allow samples to be loaded.

3. The larger portion of the gel and the glass plate were covered with plastic wrap to retain moisture, and the whole assembly was placed on a flat surface of crushed ice for loading with samples.

4. A cloth wick (one piece of "chuxcloth" folded in half to form a rectangle of size 19cm x 10cm) and approximately 200mls of electrode buffer were placed in each of the buffer trays.

5. Sample wicks (1cm high x 2-3mm wide) made from thick chromatography paper were soaked in the sample supernatent, blotted on a clean, absorbent paper towel then placed against the larger portion of the gel at the origin. A wick soaked in cochineal, a red food colouring, was placed at the beginning of the run, following every tenth sample and at the end. These dye markers made it easy to judge how far

Table 6.3. The buffer systems used in both the pilot study examining seed and tuber of *Bulbinella*, and tissues (leaf, root and stem) of related genera; and the secondary study examining the full range of *Bulbinella* tissues. Legend: AC= Amine-citrate, PH= Phosphate, TC= TRIS/Citrate, PK= Poulik and RW= Ridgway.

Buffer system	Gel buffer pH	Electrode buffer pH	Voltage	Buffer front distance (cm)
AC	6.0	6.1	170	6
PH	6.7	6.7	150	5
TC-1	6.7	6.3	100	5
TC-2	8.0	8.0	100	5
TC-3	7.0	7.0	100	5
PK	8.7	8.2	300	4
RW	8.5	8.1	300	4

the buffer front had moved. Gaps of 1-2mm were left between adjacent sample wicks (fig. 6.1). About 40-50 samples could be applied to each gel.

6. The two portions of the gel were replaced together and the run was started. Samples ran from cathode (-) to anode (+). The entire assembly was covered with plastic wrap (fig. 6.2) before the power was turned on and the voltage was adjusted according to the buffer system used (table 6.3).

7. Sample wicks were removed after 10-15 minutes. Trays of solid ice were placed over the entire gels (fig. 6.3). When using the PK and RW systems, a small tray of ice was placed on the cloth wick on the cathodal side to reduce heat buildup.

8. The gels were allowed to run for the time required to achieve the optimum buffer front distance (table 6.3), then enclosed in plastic wrap and placed in the refrigerator for a minimum of 10 minutes. The gels became firm and easier to slice.

9. The gels were trimmed using a straight-edge and scalpel, then sliced horizontally using a nylon thread to yield about 6-7 slices each approximately 1mm thick. Each slice could be histochemically stained for a different enzyme.

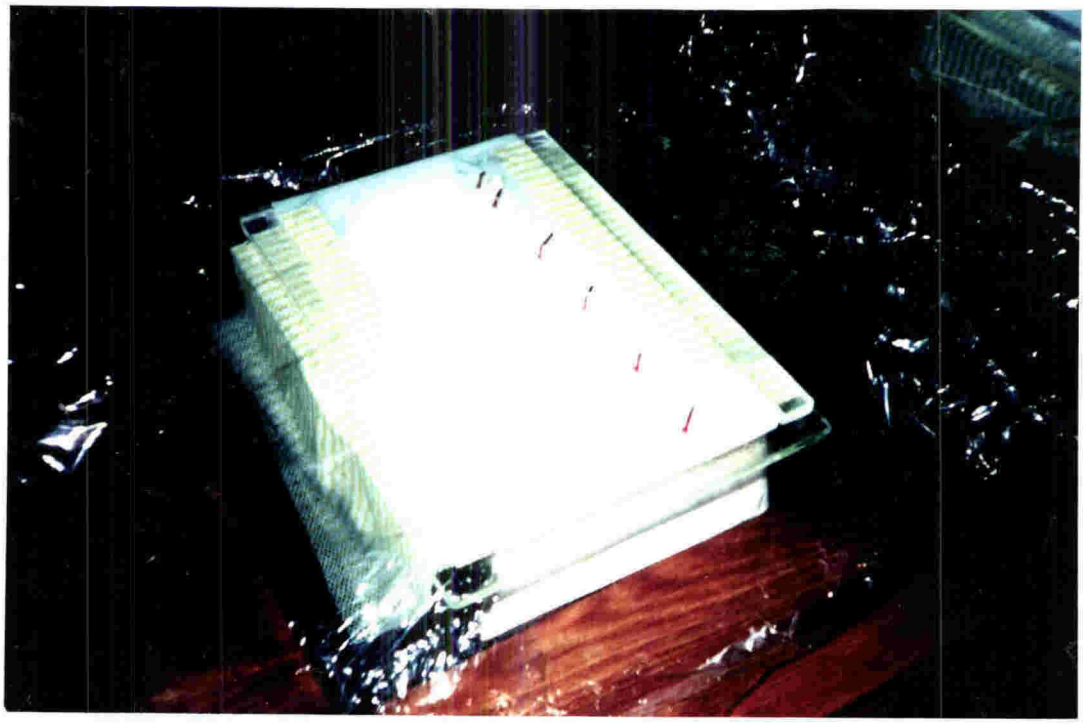
6.2.5.2 The staining of gel slices

The staining solution contained the substrate on which the enzyme acted to form a coloured end product which could be detected visually. Coloured bands formed only when the enzyme was present. The gel slices were incubated in the stain mixture at 37°C. The enzyme stains selected for trial included those in Allendorf et al. (1977) which were within budget limitations.

Enzymes were named using a standard abbreviation of their systematic or trivial name (table 6.4). The following cytochemical stains (table 6.4) were attempted in the pilot study using *Bulbinella* seed and tuber and tissues (leaves, root

Figure 6.1. Loaded gel on ice. Note 1mm gaps between consecutive sample wicks and red dye marker wicks at intervals.

Figure 6.2. Loaded gel ready to begin run. The entire assembly (gel, chuxcloth buffer wicks and trays of electrode buffer) was covered with plastic wrap.



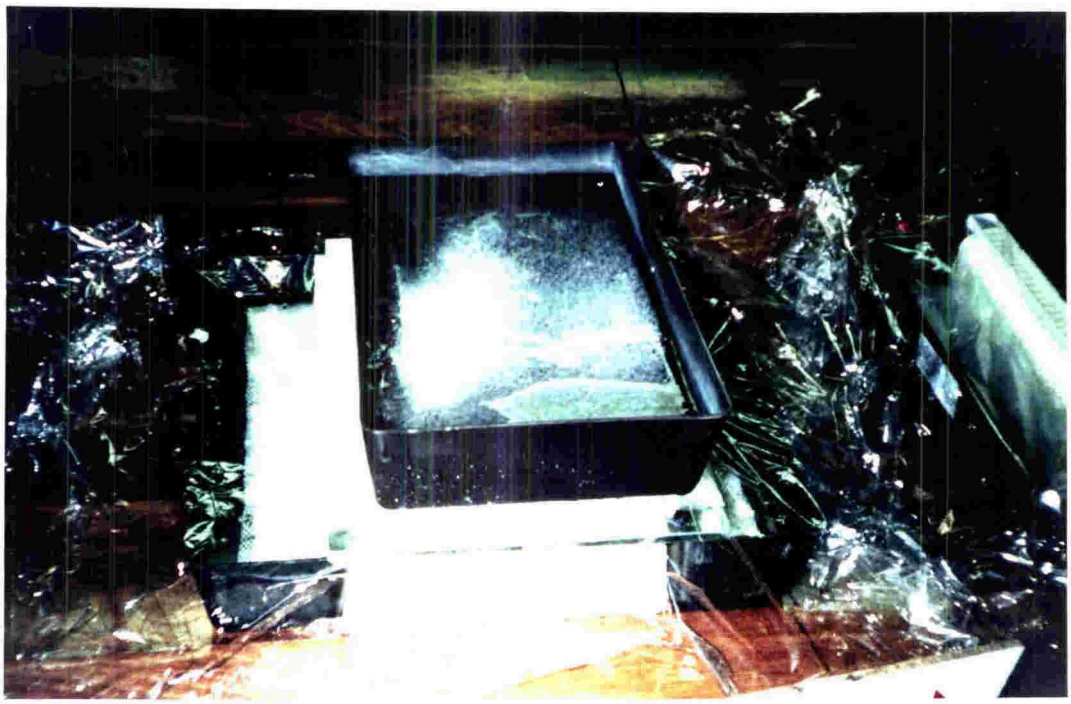


Table 6.4. Enzyme Commission (E.C. 1973) names and numbers of enzymes examined in the pilot study using *Bulbinella* seed and tuber and tissues (leaf, root and stem) from related genera, and the secondary study using the full range of *Bulbinella* tissues. Legend: The recipes for the staining protocols were taken from the following references: [1] = Allendorf et al. 1977, [2] = Shaw and Prasad 1970, [3] = Harris and Hopkinson 1976, [4] = Richardson et al. 1986, [5] = Cheliak and Pitel 1984, [6] = Soltis et al. 1983, [7] = Shields et al. 1983 and [8] = modified from Merril et al. 1982.

Abbrev-iation	Name / Reference	E.C. number
AAT / GOT	glutamate-oxaloacetate transaminase / aspartate aminotransferase[1]	2.6.1.1
ACON	aconitase[3]	4.2.1.3
ACP	acid phosphatase[2]	3.1.3.2
ADH	alcohol dehydrogenase[1]	1.1.1.1
AK	adenylate kinase[1]	2.7.4.3
ALD	aldolase[1]	4.1.2.13
ALP	alkaline phosphatase[3]	3.1.3.1
AMY	α-amylase[3]	3.2.1.1
AO	aldehyde oxidase[4]	1.2.3.1
CAT	catalase[3]	1.11.1.6
CPK	creatine phosphokinase[3]	2.7.3.2
DIA	diaphorase[1]	1.6.*.*
EST	esterases[1]	3.1.1.-
FUM	fumarase[1]	4.2.1.2
GAL	β-galactosidase[3]	3.2.1.23
GAP	glyceraldehyde-3-phosphate dehydrogenase[1]	1.2.1.12
GDH	glutamate dehydrogenase[1]	1.4.1.3
GLUDH	glucose dehydrogenase[4]	1.1.1.47
GO	glucose oxidase[7]	1.1.3.4
GP	general proteins[4]	-
G3P	glycerol-3-phosphate dehydrogenase[3]	1.1.1.8
G6PDH	glucose-6-phosphate dehydrogenase[1]	1.1.1.49
GPI	glucose-phosphate isomerase[1]	5.3.1.9
GPT	glutamate pyruvate transaminase / alanine aminotransferase[1]	2.6.1.2

Table 6.4 continued

Abbrev-iation	Name / Reference	E.C. number
GR	glutathione reductase[2]	1.6.4.2
GUS	β-glucuronidase[1]	3.2.1.31
HBDH	β-hydroxybutyrate dehydrogenase[4]	1.1.1.30
HK	hexokinase[1]	2.7.1.1
ICD	isocitrate dehydrogenase[1]	1.1.1.42
LAP	leucine aminopeptidase[1]	3.4.11.1
LDH	lactate dehydrogenase[1]	1.1.1.27
MDH	malate dehydrogenase[1]	1.1.1.37
ME	malic enzyme[1]	1.1.1.40
MNR	menadione reductase[5]	1.6.99.2
MO	monoamine oxidase[5]	-
MPI	mannose-phosphate isomerase[1]	5.3.1.8
NADHDH	nicotinamide adenine dinucleotide dehydrogenase[5]	1.6.99.3
NP	nucleoside phosphorylase[1]	2.4.2.1
PC	phosphoenolpyruvate carboxylase[5]	4.1.1.31
PEP	peptidases[1]	3.4.11 or 13
PEPS	pepsinogen[2]	3.4.23.*
PER	peroxidase[2]	1.11.1.7
6PGD	6-phosphogluconate dehydrogenase[1]	1.1.1.44
PGK	phosphoglycerate kinase[1]	2.7.2.3
PGM	phosphoglucomutase[1]	2.7.5.1
PHOS	phosphorylase[7]	2.4.1.1
SDH	sorbitol dehydrogenase[1]	1.1.1.14
silver	sensitive general protein stain[8]	-
SKDH	shikimic acid dehydrogenase[6]	1.1.1.25
SOD	superoxide dismutase[1]	1.15.1.1
SUCDH	succinate dehydrogenase[1]	1.3.99.1
XDH	xanthine dehydrogenase[1]	1.2.1.37

tips and stems) from other members of Liliaceae: AAT, ACON, ACP, ADH, AK, ALD, CPK, DIA, EST, FUM, GAP, GDH, GP, G3P, G6PDH, GPI, HK, ICD, LAP, LDH, MDH, ME, MPI, NP, PEP, PER, 6PGD, PGM, SDH, SKDH, SOD, SUCDH and XDH. The following cytochemical stains (table 6.4) were attempted in the secondary study using the full range of *Bulbinella* tissues: AAT, ACON, ACP, ADH, AK, ALP, AMY, AO, CAT, DIA, EST, FUM, GAL, GAP, GDH, G3P, G6PDH, GLUDH, GO, GP, GPI, GPT, GR, GUS, HBDH, ICD, LAP, LDH, MDH, ME, MNR, MO, MPI, NADHDH, PC, PEP, PEPS, PER, 6PGD, PGK, PGM, PHOS and silver. A number of stain recipes [selected from Allendorf et al. 1977, Brewer 1970, Cheliak and Pitel 1984, Conkle et al. 1982, Harris and Hopkinson 1976, Littler and Littler 1985, Richardson et al. 1986, Shaw and Prasad 1970, Shields et al. 1983, Siciliano and Shaw 1976, Soltis et al. 1983 and Stuber and Goodman 1983] were tested for staining quality for each of the following enzymes: ADH, GDH, G6PDH, GPI, ICD, MDH, 6PGD and PGM. When differences in staining quality were demonstrated, the recipe giving the best resolution of loci and staining most intensely in the shortest time was adopted for routine use (appendix VII). When staining quality was considered equally good using more than one recipe, the cheapest one was adopted. Gels with the buffer systems AC, TC-2, TC-3 and RW (table 6.3) which had been loaded with *Bulbinella* flower samples were used to test the stain recipes.

6.2.5.3 Precautions taken during the laboratory procedures

As a large proportion of the chemicals used in different stages of the technique were toxic, possible carcinogens, or of unknown status; plastic, disposable gloves

were worn when handing chemicals or gels. Disposable masks were used when working with powders which tended to form an aerosol or irritating dust.

The power packs were turned off when gels were handled or adjusted, for example during the removal of sample wicks.

6.2.5.4 Recording

Activity (+) or lack of activity (-), were the only data recorded from the pilot study involving analysis of *Bulbinella* seed and tuber and tissues (leaf, root and stem) of related genera. The banding pattern for each successful stain was sketched, and the buffer system(s) producing the best resolution of loci were recorded from the secondary study involving the full range of *Bulbinella* tissues.

6.2.5.5 Storage of gels

Gels could remain in a 1:1 destaining solution (appendix VII): water mixture in the stain trays for several months without fading of the bands occurring. For more permanent storage of a large number of gels, plastic bags were used. The gels were blotted between paper towels to remove excess moisture and then placed into suitably sized plastic bags. The open edges were then sealed using a strip heat sealer. The bags were labelled and placed in a refrigerator, on a flat surface, under a heavy glass plate. Gels remained useful for reference for a year or sometimes longer under these conditions.

6.3 Results and discussion

6.3.1 Results of the pilot study using *Bulbinella* seed and tuber and tissues (leaf, root and stem) from related genera

Activity was detected in one or more of the five tissues for 17 of the 33 stains attempted (table 6.5). The following enzyme stains produced no activity with any of the tissues examined: AAT, ACON, AK, ALD, CPK, FUM, GP, HK, LAP, LDH, ME, NP, PER, SKDH, SUCDH, and XDH.

6.3.1.1 Tubers

Tuber tissue was tough and very difficult to grind and the resulting supernanant was extremely viscous. Enzyme activity, when present (table 6.5), appeared as poorly resolved stained areas of gel. Neither grinding buffer was superior in resolution. No clear bands which could be interpreted as Mendelian loci were obtained for any of the enzyme stains.

6.3.1.2 Seeds

Enzyme activity was detected in seeds for 13 of the 36 enzyme stains attempted (table 6.5). Of the stains which showed activity, some were sporadic (appeared irregularly) or faint. Those enzymes which stained consistently and were therefore the most useful were: ADH, GDH, GPI, ICD, MDH, 6PGD and PGM. Usually the stains ADH, GDH, ICD and 6PGD required overnight incubation before the loci became visible. One fixed difference was established between the two species *(B. hookeri* and *B. gibbsii)* analysed. Results obtained using the two grinding buffers were equally clear and both were superior to those obtained using distilled water.

Table 6.5. Results of the pilot study of enzyme activity in Liliaceae. Legend: + denotes actvity, and - denotes no activity detected.

Stain	Tissue				
	Bulbinella		Taxa other than *Bulbinella*		
	Seed	Tuber	Leaf	Root tips	Stems
ACP	+	+	-	+	-
ADH	+	+	-	+	+
DIA	-	+	-	-	-
EST	-	+	-	-	-
GAP	+	-	+	+	-
GDH	+	-	+	+	+
G3P	+	+	-	-	-
G6PDH	+	-	+	+	+
GPI	+	+	+	+	+
IDH	+	+	+	+	+
MDH	+	+	+	+	+
MPI	+	-	+	+	+
PEP	+	+	-	-	-
6PGD	+	+	+	+	+
PGM	+	-	+	+	+
SDH	-	+	-	-	-
SOD	-	+	-	-	-

Prepared seed samples could be used for one day's scheduled run only. Loci were very faint or completely absent if the samples were subjected to a further freeze/thaw cycle.

6.3.1.3 Related genera

Darkly-staining bands which appeared the same day were obtained from tissues (young leaves, root tips and stems) of other members of Liliaceae, especially from young leaves and root tips (table 6.5). Thus it was useful to include these tissues in the secondary study using only *Bulbinella* material. Results obtained using either of the grinding buffers were superior to those obtained using distilled water. Prepared samples withstood a further freeze/thaw cycle to give bands with identical mobilities on a subsequent run.

6.3.2 Results of the secondary study using *Bulbinella* tissues

For 10 of the 43 stains attempted, activity was detected in one or more of the seven tissues analysed (table 6.6). Activity which could not be resolved sufficiently or which was too faint to be useful was found for ACON, ALP, EST and PEP. No activity was found for the following enzyme stains: AAT, ACP, AK, AMY, AO, CAT, DIA, FUM, GAL, GLUDH, GO, GP, G3P, GPT, GR, GUS, HBDH, LAP, LDH, ME, MNR, MO, NADHDH, PC, PEPS, PER, PGK, PHOS and silver.

All the loci observed in young leaf buds, bracts, root tips and other tissues were also demonstrated in flower buds, in which additional loci were detected (table 6.6). Therefore of all the tissues screened (table 6.6) it was neccessary to analyse only flower buds to obtain the full range of detectable loci for each individual to be genotyped. Both of the grinding buffers and distilled water produced loci with

Table 6.6. The allozyme activity and loci resolved in New Zealand *Bulbinella*. Legend: + denotes activity and - denotes no activity detected. The number in parentheses denotes the number of putative loci.

Tissue	Stain									
	ADH	**GAP**	**GDH**	**G6PDH**	**GPI**	**ICD**	**MDH**	**MPI**	**6PGD**	**PGM**
mature leaf	-	-	-	-	-	-	-	-	-	-
young leaf	-	-	+(1)	-	+(2)	+(1)	+(2)	+(1)	-	+(1)
root tip	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(3)	+(1)	+(1)	+(1)
stem	-	-	+(1)	-	+(2)	+(1)	+(2)	+(1)	-	+(1)
pedicel	-	-	+(1)	-	+(2)	+(1)	+(2)	+(1)	-	+(1)
bract	-	-	+(1)	-	+(2)	+(1)	+(2)	+(1)	-	+(1)
flower	+(1)	+(1)	+(1)	+(1)	+(2)	+(2)	+(3)	+(1)	+(2)	+(2)
large bud	+(1)	+(1)	+(1)	+(1)	+(2)	+(2)	+(3)	+(1)	+(2)	+(2)
small bud	+(1)	+(1)	+(1)	+(1)	+(2)	+(2)	+(3)	+(1)	+(2)	+(2)
pollen	-	-	-	-	-	-	-	-	-	-
seedling leaf	-	-	+(1)	-	+(2)	+(1)	+(2)	+(1)	-	+(1)
seedling root	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(3)	+(1)	+(1)	+(1)

identical mobility, resolution and staining intensity when used for sample preparation. The cheaper phosphate buffer was selected for routine use. Although results were obtained using distilled water, it was considered good practice to use a buffer to give samples of dubious quality or dilute ones the best chance of producing a result.

The mobilities of corresponding loci from the same individual using flowers of different ages were identical. Open (older) flowers gave fainter results sometimes. Loci observed in young vegetative buds from adult plants were also present but fainter and less clearly resolved in bracts, pedicels and peduncles from the same individual. The loci present in leaves and roots of newly germinated seedlings showed the same mobility and clarity as those in the corresponding tissues of adult plants. Because of the additional difficulty and time delay involved in germinating seed (Chapter 4) the use of seedling tissue was not pursued.

6.3.3 Conclusions pertaining to future analysis of *Bulbinella* tissue

The most suitable tissue for analysis in *Bulbinella* proved to be flowers. Therefore inflorescences were the only samples required to be collected from wild populations for allozyme analysis. It was considered good practice to use flower **buds** for the analyses when there was a choice of material. Large buds gave the best results and were easier to handle. Where open flowers were the only material available the youngest ones were selected.

Because the genetic basis of polymorphism has been proven repeatedly (for example Rajora 1990, Parks et al. 1990, Samuel et al. 1990 and Tsmura et al. 1989) for other plant taxa studied, this was also assumed to apply to the variation detected in *Bulbinella*.

During the next few scheduled laboratory runs, loci which were smeared or faint, depended on the concentration of the sample, or could not be consistently resolved in all populations were discarded. The presumptive loci Gap-1 and Mpi-1 appeared inconsistently and were therefore omitted from subsequent analyses.

A standard laboratory protocol to be used for screening the plant populations was compiled (table 6.7). The buffer system(s) producing the best resolution of loci and alleles for each enzyme stain were selected for routine use. Some loci could be resolved from gels other than those indicated (*S). The marked (*S) gels were preferred because they were the clearest and the easiest to assess for the particular enzyme. It was necessary to use four buffer systems every time laboratory work was scheduled in order to resolve all the detectable loci. A minimum of two slices was routinely stained for each enzyme because occasional samples gave no result on one of the slices. Eight stains giving 14 consistently clear loci were to be employed on a regular basis.

Of the stain recipes tested, the one with which the best (clearest and fastest) result was obtained (appendix VII) was to be used for subsequent analyses.

Loci with the same mobility, resolution and intensity of staining were obtained using prepared flower samples which had undergone a further freeze/thaw cycle. Such samples could therefore be confidently used as controls for a later run.

Table 6.7. Standard laboratory protocol for the routine analysis of *Bulbinella* flower tissue. Legend: * indicates that a slice of this gel was stained for this enzyme; *S indicates that the clearest resolution for this enzyme system was produced on this slice.

Stain	Buffer	System	
	AC	TC-2	TC-3	RW
ADH	*			*S
GDH	*		*	*S
G6PDH	*		*	*S
GPI	*		*	*S
ICD	*S		*	*
MDH	*	*S	*S	
6PGD		*S		
PGM	*S	*		

CHAPTER 7: GEOGRAPHIC VARIATION in ALLOZYMES of *BULBINELLA* in NEW ZEALAND

7.1 Introduction

The last systematic review of *Bulbinella* was conducted in the 1960s (Moore 1964) based on techniques in use since at least the nineteenth century. Since this time new techniques for both data collection and analysis have been developed which are now used routinely in systematics. The widespread use of these modern methods of data collection and analysis has removed much of the subjectiveness encountered previously. Thus, two workers given the same data set can be expected to arrive at similar results.

A relevant assessment of conservation status requires good systematics (Avise 1989). An understanding of the way in which genetic variation is partitioned among populations is of prime importance for the conservation of genetic diversity of species. Surveys of allozyme variation provide data that are critical for the establishment of management strategies designed to preserve genetic diversity (Hamrick and Godt 1990).

The aim of this section of the study is to describe the patterns of geographic variation in allozymes for as many populations of *Bulbinella* in New Zealand as possible, and to relate these patterns to present taxonomy. This was achieved by:

(1) locating plant populations and assessing the best time of the year in which to collect samples,

(2) obtaining samples of the most suitable tissue (inflorescences, table 6.6) for analysis,

(3) maintaining the enzymes in these samples during extensive field work and

(4) preparing and analysing the samples following the guidelines established in Chapter 6.

7.2 Materials and methods

7.2.1 Samples

The populations from which inflorescences were collected were the same as those used to study habitat reduction (Chapter 3, fig. 3.1). Inflorescence material was obtained from a total of 61 populations. The number of samples (the number of individual plants from which floral material was obtained) collected from each population varied from 1 - 51 (table 7.1). While some pairs of populations of different species are geographically relatively close (fig. 3.1), they were physically separated by considerable distances (in most cases) and by steep terrain. Examples of such pairs of populations were Purity Hut (population number 9), altitude 1120m, and Whanahuia Range (17), 1500m, which were separated by 10km and several deep gullies; Gouland Downs (35), 576m, and Cobb Valley (10), 819m, which were separated by 35km and several mountain ranges, and Mount Fox (22), 1213m, and Okarito Swamp (33), almost at sea level, which were separated by 35km and steep terrain.

Floral material from South African plants was not analysed because the *Bulbinella* plants sent to New Zealand from Africa died before this section of the study was undertaken.

Table 7.1. Genetic variability at 14 loci in 61 natural populations of *Bulbinella* in New Zealand. The population numbers correspond to those in table 3.1. Legend: The taxa are: [h] = *B. hookeri*, [b] = *B. gibbsii* var. *balanifera*, [g] = *B. gibbsii* var. *gibbsii*, [r] = *B. rossii*, [m] = *B. modesta*, [t] = *B. talbotii* and [a] = *B. angustifolia*. %P = percent polymorphic (variable) loci (0.05 criterion). n = mean or average number of alleles per locus. $\hat{H}$ = unbiased estimate of heterozygosity (Nei 1978).

Population number & species identity	Number of plants examined	Polymorphic loci (%P)	Mean number of alleles / locus (n)	Unbiased heterozygosity ($\hat{H}$)
1[h]	11	21	1.21	0.06
2[h]	18	21	1.21	0.08
3[h]	10	21	1.21	0.08
4[h]	10	21	1.21	0.08
5[h]	11	7	1.29	0.06
6[h]	09	21	1.21	0.06
7[h]	15	7	1.14	0.04
8[h]	08	7	1.07	0.01
9[h]	17	29	1.36	0.09
10[h]	34	36	1.50	0.15
11[h]	05	14	1.14	0.05
12[h]	01	0	1.00	0
13[h]	15	21	1.21	0.09
14[h]	06	29	1.29	0.09
15[h]	03	21	1.29	0.12
16[h]	41	14	1.29	0.03
17[b]	20	36	1.36	0.10
18[b]	04	29	1.29	0.07
19[b]	16	14	1.21	0.05
20[b]	05	0	1.0	0
21[b]	07	14	1.14	0.02
22[b]	02	0	1.0	0
23[b]	16	0	1.0	0
24[b]	12	29	1.36	0.09

Table 7.1 continued

Population number & species identity	Number of plants examined	Polymorphic loci (%P)	Mean number of alleles / locus (n)	Unbiased heterozygosity ($\hat{H}$)
25[b]	14	29	1.43	0.11
26[b]	19	36	1.43	0.15
27[b]	03	7	1.07	0.04
28[b]	11	21	1.21	0.07
29[g]	01	0	1.00	0
30[r]	06	14	1.14	0.07
31[m]	26	36	1.64	0.14
32[m]	51	36	1.57	0.10
33[m]	25	14	1.36	0.08
34[m]	19	29	1.29	0.12
35[t]	18	21	1.36	0.08
36[t]	07	14	1.14	0.04
37[a]	17	21	1.21	0.05
38[a]	12	7	1.07	0.02
39[a]	16	14	1.21	0.03
40[a]	09	0	1.0	0
41[a]	10	0	1.0	0
42[a]	13	0	1.0	0
43[a]	12	0	1.07	0.01
44[a]	08	0	1.0	0
45[a]	25	7	1.07	0.01
46[a]	21	7	1.14	0.02
47[a]	29	21	1.21	0.08
48[a]	23	7	1.14	0.02
49[a]	17	14	1.14	0.05
50[a]	21	14	1.21	0.04

Table 7.1 continued

Population number & species identity	Number of plants examined	Polymorphic loci (%P)	Mean number of alleles / locus (n)	Unbiased heterozygosity ($\hat{H}$)
51[a]	04	14	1.21	0.07
52[a]	23	14	1.29	0.04
53[a]	25	21	1.36	0.07
54[a]	11	21	1.29	0.06
55[a]	12	14	1.21	0.06
56[a]	10	36	1.36	0.12
57[a]	08	21	1.21	0.06
58[a]	04	21	1.21	0.05
59[a]	13	21	1.21	0.08
60[a]	20	29	1.36	0.08
61[a]	11	14	1.21	0.06

7.2.1.1 Collection and treatment

Most of the inflorescence samples obtained for the study of geographic variation were collected from wild plants. At some locations poor weather conditions made sites unsafe to revisit for the collection of inflorescences [Mount Fox (22)]. As actual flowering times varied both within and between species (Chapter 2), some populations were not in flower or even in bud when revisited for inflorescence collection [Carroll Hut (20) and Red Hills (11)]. In situations such as these, inflorescences from plants growing in cultivation (but originally obtained from wild populations) were the only material available for analysis.

When inflorescences were collected from wild plants, the stems were cut with scissors and the flower heads placed in a vessel with damp paper in the base. An old plastic bottle with an opening cut in the side was suitable. Flower material could be kept fresh like this for more than 24 hours if necessary, but samples were normally processed well before that. Upon return to base, the stem was removed and each inflorescence was placed in one or more labelled AA tubes. Invisible tape was used to keep the lids securely on the tubes, which were then placed in a dewar of liquid nitrogen. The samples remained in this dewar for the duration of the field work. Upon returning to the laboratory, the samples from liquid nitrogen were sorted on crushed ice to reduce sudden warming.

Inflorescences obtained from plants in cultivation were placed in labelled AA tubes and kept in a refrigerator (4°C) for up to one hour then placed in a vacuum flask with crushed ice for transport to the laboratory. In the laboratory inflorescences were stored ultra-frozen at -80°C until required.

7.2.1.2 Preparation for analysis

Samples were prepared on a tray of crushed ice in order to reduce the rate of denaturation (fig. 7.1). Individual buds were carefully removed from each inflorescence using forceps and placed in another labelled AA tube. The largest unopened flowers were preferred since they contained the greatest amount of tissue. Five or six large buds plus three drops of grinding buffer gave a sample of a suitable size. The volume of grinding buffer could easily be reduced if less plant material was available. Approximately equal volumes of tissue and buffer were used. The flower buds were ground with the buffer in the AA tubes using a polystyrene instrument (Chapter 6). The prepared samples were stored ultra-frozen in these AA tubes until required. After the selected buds had been removed, the remainder of the inflorescence was returned to the -80°C freezer. If further samples were required, the process could be repeated.

7.2.2 Allozyme analysis

7.2.2.1 Basic work pattern

All individuals sampled from all populations of each taxon were analysed before material from other taxa was attempted. This protocol reduced confusion and hence the possibility of scoring mistakes (assigning the wrong genotype to an individual). Most variation is found between rather than within taxa. The taxa were analysed in the following order: *B. hookeri, B. gibbsii* var. *balanifera, B. gibbsii* var. *gibbsii, B. rossii, B. modesta, B. talbotii* and *B. angustifolia.* The populations belonging to each taxon were analysed in a north to south direction according to their geographical location (table 7.1 and fig. 3.1). This orderly analysis made comparison of the results more simple because controls were easier to arrange on gels.

7.2.2.2 Modifications made to technique

The following modifications were found to be useful in relation to the laboratory work.

1. The use of four layers of chuxcloth instead of two increased the current and hence the speed of the run. Results were comparable to those obtained using two layers of cloth. Excessive heat buildup did not occur.

In some cases the use of thicker wicks enabled the gels to be run the standard distance or further within a convenient time period (four hours). This was particularly useful for PK, RW and TC3, which took about six hours or longer to run the prescribed distance when using only two layers of cloth.

2. Individual gel slices could be enclosed in plastic wrap unstained, ultra-frozen and stained at a future time. A disadvantage compared to staining the fresh slice was that one further freeze/thaw cycle was applied to the enzymes. The different stains responded variously to this treatment with some better than fresh slices and some not as clear. However, most frozen slices were scorable after staining. The method nearly always avoided repeating an entire day's work because one stain failed.

7.2.2.3 Recording of results

The banding patterns for each stain were sketched as soon as they were clearly visible. Interpretation of the patterns (number of loci and number of alleles per locus) for each stain was achieved following a literature search to determine what had been found in previous plant studies for comparison with the patterns obtained for *Bulbinella*. Individual bands of the same mobility in different individuals were considered to represent the same Mendelian allele. Single-banded individuals were recorded as homozygotes, while individuals in which multiple bands were detected

were recorded as heterozygotes. The banding pattern obtained in heterozygotes for any one locus depended on the quaternary structure (number of component subunits) of the enzyme [tetramer(4), dimer(2) or monomer(1)] (Richardson et al. 1986 p. 23) and the distance separating the two component alleles on the gel. In heterozygotes for a dimeric enzyme, three bands (the two alleles and their interaction product) were produced on a gel following electrophoresis. If the component alleles were sufficiently separated by distance on the gel, three clear bands were visible. However, if the two alleles had similar mobilities, resolution was not sufficiently sharp to detect three distinct bands. All the enzymes examined in this study were dimers, except PGM which was monomeric. Loci were scored tentatively following analysis of the first few populations. Sometimes interpretations changed as new variations were detected, in which case all previously scored individuals were rescored according to the latest system.

As a permanent record, photographic transparencies were taken of most gels after they had been in destaining solution for about 10 minutes, when the background was palest and the banding pattern darkest.

7.2.2.4 Quality control

(A) Sample wicks were blotted thoroughly between two sheets of absorbent paper towel to remove excess liquid and any pieces of plant tissue which had adhered. This reduced the chance of material from one sample contaminating the neighbouring ones (carryover).

(B) A previously analysed sample of established genotype (control) was placed in the middle of every group of 10 samples for routine screening (that is between samples 5 and 6, 15 and 16 and so on) for all runs. This compensated to a certain

extent for a number of variables associated with the technique such as slight differences in the pH of buffers between successive runs. The time taken for the buffer front to travel the set distance varied, and the buffer front was often slightly uneven. The inclusion of controls compensated for these factors.

(C) Within a run, some of the samples (usually about five) from the beginning of the gel were placed again near the end. This was useful to compensate for differences in the distance travelled by the buffer front at the two ends of the gel.

(D) A large number of samples were analysed more than once during the course of the study. Some were used as controls in (B) above. The results of these were checked against the original genotype and any discrepancies were resolved. Other samples were rerun because they gave only faint bands on the first occasion, or because their genotype was in some way different from the rest of their population.

(E) Line-up (control) gels were run at intervals to check tentative scoring. Individuals with the same presumptive genotype for a particular locus, determined by a previous run, were placed side by side to confirm that they gave identical results. Samples were loaded with those having the most cathodal alleles at the beginning of the gel, the least cathodal at the end and any heterozygotes in the middle. Control gels were essential to resolve difficulties in scoring.

7.2.3 Analysis: statistical methods

Statistical calculations were performed using the BIOSYS-1 computer programme of Swofford and Selander (1981).

Three measures of genetic variability which have been of value in other plant work (Morikawa and Leggett 1990) were used in this study: the degree of polymorphism (% variable loci), mean number of alleles per locus and

heterozygosity. The degree of polymorphism, P, is the proportion of loci which are polymorphic, expressed as a percentage. A polymorphic locus was defined as one in which the frequency of the most common allele was <0.95. The number of alleles per locus, n, is the total number of alleles detected, divided by the number of loci.

Heterozygosity at one locus is the estimated proportion of individuals having two alleles at that locus. Mean heterozygosity over all loci, H, the most commonly used measure of heterozygosity in a population, is the mean proportion of heterozygous loci per individual, or the proportion of individuals heterozygous over all loci. H can be calculated directly from the observed proportion of heterozygous individuals ("direct count" or "observed heterozygosity", Ho,) or as the expected level of heterozygosity (H_s) calculated from gene frequencies, p_i, and assuming the population to be in Hardy-Weinberg equilibrium ($H_s = 1-\sum p_i$). Nei (1978) recommended that an alternative expected heterozygosity, the unbiased estimate, $\hat{H}$, should be used when the number of individuals sampled is small. This was considered appropriate for *Bulbinella* since Hardy-Weinberg proportions could not be assumed.

Regression analysis was used to test for a relationship between the number of plants sampled and the unbiased heterozygosity.

Chi square tests were performed to check for conformity to the Hardy-Weinberg equilibrium where the sample size was large enough (20 or more).

The following F-statistics (fixation indices, Nei 1977) were calculated. F_{IS}, the inbreeding coefficient, is near zero for a panmictic population, positive for an inbreeding population and negative for an outcrossing population. F_{ST} measures the amount of differentiation among sub-populations. Various taxonomic groupings were defined as 'sub-populations':

(1) The F_{IS} value was calculated for six of the seven taxa described by Moore (1964).

(2) Pairwise F_{ST} values were obtained between the seven taxa described by Moore (1964).

(3) The F_{IS} value was calculated for each of the taxonomic groupings inferred from the allozyme data.

(4) Pairwise F_{ST} values were obtained between the taxonomic groupings in (3).

From the available BIOSYS selection, the methods of Nei (1978) were employed to calculate unbiased genetic similarity (I) and distance (D) coefficients. For any two populations I can range from 0.0 - 1.0. No alleles in common gives a value of 0.0, whereas the same alleles in the same frequencies produce a value of 1.0. Therefore I is the identity of genes between two populations, with $D = - \ln I$. D can range from zero to infinity. Nei's unbiased estimates correct for small sample size. The distribution of D values at different taxonomic levels was examined.

Phenetic cluster analysis was used to further define genetic and taxonomic affinities of all the 61 study populations. Weighted pair-group arithmetic average linkage clustering (WPGMA; Sneath and Sokel 1973) was applied to Nei's (1978) unbiased genetic distances.

7.3 Results and discussion

7.3.1 Variation at individual loci

Fourteen presumed genetic (allozyme) loci could be consistently resolved in all populations (table 7.2). Three (21%) of the loci (G6pdh-1, Gpi-2 and Pgm-2) showed no variation. Variable loci possessed between two and five alleles.

Table 7.2. Number of alleles per locus following starch gel electrophoresis of *Bulbinella* flower extracts.

Locus	Number of alleles
Adh-1	2
Gdh-1	3
G6pdh-1	1
Gpi-1	5
Gpi-2	1
Icd-1	3
Icd-2	2
Mdh-1	4
Mdh-2	4
Mdh-3	4
6Pgd-1	4
6Pgd-2	3
Pgm-1	4
Pgm-2	1

7.3.1.1 Adh-1

Two electromorphs were found (fig. 7.2). In heterozygotes these two alleles and their interaction product appeared two-banded with a fuzzy area between as drawn. However, if gels were run further than the standard distance of 4cm (for example 6cm), three distinct bands were visible in heterozygotes. No Adh-1(bb) homozygote was found.

7.3.1.2 Gdh-1

Three electromorphs were detected (fig. 7.3). In the heterozygotes Gdh-1(ab) and Gdh-1(ac) resolution was not sharp. Because the alleles Gdh-1(b) and Gdh-1(c) were never found in the same population, the heterozygote Gdh-1(bc) was not seen.

7.3.1.3 G6pdh-1

Only one electromorph was detected (fig. 7.4).

7.3.1.4 Gpi-1

Five electromorphs were detected (fig. 7.5). In the heterozygotes Gpi-1(ae) and Gpi-1(ac) three clear bands were always observed, while Gpi-1(bc), Gpi-1(ad) and Gpi-1(ab) sometimes produced three distinct bands but sometimes the resolution was not sufficiently sharp. No other combinations of the five alleles were observed in heterozygotes.

7.3.1.5 Gpi-2

Only one electromorph was detected (fig. 7.5).

Figures 7.2. to 7.6. Schematic representation of the observed banding patterns and allelic and genotypic designations for allozyme loci (indicated by numbers) coding for five of the eight enzyme systems examined in floral tissue of *Bulbinella*. Legend: The line below the enzyme name represents the distance travelled by the buffer front. For ADH, GDH, G6PDH and GPI this was 4cm (RW buffer system), and for ICD this was 6cm (AC buffer system). A "+" indicates the anodal direction. Stippled areas represent diffuse areas of stain. Lower case "o" represents the origin. L = locus and the pairs of lower case letters below the origin are the genotypes assigned to an individual with the banding pattern above. All the banding patterns observed have been sketched. Details of particular loci are explained in the text. Photographs of some of the enzymes are in appendix VIII.

Figure 7.2.

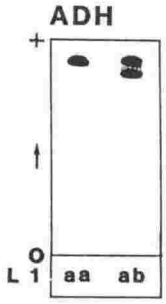


Figure 7.3.

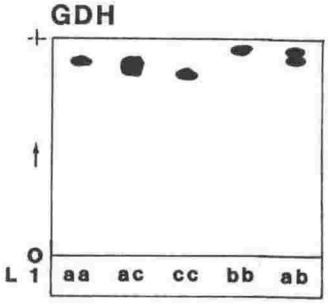


Figure 7.4.

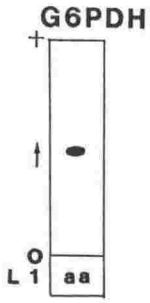


Figure 7.5.

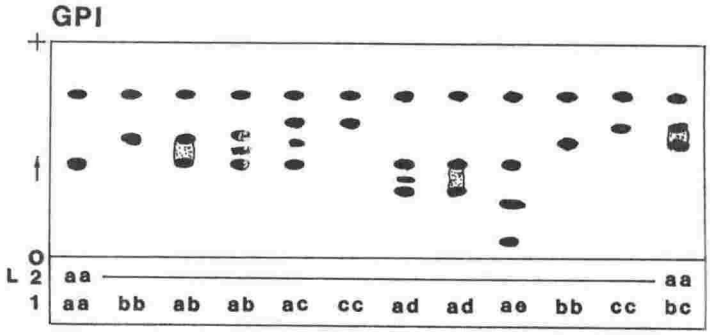
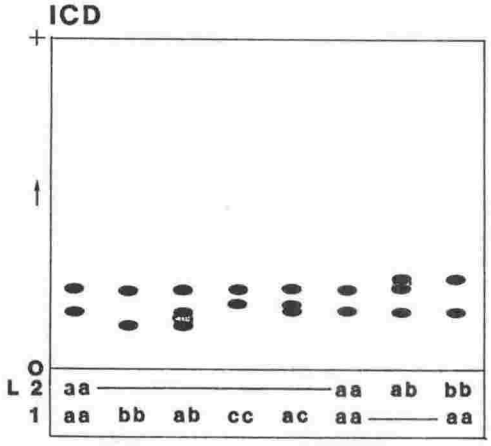


Figure 7.6.



7.3.1.6 Icd-1

Three electromorphs were detected (fig. 7.6). In the heterozygotes Icd-1(ab) and Icd-1(ac) resolution was not sharp. No heterozygotes Icd-1(bc) were seen.

7.3.1.7 Icd-2

Two electromorphs were detected (fig. 7.6). In the heterozygote Icd-2(ab) resolution was poor.

7.3.1.8 Mdh-1, Mdh-2 and Mdh-3

Usually, four zones of activity for MDH were detected (fig. 7.7). Three were interpreted as representing Mendelian loci, and the fourth as an interaction product between loci two and three. This interlocus interaction product was present in all samples with one exception. One plant in one population (Molesworth) was scored as homozygous for Mdh-3(d), which was either a null allele (an allele producing an inactive protein) or an allele which migrated to the same position as Mdh-2(a). Either interpretation accounted for the lack of interaction product in this one plant. Although the other five plants sampled in this population were all assigned the genotype Mdh-3(aa) [no Mdh-3(ad) heterozygotes were detected], some may have in fact been Mdh-3(ad) since according to Hurka (1980) null alleles may be recessive in some cases.

Four electromorphs were detected for each of the three loci (fig. 7.7). In heterozygotes observed for any pairs of alleles for any of the three MDH loci, resolution was not sharp. No three-banded heterozygotes were seen.

Figures 7.7. to 7.9. Schematic representation of the observed banding patterns and allelic and genotypic designations for allozyme loci (indicated by numbers) coding for three of the eight enzyme systems examined in floral tissue of *Bulbinella*. Legend: The line below the enzyme name represents the distance travelled by the buffer front. For MDH and 6PGD this was 5cm (TC buffer system) and for PGM this was 6cm (AC buffer system). A "+" indicates the anodal direction. Stippled areas represent diffuse areas of stain. "I" = interlocus interaction product. Lower case "o" represents the origin. L = locus and the pairs of lower case letters below the origin are the genotypes assigned to an individual with the banding pattern above. All the banding patterns observed have been sketched. Details of particular loci are explained in the text. Photographs of some of the enzymes are in appendix VIII.

Figure 7.7.

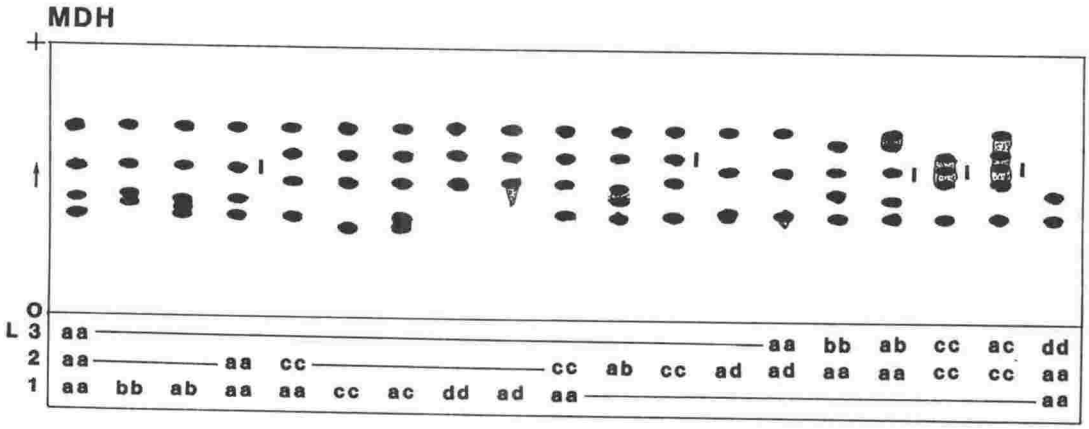


Figure 7.8.

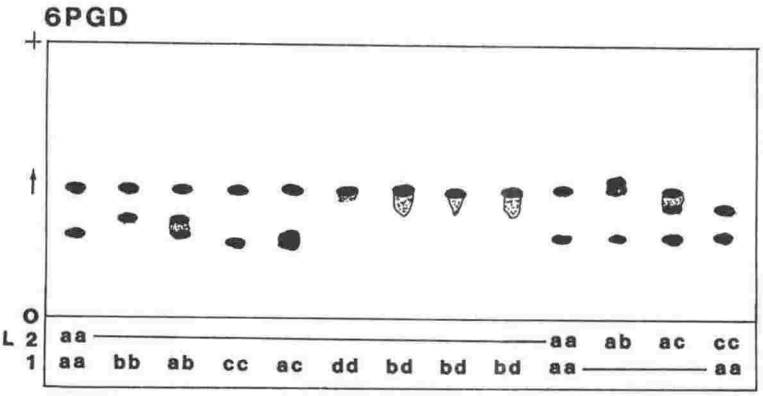
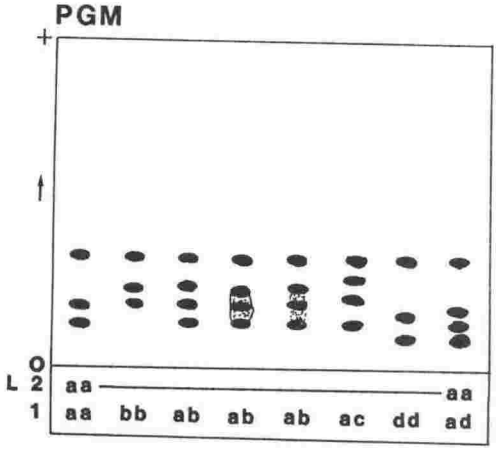


Figure 7.9.



7.3.1.9 6Pgd-1

Four electromorphs were detected (fig. 7.8). In none of the heterozygotes observed was resolution sharp.

7.3.1.10 6Pgd-2

Three electromorphs were detected (fig. 7.8). Resolution was poor in all heterozygotes.

7.3.1.11 Pgm-1

Four electromorphs were detected (fig. 7.9). A degradation product between locus 1 and locus 2 but closer to locus 1 was always observed. In homozygotes for Pgm-1, a total of three bands per sample was present [Pgm-1(homozygote), degradation product and Pgm-2(aa)]. In plants heterozygous for Pgm-1, four bands were seen altogether [Pgm-1(allele 1), Pgm-1(allele 2) degradation product and Pgm-2(aa)]. All the heterozygotes observed therefore appeared three-banded, as drawn, because of the presence of this degradation product.

7.3.1.12 Pgm-2

Only one electromorph was detected (fig. 7.9).

7.3.2 Genetic variability

Over all populations the % polymorphic loci (%P) ranged from 0 - 36%, the mean number of alleles (n) ranged from 1.0 - 1.64 and the unbiased heterozygosity ($\hat{H}$) ranged from 0 - 0.15 (table 7.1). Mean values of $\hat{H}$ for each species ranged from 0 - 0.11 (table 7.3), lower than the mean for 28 species of grasses found by Hamrick

Table 7.3. Mean genetic variability in *Bulbinella* in New Zealand. Legend: The number in brackets is the number of populations examined. %P = percent polymorphic (variable) loci (0.05 criterion). n = mean or average number of alleles per locus. $\hat{H}$ = unbiased estimate of heterozygosity (Nei 1978). The data for Monocotyledoneae (all grasses) were obtained from a review where the mean number of loci was 11.6, with a range of 3-28 (Hamrick et al. 1979).

Species or Group Name	**Total number of plants examined**	**Mean %P**	**Mean n**	**Mean $\hat{H}$**
B. hookeri (16)	214	18	1.23	0.07
B. gibbsii var. *balanifera* (12)	129	18	1.21	0.06
B. gibbsii var. *gibbsii* (1)	1	0	1.00	0
B. rossii (1)	6	14	1.14	0.07
B. modesta (4)	121	23	1.47	0.11
B. talbotii (2)	25	18	1.25	0.06
B. angustifolia (25)	374	14	1.18	0.04
Monocotyledoneae	number of species = 28	40	2.11	-

et al. (1979). For all parameters, *B. modesta* showed the highest and *B. angustifolia* the lowest value, again lower than found in grasses.

Genetic variation in plants varies non-randomly, being associated with certain ecological and life history characteristics (Hamrick et al. 1979). Species characterised by large ranges, high fecundities, an outcrossing mode of reproduction, wind pollination, a long generation time and from habitats representing later stages of succession have more genetic variation than do species with contrasting characteristic profiles. Gymnosperms were more variable than monocots or dicots (Hamrick et al. 1979), probably because their life histories demonstrate a number of the factors associated with high variability. The 28 monocots reviewed (Hamrick et al. 1979), which were all grasses, showed a higher variability than the dicots reviewed. The mean number of loci examined in these 28 grasses was 11.6. with a range of 3 - 28. However, the higher variability of grasses (which are almost invariably wind pollinated) may not be representative of monocots as a whole. These mean parameters (for grasses) were higher than those obtained in the current study (table 7.1), in which four of the seven *Bulbinella* taxa are hermaphroditic, and all seven taxa are pollinated by insects primarily (Chapter 2). Some species (notably *B. angustifolia* and *B. modesta*) have had their ranges reduced over the past three decades (Chapter 3). Some populations of the taxon *B. gibbsii* var. *balanifera* do not set seed every season (Chapter 2), thus reducing their fecundity. The lower variability found in *Bulbinella* reflects these life history characteristics which differ from those of plants which typically display a higher variability.

Genetic variation within natural plant populations has a patchy distribution, which is sometimes associated with environmental factors, may be influenced by selection and sometimes results from limited pollen or seed dispersal (Hamrick and

Godt 1990). It is likely that pollen and seed dispersal is limited in *Bulbinella* (Chapter 2). According to Hamrick et al. (1979) plants display levels of variability roughly equivalent to those of invertebrate animals, but considerably higher than those of vertebrate species. Other reviews (for example Brown 1978) indicate that generally a high level of polymorphism is observed in most plant species. Monomorphic natural populations are more common among inbreeding colonisers or at extreme limits of distribution.

Bayer and Crawford (1986) who studied five species of *Antennaria* (dicot), found lower levels of variability (P and n) than those associated with other dicots, more comparable to those detected in *Bulbinella*. *Antennaria* is also an herbacious perennial. However, all the diploid species of this genus were dioecious in contrast to *Bulbinella* where most species are hermaphroditic.

Regression analysis revealed a weak positive correlation between sample size and unbiased heterozygosity (fig. 7.10, correlation coefficient [r] = 0.34). The regression coefficient (slope of the line) was 1.45×10^{-3}. The significance of the regression coefficient was tested: $t_s = 2.7566$. Since $t_{0.001[60]} = 3.460$ ($P < 0.001$), the slope of the regression line did not differ significantly from zero. Therefore no correlation between sample size and unbiased heterozygosity was found in this study.

7.3.3 Conformity of data to Hardy-Weinberg proportions

Three of the 53 loci tested, from populations where the sample size was 20 or more, were not in Hardy-Weinberg equilibrium (table 7.4). These three loci were contained in three populations, all of which were from one of the four hermaphroditic species (*B. angustifolia*). In each case the disequilibrium was due to a deficiency in the observed numbers of heterozygotes. However, one should note

Figure 7.10. The relationship between variability (unbiased heterozygosity) and sample size in 61 natural populations of *Bulbinella* in New Zealand.

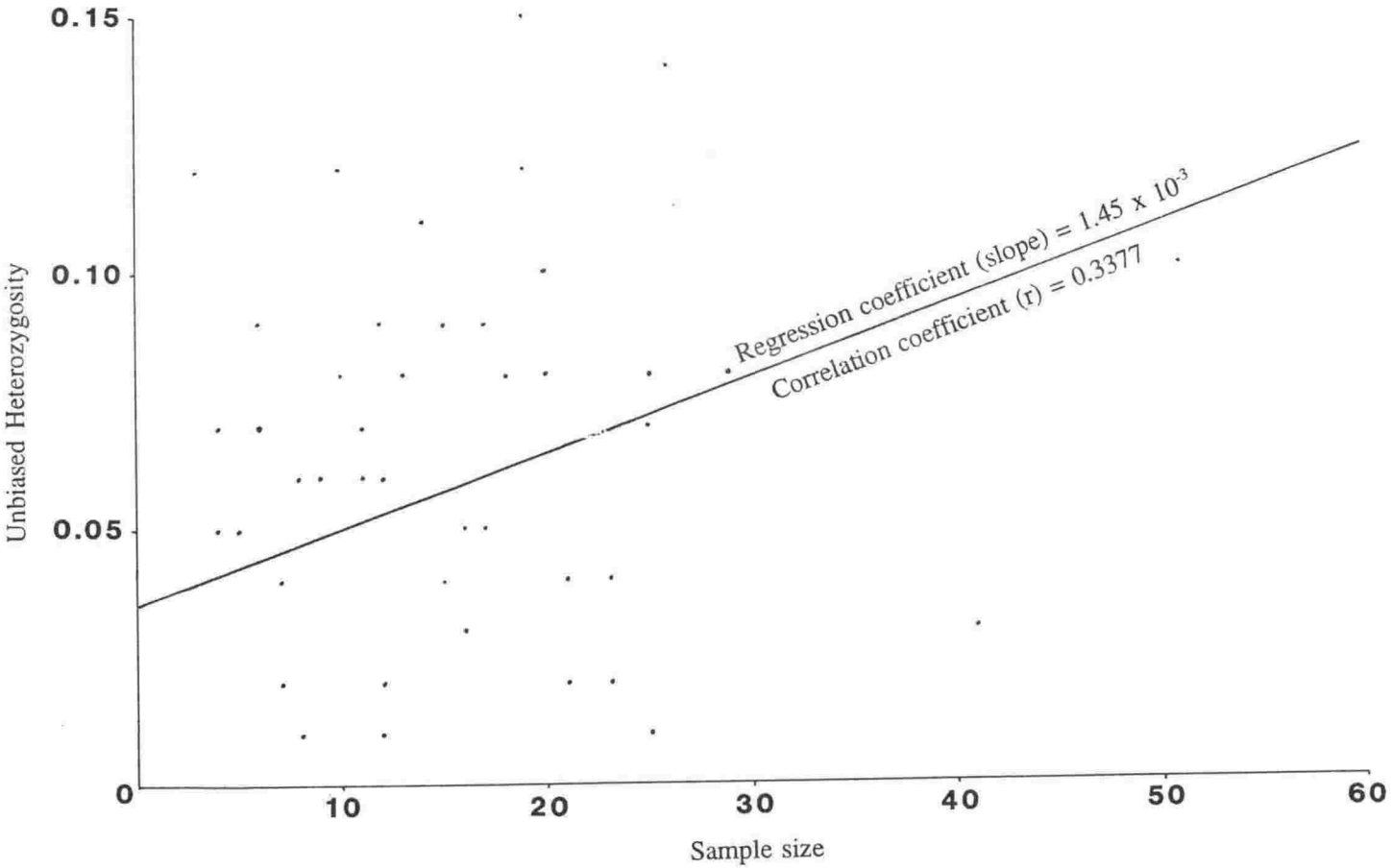


Table 7.4. The 14 populations in which 20 or more individuals were sampled which were tested for conformity to Hardy-Weinberg proportions. In three of the 53 loci tested, phenotypic distribution did not conform to Hardy-Weinberg expectations. The disequilibrium in each case was due to a deficiency in the observed numbers of heterozygotes. Legend: The taxa are: [h] = *B. hookeri*, [b] = *B. gibbsii* var. *balanifera*, [m] = *B. modesta* and [a] = *B. angustifolia*. The number in brackets after the locus name is the number of alleles detected in the locus concerned. O = observed number of heterozygotes, E = expected number of heterozygotes. ♦ = locus does not conform to Hardy-Weinberg expectations.

Population (number) name	Number of plants sampled	Locus [number of alleles]	Heterozygotes	Comments
(10) Cobb Valley[h]	34	Gdh-1 [2] Icd-2 [2] 6Pgd-1 [2] Pgi-1 [3] Pgm-1 [3]	O=11, E=16 O=7, E=8 O=18, E=17 O=20, E=20 O=9, E=8	hermaphroditic, large population
(16) Hanmer[h]	41	Gdh-1 [2] 6Pgd-1 [2] Pgm-1 [3]	O=7, E=6 O=2, E=2 O=10, E=11	hermaphroditic, large population
(17) Whanahuia Range[h]	20	Gdh-1 [2] Icd-1 [2] Mdh-3 [2] 6Pgd-1 [2] Pgi-1 [2]	O=3, E=3 O=7, E=8 O=9, E=10 O=2, E=2 O=6, E=5	gynodioecious, large population, in pockets
(31) Charming Creek[m]	26	Icd-1 [2] Icd-2 [2] Mdh-2 [2] 6Pgd-1 [2] Pgi-1 [3] Pgm-1 [4]	O=8, E=13 O=3, E=5 O=2, E=2 O=8, E=8 O=10, E=13 O=6, E=8	hermaphroditic, several areas of plants
(32) Atarau[m]	51	Icd-1 [2] Icd-2 [2] Mdh-1 [2] 6Pgd-1 [3] 6Pgd-2 [2] Pgi-1 [2] Pgm-1 [2]	O=13, E=13 O=5, E=5 O=7, E=8 O=13, E=13 O=1, E=1 O=13, E=13 O=12, E=16	hermaphroditic, population reduced to small areas of plants of which 2 were sampled
(33) Okarito Swamp[m]	25	Icd-1 [3] 6Pgd-2 [2] Pgi-1 [2] Pgm-1 [2]	O=7, E=11 O=2, E=2 O=2, E=2 O=10, E=12	hermaphroditic, patchy distribution
(45) Clayton Wetlands[a]	25	Pgm-1 [2] ♦	O=0, E=4	hermaphroditic, plants concentrated in wet areas
(46) Backline Road[a]	21	Mdh-1 [2] 6Pgd-1 [2]	O=4, E=5 O=1, E=1	hermaphroditic, pockets of plants
(47) Lake Ohau[a]	29	Mdh-1 [2] 6Pgd-1 [2] Pgm-1 [2]	O=8, E=8 O=9, E=12 O=10, E=12	hermaphroditic, plants grouped around seepages
(48) Lindis Pass[a]	23	Mdh-1 [2] 6Pgd-1 [2]	O=6, E=5 O=1, E=1	hermaphroditic, patchy distribution
(50) Danseys Pass[a]	21	Mdh-1 [2] ♦ 6Pgd-1 [2] Pgm-1 [2]	O=4, E=8 O=1, E=1 O=3, E=3	hermaphroditic, large population, sampled at intervals
(52) Macraes Flat[a]	23	Mdh-1 [2] 6Pgd-1 [2] Pgi-1 [2] Pgm-1 [2]	O=3, E=3 O=8, E=7 O=2, E=2 O=1, E=1	hermaphroditic, fragmented population
(53) Rock & Pillar Range[a]	25	Mdh-1 [2] 6Pgd-1 [2] ♦ Pgi-1 [3] Pgm-1 [2]	O=1, E=1 O=7, E=13 O=4, E=4 O=8, E=7	hermaphroditic, large area, sampled one slope
(60) Lilburn Valley[a]	20	Mdh-3 [2] 6Pgd-1 [2] Pgi-1 [3] Pgm-1 [2]	O=5, E=6 O=2, E=2 O=7, E=6 O=7, E=9	hermaphroditic, extensive population

that in 18 tests the expected number of heterozygotes was below five, which violates assumptions used in the chi-square test. In loci where more than two alleles were detected in a population, making the presence of heterozygotes of more than one genotype possible, heterozygote classes were combined to eliminate low expected numbers as far as possible.

Failure to find expected numbers of heterozygotes could reflect artifacts such as sampling errors (for example when not all plants possessed inflorescences sampling was restricted to those which did), mislabelled or misscored samples. In cases where it was suspected that this might have occurred, a new sample was prepared from the ultra-frozen inflorescence and rerun. Any obvious misfits (for example inflorescences which had been replaced in the wrong AA tube when sorting from liquid nitrogen) were discarded before computer analysis.

On the other hand, the paucity of heterozygotes in some populations suggests that these populations may be split into small breeding groups, perhaps associated with self-fertilisation, having fixed differences between them and few or no heterozygotes (for example Danseys Pass). This is known as the Wahlund effect (Spiess 1977, p. 351).

However, in some localities where 'pockets' of plants were physically separated by several kilometres (for example Backline Road), and where variable loci were detected, phenotypic distribution conformed to Hardy-Weinberg proportions.

Because of the alternative insect or wind/self pollination mechanism of most species of the study genus (Chapter 2), random mating was considered unlikely to occur in most populations. As seeds were more likely to be dispersed into the vicinity of the parent plant than further away (Chapter 2), pollen transferred between adjacent or nearby plants would often be passed among genetically related

individuals, resulting in fewer heterozygotes. This was especially applicable to populations where plants were physically separated into small breediing groups or 'pockets' by the nature of their preferred habitat (seepages).

7.3.4 Allelic frequencies

In this study comparatively few alleles were detected per locus over the seven taxa. In *Bulbinella* usually 2 or sometimes 3 alleles predominate over the genus. No single allele was fixed (frequency = 1.0) and diagnostic for any one taxon (table 7.5). Some alleles, however, were fixed in some species but not diagnostic.

7.3.4.1 **Adh-1** (fig. 7.11)

Adh-1(a) was fixed in all but one species. *B. talbotii* was the only species where the alternative allele, Adh-1(b), was found. In both populations [Gouland Downs (35) and Saxon Hut (36)] Adh-1(b) occurred with equal frequency (0.25, table 7.5).

7.3.4.2 **Gdh-1** (fig. 7.12)

Gdh-1(a) was the most common allele in all species except *B. angustifolia* and *B. hookeri*. Gdh-1(b) was fixed in all populations of *B. angustifolia*. Gdh-1(b) also occurred with moderate frequency (0.18 to 0.29) in three populations of *B. gibbsii* var. *balanifera* [Lake Marian (24), Homer (26) and Lake Harris (25)]. Apart from one population of *B. gibbsii* var. *balanifera* [Whanahuia Range (17)] Gdh-1(c) was confined to some populations of *B. hookeri*, where the frequency varied from 0.06 to 0.91.

Table 7.5. The allelic frequencies in the 11 variable loci detected in 61 natural populations of *Bulbinella*. The numbers of the populations correspond to those in table 7.1. The sample size = N (number of individual plants) except for occasional missing data points. The lower case letters under names of the loci represent the alleles detected. The taxa are: [h] = *B. hookeri*, [b] = *B. gibbsii* var. *balanifera*, [g] = *B. gibbsii* var. *gibbsii*, [r] = *B. rossii*, [m] = *B. modesta*, [t] = *B. talbotii* and [a] = *B. angustifolia*.

	Population Number										
Locus	1[h]	2[h]	3[h]	4[h]	5[h]	6[h]	7[h]	8[h]	9[h]	10[h]	11[h]
N=	11	18	10	10	11	09	15	08	17	34	05
Adh-1											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
Gdh-1											
a	0.09	0.94	0.90	0.39	0.45	0.28	1.00	1.00	0.82	0.47	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	0.91	0.06	0.10	0.61	0.55	0.72	-	-	0.18	0.53	-
Gpi-1											
a	0.09	0.36	0.35	0.05	0.05	0.78	0.03	-	0.47	0.19	0.50
b	0.91	0.64	0.65	0.95	0.95	0.22	0.97	1.00	0.50	0.59	0.50
c	-	-	-	-	-	-	-	-	0.03	0.22	-
d	-	-	-	-	-	-	-	-	-	-	-
e	-	-	-	-	-	-	-	-	-	-	-
Icd-1											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
Icd-2											
a	0.59	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	0.87	1.00
b	0.41	-	-	-	0.05	-	-	-	-	0.13	-
Mdh-1											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
Mdh-2											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
Mdh-3											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
6Pgd-1											
a	1.00	1.00	1.00	0.55	0.95	0.94	1.00	1.00	0.78	0.53	1.00
b	-	-	-	0.45	0.05	0.06	-	-	0.22	0.47	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
6Pgd-2											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
Pgm-1											
a	1.00	0.61	0.75	1.00	1.00	1.00	0.70	0.94	0.94	0.12	0.90
b	-	-	-	-	-	-	-	-	-	0.87	0.10
c	-	-	-	-	-	-	-	-	-	-	-
d	-	0.39	0.25	-	-	-	0.30	0.06	0.06	0.01	-

Table 7.5 continued

Locus	Population										
	12[h]	13[h]	14[h]	15[h]	16[h]	17[b]	18[b]	19[b]	20[b]	21[b]	22[b]
N=	01	15	06	03	41	20	04	16	05	07	02
Adh-1											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
Gdh-1											
a	1.00	1.00	0.70	1.00	0.92	0.93	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	0.30	-	0.08	0.07	-	-	-	-	-
Gpi-1											
a	1.00	0.33	0.08	0.33	1.00	0.15	0.12	0.69	-	-	-
b	-	0.67	0.92	0.67	-	0.85	0.88	0.31	1.00	1.00	1.00
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
e	-	-	-	-	-	-	-	-	-	-	-
Icd-1											
a	1.00	1.00	1.00	1.00	1.00	0.27	-	-	-	-	-
b	-	-	-	-	-	0.73	1.00	1.00	1.00	1.00	1.00
c	-	-	-	-	-	-	-	-	-	-	-
Icd-2											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
Mdh-1											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
Mdh-2											
a	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	0.12	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
Mdh-3											
a	1.00	1.00	0.83	1.00	1.00	0.38	0.88	1.00	-	-	1.00
b	-	-	-	-	-	0.62	0.12	-	1.00	1.00	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	0.17	-	-	-	-	-	-	-	-
6Pgd-1											
a	1.00	0.37	0.17	0.67	0.98	0.95	1.00	0.97	1.00	0.93	1.00
b	-	0.63	0.83	0.33	0.02	0.05	-	-	-	-	-
c	-	-	-	-	-	-	-	0.03	-	0.07	-
d	-	-	-	-	-	-	-	-	-	-	-
6Pgd-2											
a	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	1.00	0.93	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	0.12	-	-	0.07	-
Pgm-1											
a	1.00	0.83	1.00	0.66	0.85	1.00	1.00	0.94	1.00	1.00	-
b	-	0.17	-	0.17	0.06	-	-	-	-	-	1.00
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	0.17	0.09	-	-	0.06	-	-	-

Table 7.5 continued

	Population Number										
Locus	23[b]	24[b]	25[b]	26[b]	27[b]	28[b]	29[g]	30[r]	31[m]	32[m]	33[m]
N=	16	12	14	19	03	11	01	06	26	51	25
Adh-1											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
Gdh-1											
a	1.00	0.71	0.79	0.82	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	0.29	0.21	0.18	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
Gpi-1											
a	-	1.00	0.57	0.53	0.50	0.85	1.00	1.00	0.62	0.85	0.96
b	1.00	-	0.39	0.34	0.50	0.15	-	-	0.36	0.15	0.04
c	-	-	0.04	0.13	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	0.02	-	-
e	-	-	-	-	-	-	-	-	-	-	-
Icd-1											
a	-	0.04	-	-	-	0.09	1.00	1.00	0.58	0.85	0.70
b	1.00	0.96	1.00	1.00	1.00	0.91	-	-	-	-	0.02
c	-	-	-	-	-	-	-	-	0.42	0.15	0.28
Icd-2											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90	0.95	1.00
b	-	-	-	-	-	-	-	-	0.10	0.05	-
Mdh-1											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.91	1.00
b	-	-	-	-	-	-	-	-	-	0.09	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
Mdh-2											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	0.04	-	-
Mdh-3											
a	-	0.88	0.96	0.84	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	1.00	0.12	0.04	0.16	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
6Pgd-1											
a	1.00	1.00	1.00	1.00	-	0.60	1.00	0.42	0.81	0.85	1.00
b	-	-	-	-	-	-	-	0.58	0.19	0.13	-
c	-	-	-	-	1.00	0.40	-	-	-	0.02	-
d	-	-	-	-	-	-	-	-	-	-	-
6Pgd-2											
a	1.00	0.25	0.86	0.34	1.00	1.00	1.00	1.00	1.00	0.99	0.96
b	-	-	-	-	-	-	-	-	-	0.01	-
c	-	0.75	0.14	0.66	-	-	-	-	-	-	0.04
Pgm-1											
a	-	0.08	0.86	0.61	-	1.00	1.00	0.75	0.81	0.80	0.60
b	1.00	0.92	0.14	0.39	1.00	-	-	0.25	0.11	0.20	0.40
c	-	-	-	-	-	-	-	-	0.02	-	-
d	-	-	-	-	-	-	-	-	0.06	-	-

Table 7.5 continued

	Population Number										
Locus	34[m]	35[l]	36[l]	37[a]	38[a]	39[a]	40[a]	41[a]	42[a]	43[a]	44[a]
N=	19	18	07	17	12	16	09	10	13	12	08
Adh-1											
a	1.00	0.75	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	0.25	0.25	-	-	-	-	-	-	-	-
Gdh-1											
a	1.00	1.00	1.00	-	-	-	-	-	-	-	-
b	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
c	-	-	-	-	-	-	-	-	-	-	-
Gpi-1											
a	1.00	1.00	1.00	0.85	1.00	0.84	1.00	1.00	1.00	1.00	1.00
b	-	-	-	0.15	-	0.16	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
e	-	-	-	-	-	-	-	-	-	-	-
Icd-1											
a	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	0.50	-	-	-	-	-	-	-	-	-	-
Icd-2											
a	1.00	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	1.00	1.00	-	-	-	-	-	-	-	-
Mdh-1											
a	1.00	0.92	0.07	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	0.08	0.93	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
Mdh-2											
a	0.61	1.00	1.00	-	-	-	-	-	-	-	-
b	-	-	-	-	-	-	-	-	-	-	-
c	0.39	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
d	-	-	-	-	-	-	-	-	-	-	-
Mdh-3											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
6Pgd-1											
a	1.00	0.97	1.00	0.06	-	-	-	-	-	-	-
b	-	-	-	0.94	1.00	0.97	1.00	1.00	1.00	1.00	1.00
c	-	0.03	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	0.03	-	-	-	-	-
6Pgd-2											
a	0.74	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	0.26	-	-	-	-	-	-	-	-	-	-
Pgm-1											
a	0.87	0.64	1.00	0.82	0.83	0.94	1.00	1.00	1.00	0.96	1.00
b	0.13	0.30	-	0.18	0.17	-	-	-	-	0.04	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	0.06	-	-	-	0.06	-	-	-	-	-

Table 7.5 continued

	Population Number									
Locus	45[a]	46[a]	47[a]	48[a]	49[a]	50[a]	51[a]	52[a]	53[a]	54[a]	55[a]
N=	25	21	29	23	17	21	04	23	25	11	12
Adh-1											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
Gdh-1											
a	-	-	-	-	-	-	-	-	-	-	-
b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
c	-	-	-	-	-	-	-	-	-	-	-
Gpi-1											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96	0.92	0.91	1.00
b	-	-	-	-	-	-	-	0.04	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	0.06	0.09	-
e	-	-	-	-	-	-	-	-	0.02	-	-
Icd-1											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
Icd-2											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
Mdh-1											
a	1.00	0.86	0.83	0.87	0.88	0.76	0.38	0.94	0.98	0.96	0.67
b	-	-	-	-	-	-	-	-	-	-	-
c	-	0.14	-	-	-	-	-	-	-	-	-
d	-	-	0.17	0.13	0.12	0.24	0.62	0.06	0.02	0.04	0.33
Mdh-2											
a	-	-	-	-	-	-	-	-	-	-	-
b	-	-	-	-	-	-	-	-	-	-	-
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
d	-	-	-	-	-	-	-	-	-	-	-
Mdh-3											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-
6Pgd-1											
a	-	-	-	-	-	-	0.12	-	-	-	0.04
b	1.00	0.98	0.71	0.98	1.00	0.98	0.76	0.18	0.54	0.80	0.79
c	-	-	-	-	-	-	-	-	-	-	-
d	-	0.02	0.29	0.02	-	0.02	0.12	0.82	0.46	0.20	0.17
6Pgd-2											
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-	-	-	-	-
Pgm-1											
a	0.92	1.00	0.68	1.00	0.50	0.93	1.00	0.98	0.82	0.83	1.00
b	0.08	-	0.32	-	0.50	0.07	-	0.02	0.18	0.17	-
c	-	-	-	-	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-	-	-	-	-

Table 7.5 continued

	Population Number					
Locus	**56[a]**	**57[a]**	**58[a]**	**59[a]**	**60[a]**	**61[a]**
N=	10	08	04	13	20	11
Adh-1						
a	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-
Gdh-1						
a	-	-	-	-	-	-
b	1.00	1.00	1.00	1.00	1.00	1.00
c	-	-	-	-	-	-
Gpi-1						
a	0.65	0.56	0.88	0.92	0.82	0.95
b	-	-	-	-	0.03	-
c	-	-	-	-	-	-
d	0.35	0.44	0.12	0.08	0.15	0.05
e	-	-	-	-	-	-
Icd-1						
a	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-
c	-	-	-	-	-	-
Icd-2						
a	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-
Mdh-1						
a	0.85	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-
c	-	-	-	-	-	-
d	0.15	-	-	-	-	-
Mdh-2						
a	-	-	-	-	-	-
b	-	-	-	-	-	-
c	1.00	1.00	1.00	1.00	1.00	1.00
d	-	-	-	-	-	-
Mdh-3						
a	0.95	0.94	0.88	0.58	0.83	0.77
b	-	-	-	-	-	-
c	0.05	0.06	0.12	0.42	0.17	0.23
d	-	-	-	-	-	-
6Pgd-1						
a	0.80	1.00	1.00	1.00	0.95	1.00
b	0.20	-	-	-	0.05	-
c	-	-	-	-	-	-
d	-	-	-	-	-	-
6Pgd-2						
a	1.00	1.00	1.00	1.00	1.00	1.00
b	-	-	-	-	-	-
c	-	-	-	-	-	-
Pgm-1						
a	0.65	0.88	0.88	0.69	0.64	0.82
b	0.35	0.12	0.12	0.31	0.36	0.18
c	-	-	-	-	-	-
d	-	-	-	-	-	-

Figure 7.11. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand. Broken lines indicate species divisions.

Locus: Adh-1

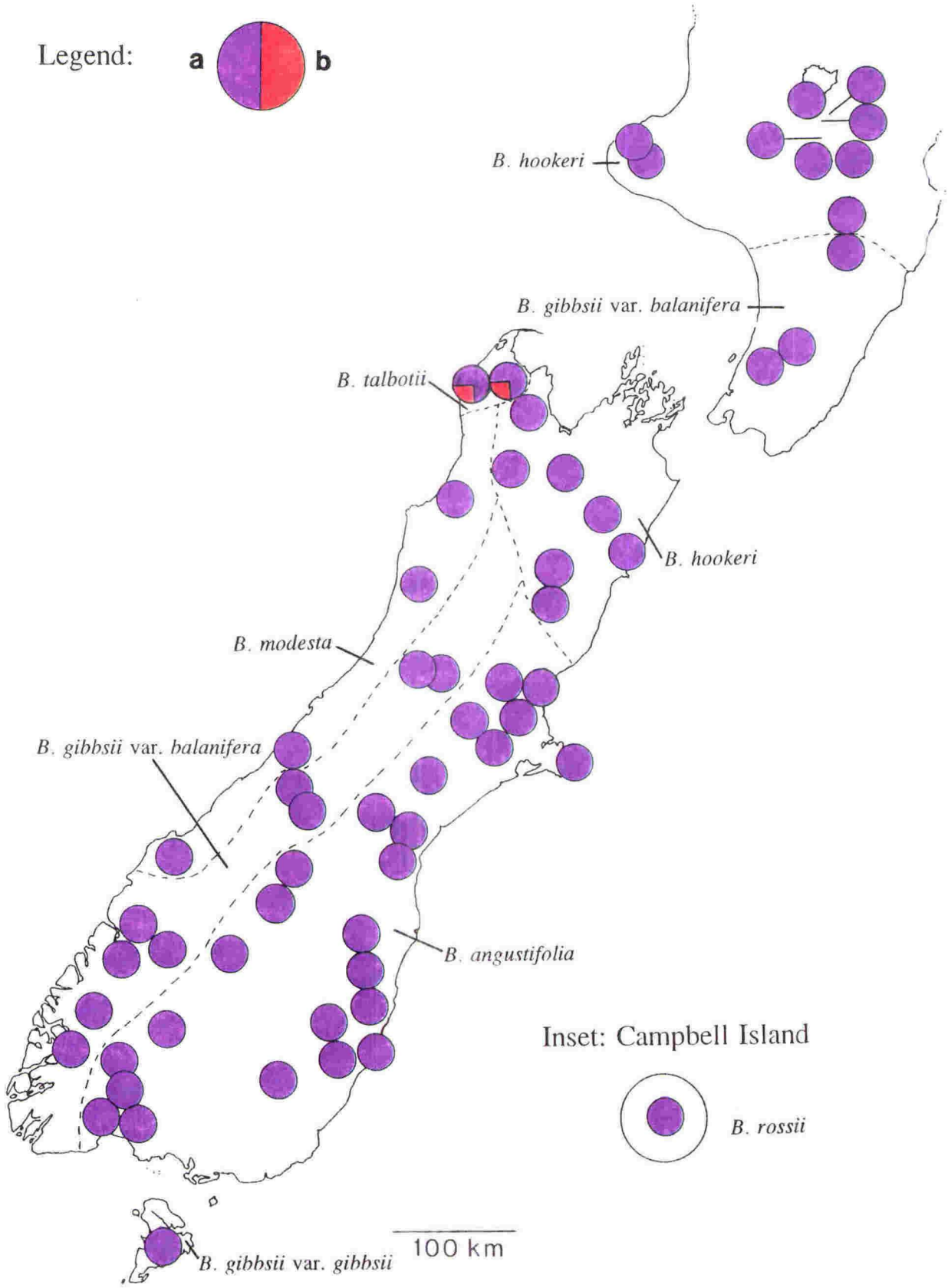
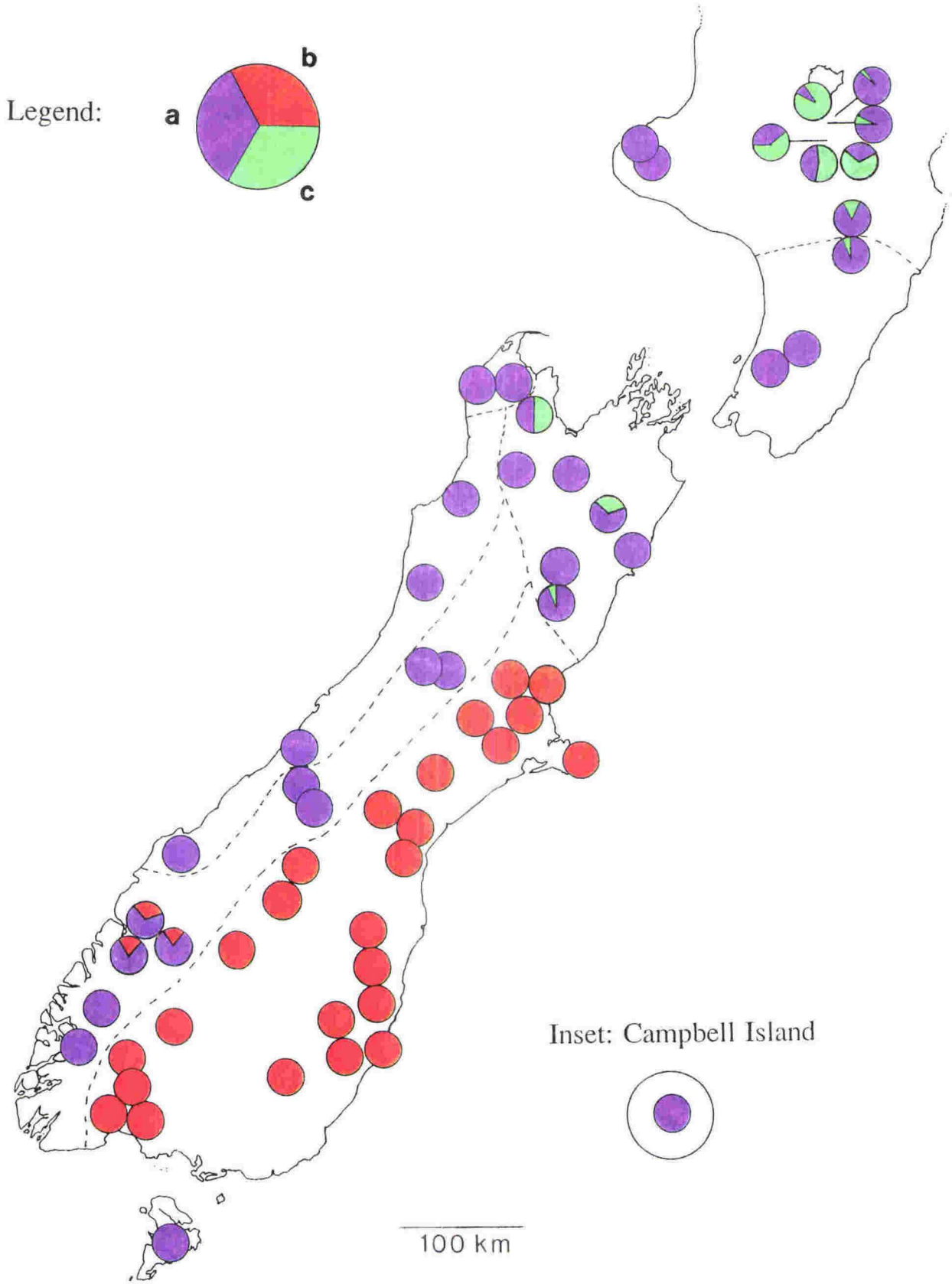


Figure 7.12. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand.

Locus: Gdh-1



7.3.4.3 G6pdh-1

G6pdh-1(a) was fixed in all species.

7.3.4.4 Gpi-1 (fig. 7.13)

Over all species except *B. hookeri* and *B. gibbsii* var. *balanifera* where Gpi-1(b) was at very high frequency, Gpi-1(a) was the most common allele. The frequency of Gpi-1(d) varied from 0.02 to 0.44, and this allele was almost completely confined to southern populations of *B. angustifolia*. Gpi-1(c) was relatively rare, being confined to two populations in each of two taxa (*B. gibbsii* var. *balanifera* and *B. hookeri*). Gpi-1(e) was rare, with a frequency of 0.02 and was detected in only one population, Rock and Pillar Range (53) [*B. angustifolia*].

7.3.4.5 Icd-1 (fig. 7.14)

The most common allele was Icd-1(a) in all populations of all species except *B. gibbsii* var. *balanifera,* where the most common (but not the only) allele detected was Icd-1(b). Icd-1(a) was found at moderate or low frequency in three populations of *B. gibbsii* var. *balanifera* [Whanahuia Range {population 17}, (frequency = 0.27), Lake Marian {24}, (0.04) and Wilmot Pass {28}, (0.09)]. Icd-1(b) was found in one population of *B. modesta,* Okarito Swamp {33}, with a frequency of 0.02. Icd-1(c) was found only in *B. modesta* varying in frequency from 0.15 to 0.50.

7.3.4.6 Icd-2 (fig. 7.15)

In *B. talbotii* Icd-2(b) was the only allele found. In all populations of the other species, Icd-2(a) was the more common allele and was usually fixed. Icd-2(b) was also found in three populations of *B. hookeri* [Desert Road {1}, (0.41), Mangaio

Figure 7.13. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand.

Locus: Gpi-1

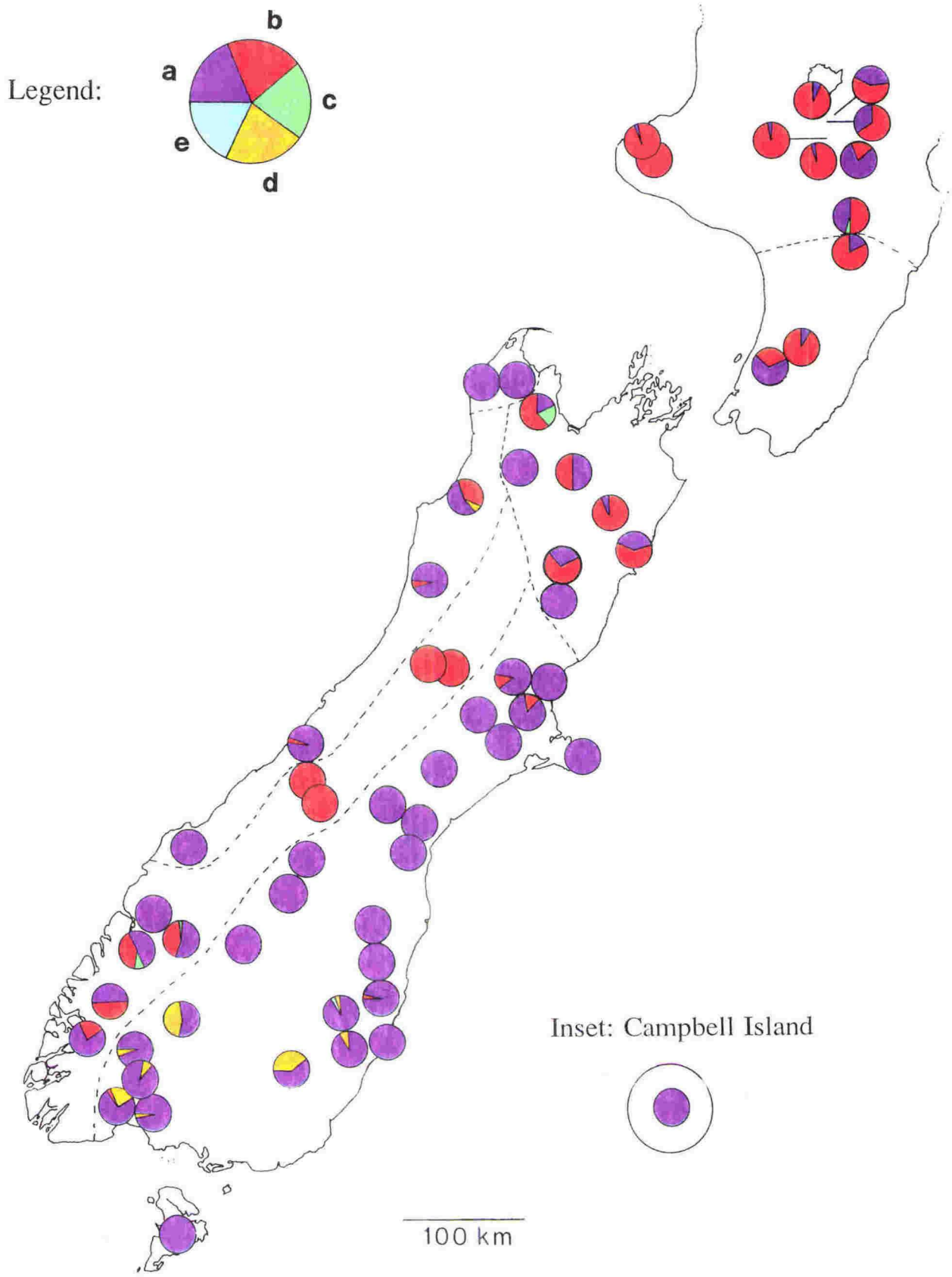


Figure 7.14. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand.

Locus: Icd-1

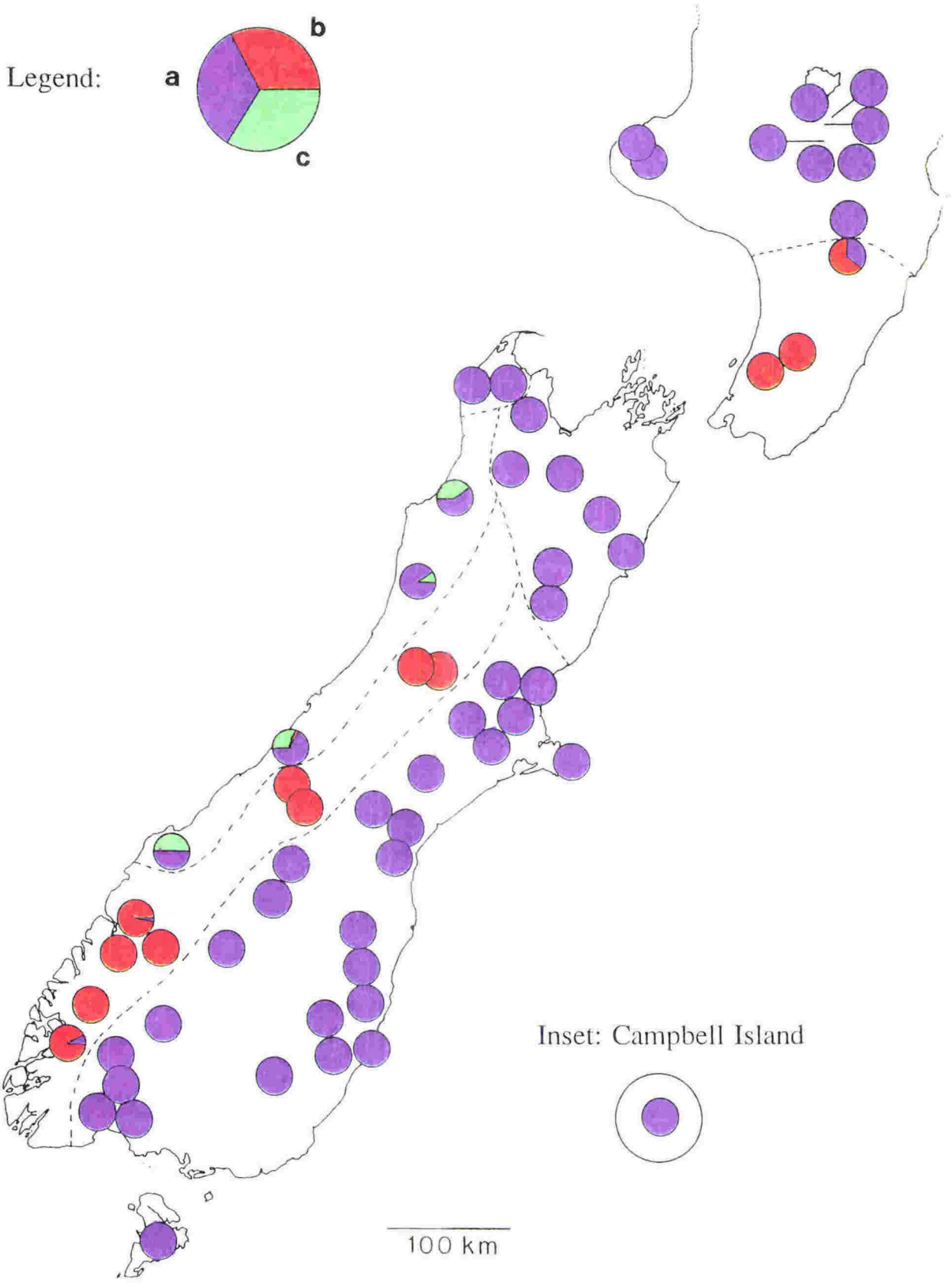
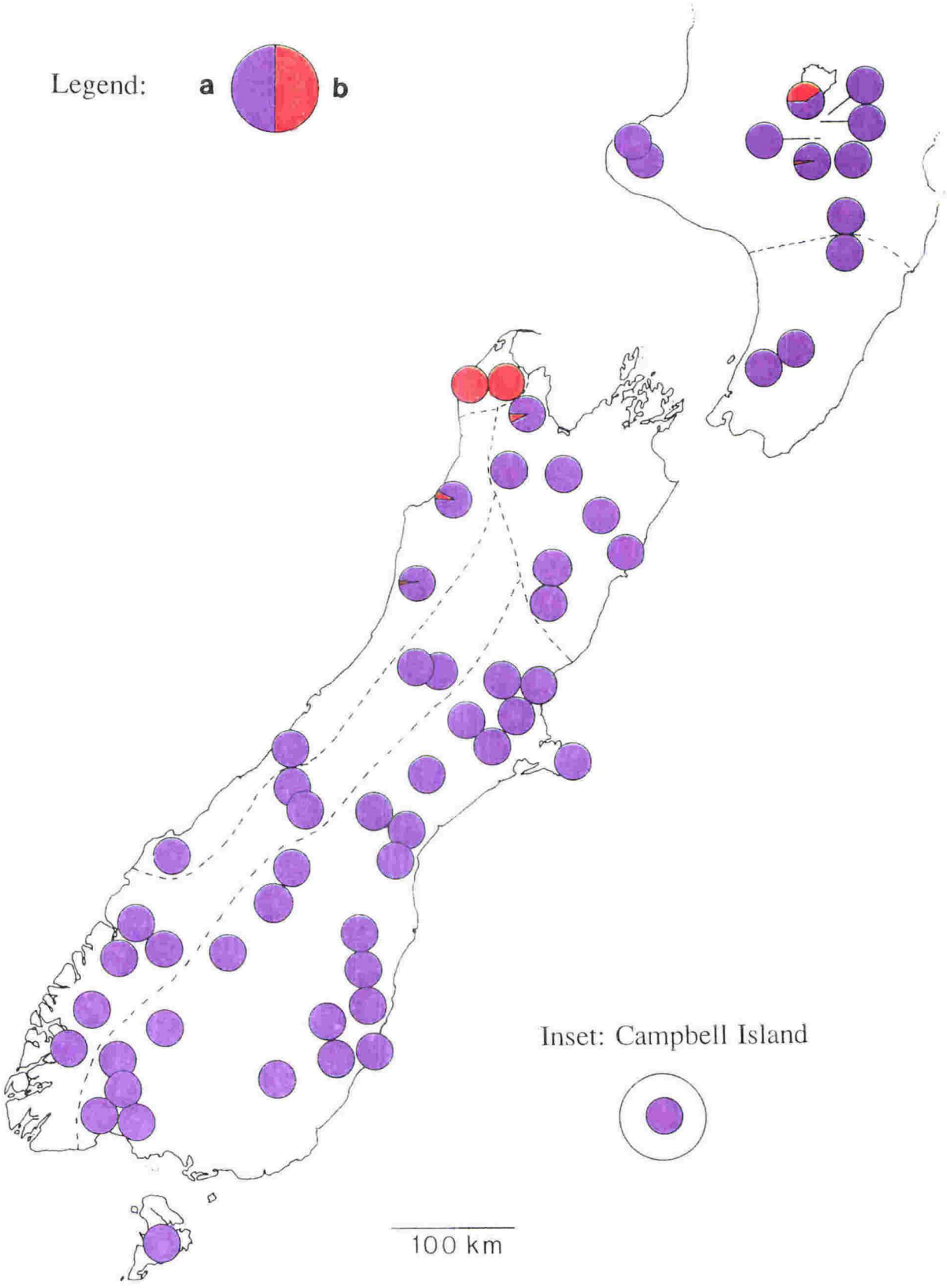


Figure 7.15. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand.

Locus: Icd-2



Stream {5}, (0.05) and Cobb Valley {10}, (0.13)] and two populations of *B. modesta* [Charming Creek {31}, (0.10) and Atarau {32}, (0.05)].

7.3.4.7 **Mdh-1** (fig. 7.16)

Mdh-1(a) was found in all species and populations and was the most common allele in most populations. Mdh-1(b) occurred in *B. talbotii* [Gouland Downs {35}, (0.93) and Saxon Hut {36}, (0.08)] and *B. modesta* [Atarau {32}, (0.09)] only. Mdh-1(c) was found only in one population of *B. angustifolia* [Clayton {45}, (0.14)]. Mdh-1(d) was present with variable frequency (0.02 to 0.62) in ten populations of *B. angustifolia* in the south-east of the South Island.

7.3.4.8 **Mdh-2** (fig. 7.17)

In all populations of all species except *B. angustifolia,* Mdh-2(a) was the most common allele. Mdh-2(c) was fixed in all populations of *B. angustifolia.* Mdh-2(c) was also recorded in one population of *B. modesta* [Lake Ellery Track (34)] at a frequency of 0.39. The rare alleles Mdh-2(b) [Mt Holdsworth {18}, (0.12)] and Mdh-2(d) [Charming Creek {31}, (0.04)] were each detected in one population only (the taxa *B. gibbsii* var. *balanifera* and *B. modesta* respectively).

7.3.4.9 **Mdh-3** (fig. 7.18)

In all populations of all species except *B. gibbsii* var. *balanifera* the most common allele was Mdh-3(a). Mdh-3(b) was detected only in some populations of *B. gibbsii* var *balanifer*a where the frequency ranged from 0.04 to 1.0. Mdh-3(c) was confined to six populations of *B. angustifolia* in the far south of the South Island, where the frequency ranged from 0.05 to 0.42. Mdh-3(d), a presumptive null

Figure 7.16. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand.

Locus: Mdh-1

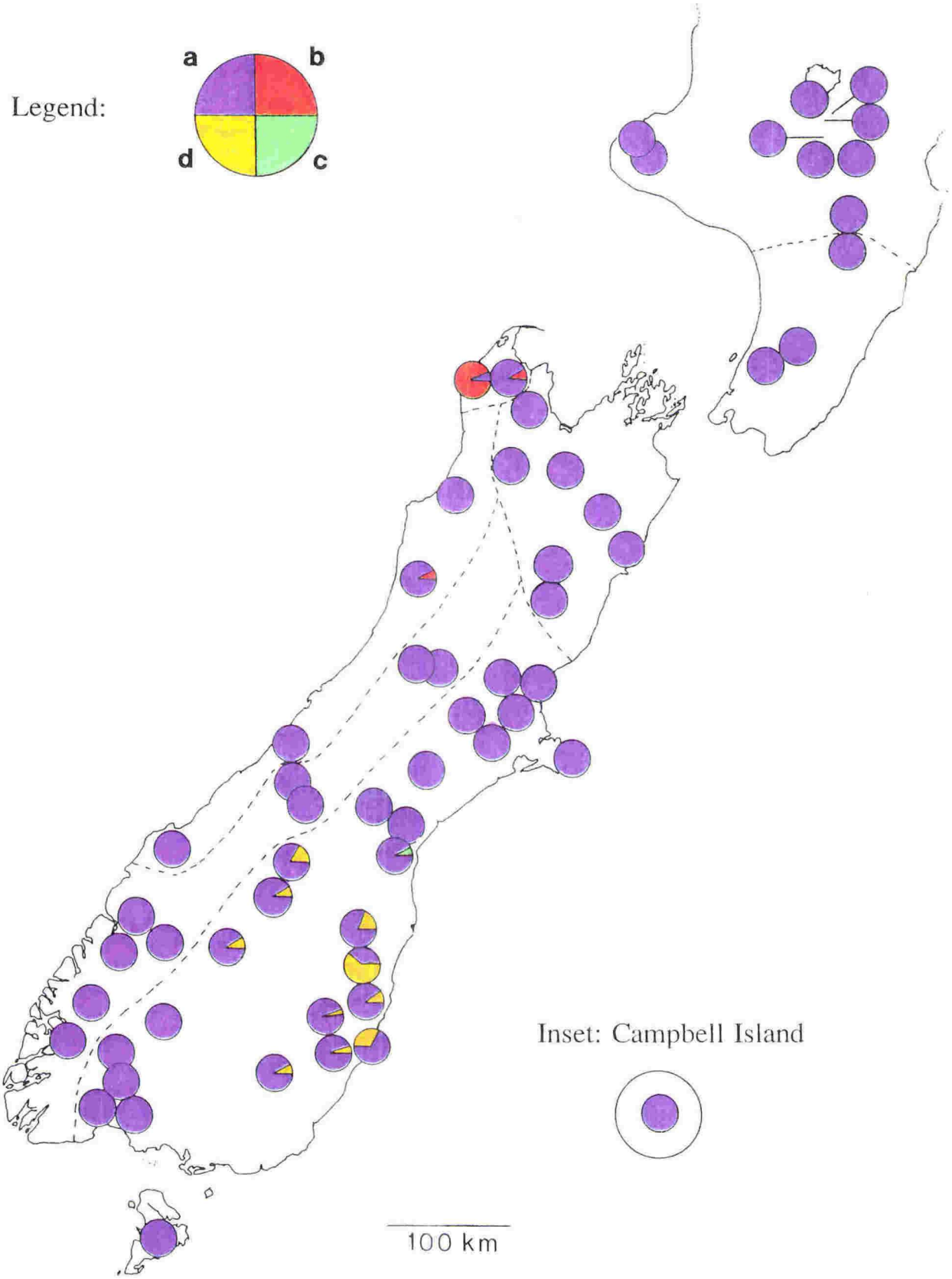


Figure 7.17. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand.

Locus: Mdh-2

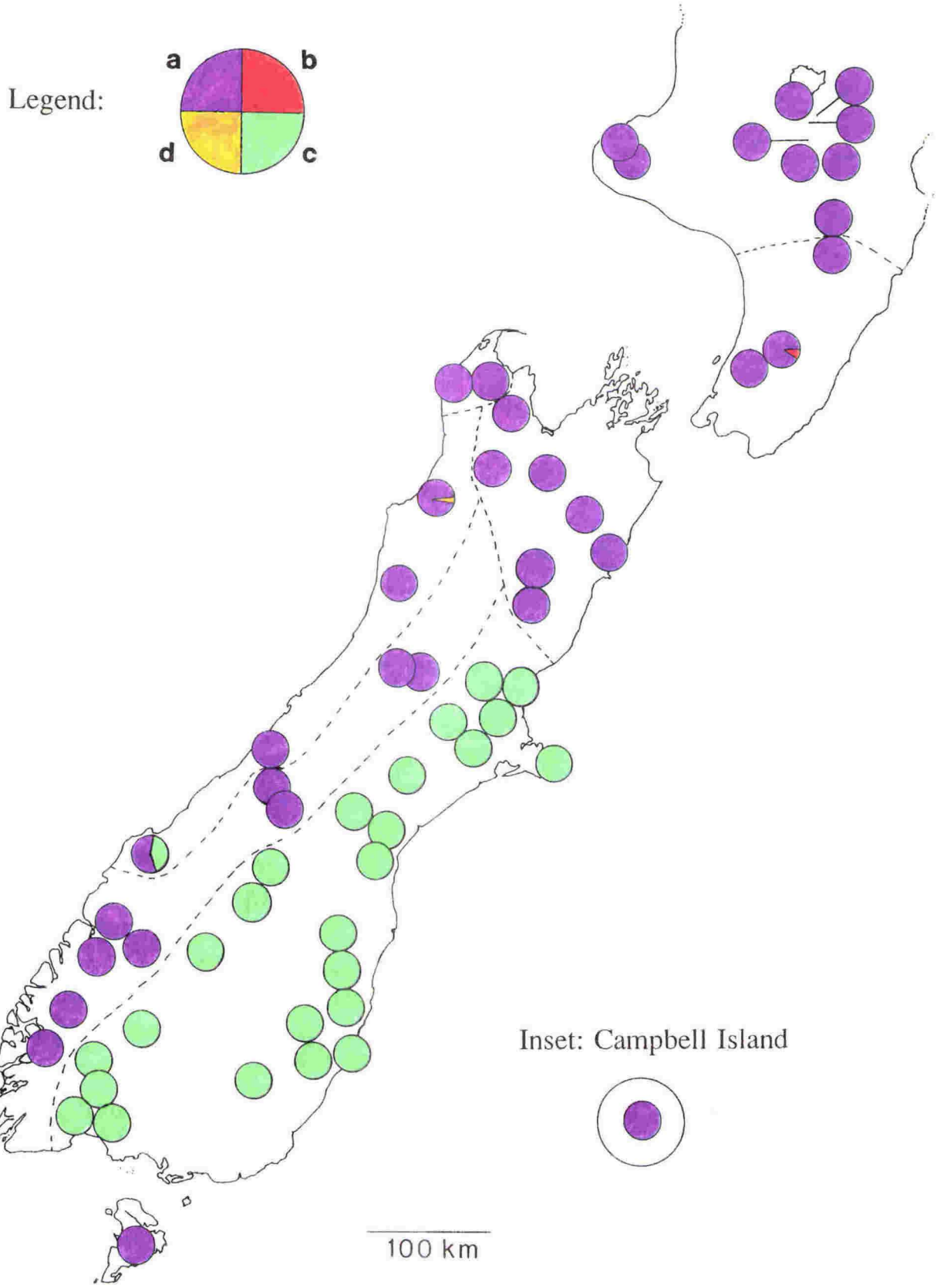
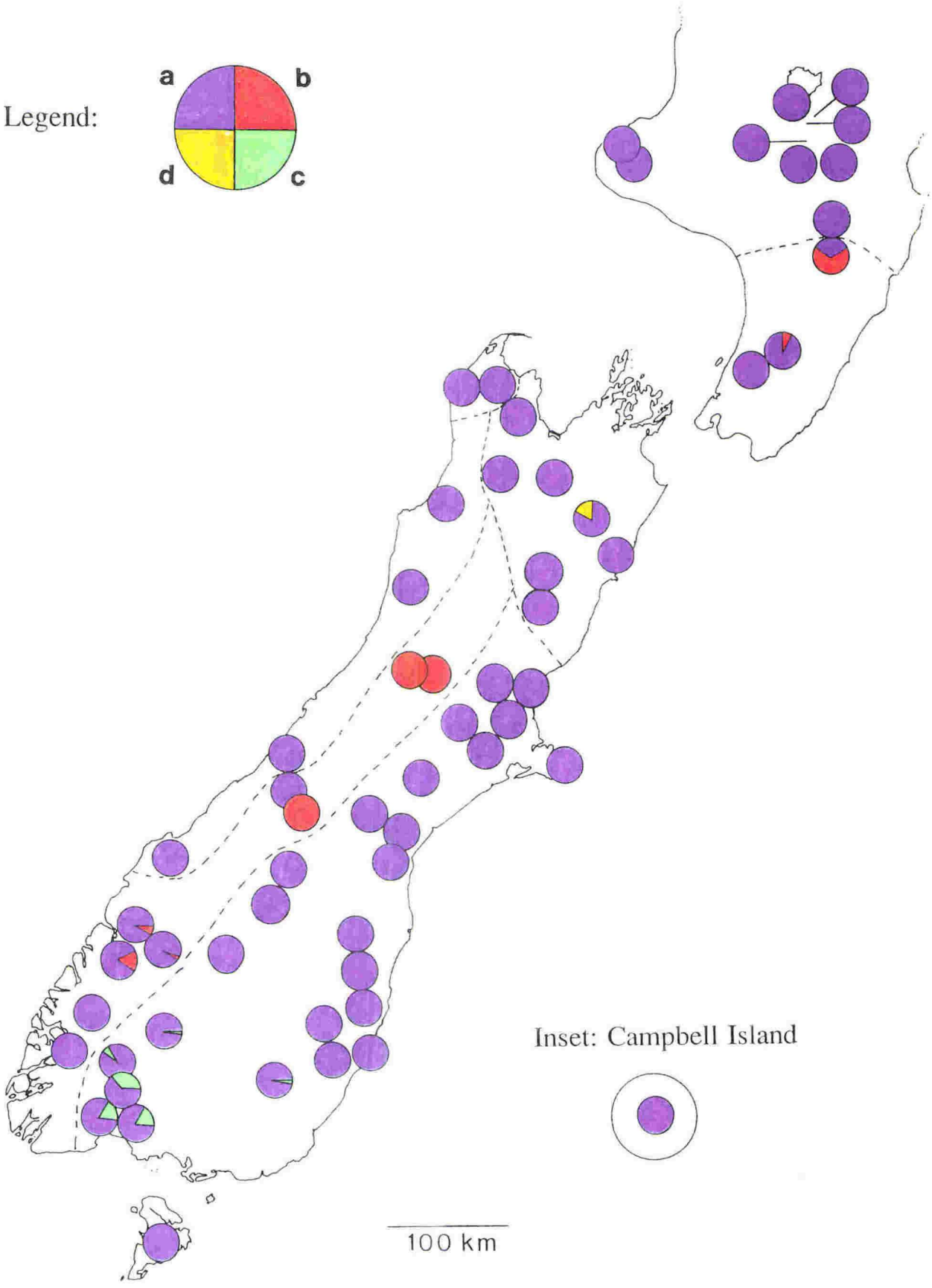


Figure 7.18. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand.

Locus: Mdh-3



allele (section 7.3.1.8), was detected in only one plant from Molesworth [14] (*B. hookeri*) in the homozygous condition.

7.3.4.10 6Pgd-1 (fig. 7.19)

The most common allele in most populations of all species except *B. angustifolia* and *B. rossii* was 6Pgd-1(a). 6Pgd-1(b) was more common in *B. rossii* and northern populations of *B. angustifolia*, and was also detected in some populations of *B. hookeri*, *B. gibbsii* var. *balanifera* and *B. modesta*. The six southern populations of *B. angustifolia* had 6Pgd-1(a) as their most common allele. 6Pgd-1(d) was detected only in some populations of *B. angustifolia* and was more common in south-eastern populations. 6Pgd-1(c) was detected in only three populations of *B. gibbsii* var. *balanifera* [Bridge peak {19}, (0.03), Murchison Mountains {27}, (1.0) and Wilmot Pass {28}, (0.4)].

7.3.4.11 6Pgd-2 (fig. 7.20)

The most common allele in all species was 6Pgd-2(a). 6Pgd-2(b) was very rare, occurring in only one population [Atarau (32), *B. modesta*] with a frequency of 0.01. 6Pgd-2(c) was detected in five populations of *B. gibbsii* var. *balanifera* (where the frequency ranged from 0.07 to 0.75) and two populations of *B. modesta* [Okarito {33}, (0.04) and Lake Ellery Track {34}, (0.26)].

7.3.4.12 Pgm-1 (fig. 7.21)

In most populations of all species Pgm-1(a) was the most common allele. Pgm-1(b) occurred with varying frequency in some populations of all taxa except *B. gibbsii* var. *gibbsii*. Pgm-1(c) was detected in low frequency (0.02) in only one

Figure 7.19. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand.

Locus: 6Pgd-1

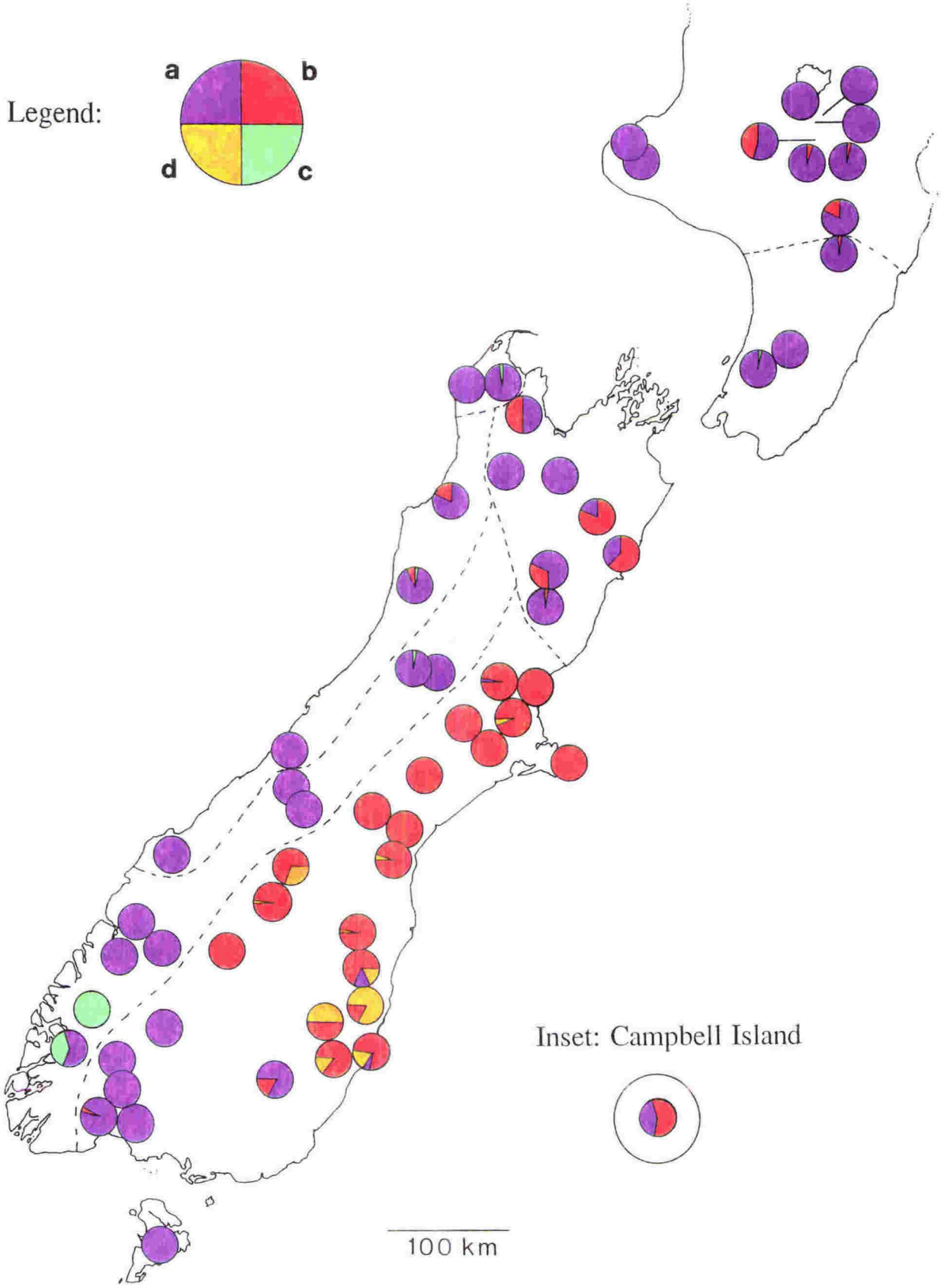


Figure 7.20. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand.

Locus: 6Pgd-2

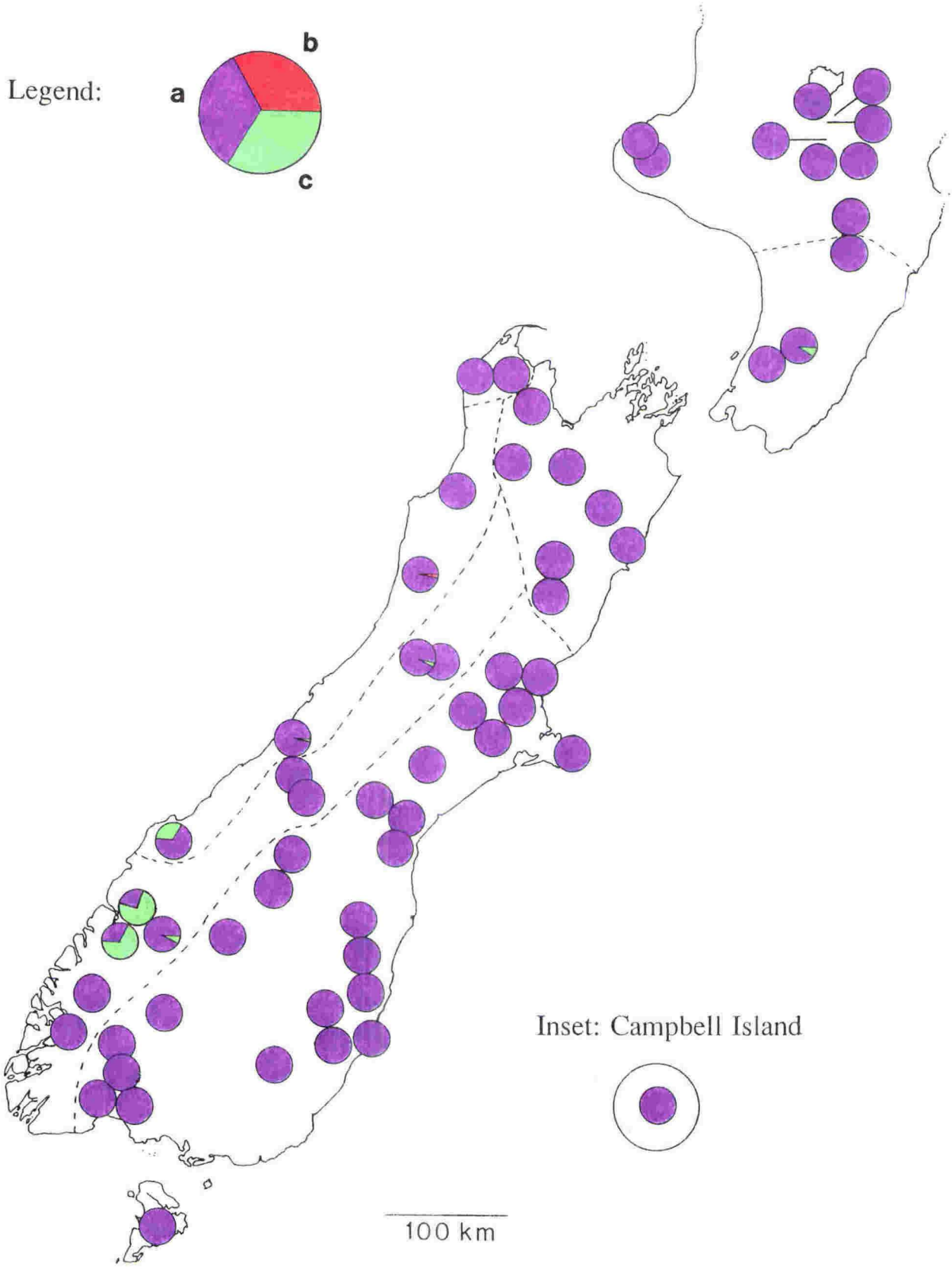
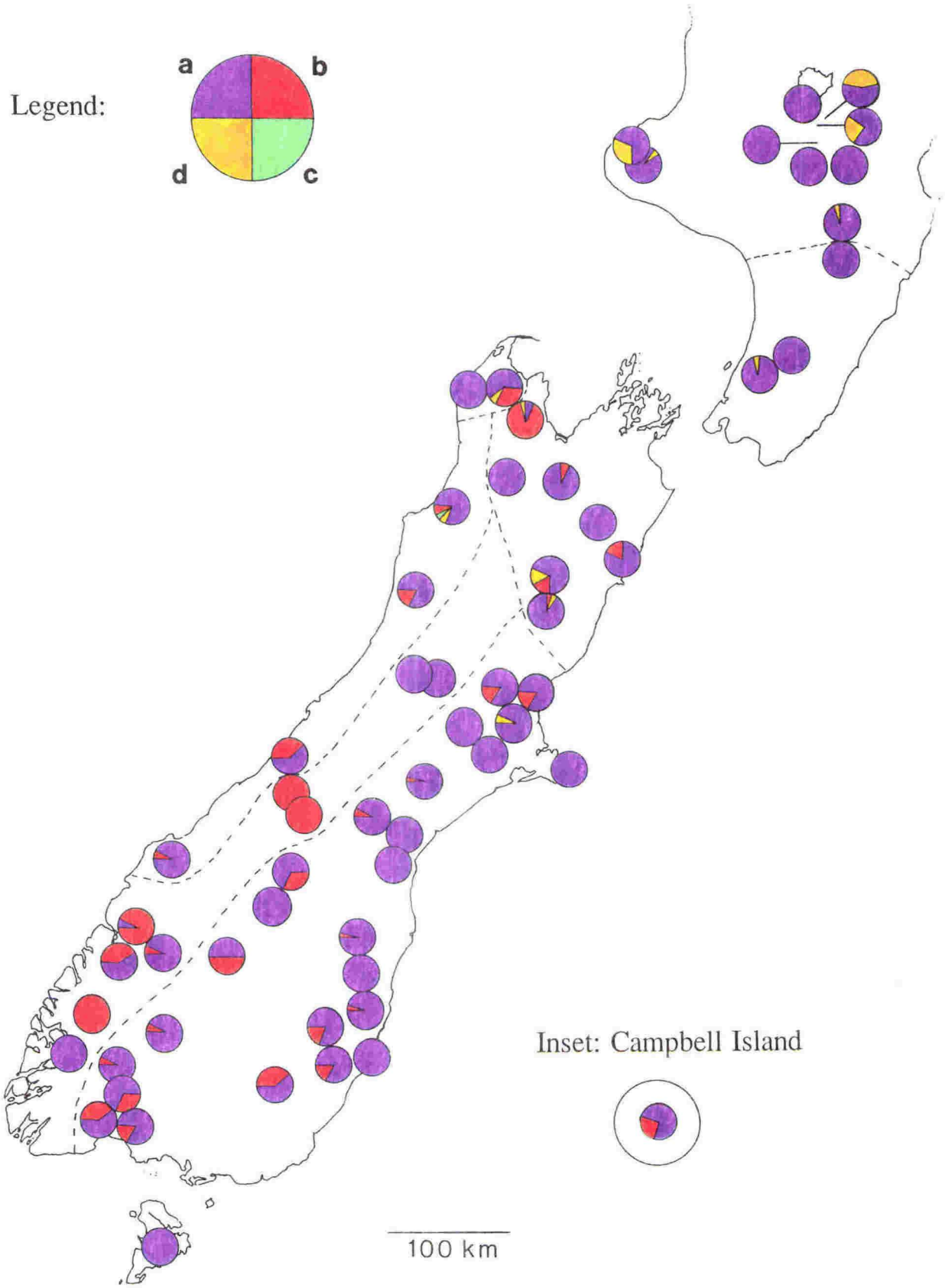


Figure 7.21. The allele frequencies detected in 61 natural populations of *Bulbinella* in New Zealand.

Locus: Pgm-1



population, Charming Creek {31}, (*B. modesta*). Pgm-1(d) was moderately common only in some populations of *B. hookeri* and occurred in low frequency in *B. gibbsii* var. *balanifera, B. talbotii, B. modesta* and *B. angustifolia.*

7.3.5 Patterns of geographic differentiation

Within populations it is likely that geographic differentiation occurs. Some populations of the genus may be divided into isolated breeding groups (possibly evidenced by lower than expected numbers of heterozygotes being detected, section 7.3.3). Some populations are physically divided because favoured sites for *Bulbinella* are seepages and other permanently wet areas, which occur intermittently throughout the habitat.

Within species (between populations) certain alleles are more common in some populations of a species than in others. Examples are *B. angustifolia*, in which Mdh-1(d) and 6Pgd-1(d) were detected only in populations from Otago in the south-east of the South Island, while Mdh-3(c) and 6Pgd-1(a) were found only in southern populations of this species. Fixed differences were found between some populations of *B. gibbsii* var. *balanifera* (Mdh-3, fig. 7.18 and Pgm-1, fig. 7.21). Some alleles in the taxon *B. hookeri* showed a wide range in frequency (for example Gpi-1, fig 7.13, 6Pgd-1, fig. 7.19 and Pgm-1, fig. 7.21). Studies by Templeton et al. (1990) indicated that in a fragmented species the partitioning of available genetic variation changed from within population to between populations. It was claimed that where habitat had been fragmented, redistribution occurred as opposed to elimination in that variation was still present as fixed differences between local populations instead of within a single larger population. In the present study of distribution of variation, such a pattern was detected between groups of populations; for example the two

groups of *B. angustifolia* mentioned above, one in Otago and the other in the southern South Island; and two groups of *B. gibbsii* var. *balanifera* between which a fixed difference was demonstrated.

Between species no completely fixed differences were found. Alleles which were fixed in one taxon were invariably present in some (usually adjacent) populations of other taxa and/or alternative alleles for the locus were detected in addition to the fixed one (table 7.6). For example, Adh-1(b) was fixed in *B. talbotii* but not diagnostic for this taxon. Gdh-1(b), which was fixed in all populations of *B. angustifolia*, was also present in three populations of *B. gibbsii* var. *balanifera* (Lake Marian, Lake Harris and Homer, fig. 7.12). Icd-1(b) was present in Okarito Swamp (*B. modesta*) and almost fixed in *B. gibbsii* var. *balanifera*, where Icd-1(a) was also detected in some populations (fig. 7.14). Icd-2(b), which was fixed in *B. talbotii*, was also present in two populations of *B. modesta* (Charming Creek and Atarau) and three populations of *B. hookeri* (Desert Road, Mangaio Stream and Cobb Valley, fig. 7.15). Mdh-2(c) was fixed in all populations of *B. angustifolia* and present also in Lake Ellery Track (*B. modesta*, fig. 7.17). The disjunct distributions (discontinuities in geographic ranges) of *B. hookeri* and *B. gibbsii* var. *balanifera* were reflected in genetic discontinuities. The only species characterised by a unique combination of alleles was *B. angustifolia*, in which Gdh-1(b) and Mdh-2(c) were fixed in all populations (table 7.6). These two alleles were not found together in any other taxon. In some other species, certain alleles were characteristic of, but not diagnostic for, a particular species. For example, Icd-2(b) was fixed in but not diagnostic for *B. talbotii*.

Introgression, shared alleles between taxa, which are common in one taxon but rare in the other(s), may provide evidence for hybridization having occurred between

Table 7.6. The alleles for each variable locus which were present in each taxon. Legend: Lower case letters without parentheses represent the alleles occurring at high or moderate degree. Lower case letters in parentheses indicate alleles rare in that taxon. The number in parentheses is the total number of plants examined. Key to taxa: B.h. = *B. hookeri*, B.gvb. = *B. gibbsii* var. *balanifera*, B.gvg. = *B. gibbsii* var. *gibbsii*, B.r. = *B. rossii*, B.m. = *B. modesta*, B.t. = *B. talbotii* and B.a. = *B. angustifolia*.

	Taxon						
Locus	**B.h.** (214)	**B.gvb.** (129)	**B.gvg.** (1)	**B.r.** (6)	**B.m.** (121)	**B.t.** (25)	**B.a.** (374)
Adh-1	a	a	a	a	a	a,b	a
Gdh-1	a,c	a [b,c]	a	a	a	a	b
Gpi-1	a,b [c]	a,b [c]	a	a	a,b [d]	a	a,d [b,e]
Icd-1	a	b [a]	a	a	a,c [b]	a	a
Icd-2	a [b]	a	a	a	a [b]	b	a
Mdh-1	a	a	a	a	a [b]	a,b	a,d [c]
Mdh-2	a	a [b]	a	a	a,c [d]	a	c
Mdh-3	a [d]	a,b	a	a	a	a	a,c
6Pgd-1	a,b	a,c [b]	a	a,b	a,b [c]	a [c]	a,b,d
6Pgd-2	a	a,c	a	a	a,c [b]	a	a
Pgm-1	a,b,d	a,b [d]	a	a,b	a,b [c,d]	a,b [d]	a,b [d]

adjacent populations of different taxa. Examples are Gdh-1(b), which is fixed in *B. angustifolia* and also present in *B. gibbsii* var. *balanifera* (fig. 7.12) and Icd-2(b) which is fixed in *B. talbotii* and also found in some populations of *B. modesta* and *B. hookeri* (fig. 7.15). That this allele was detected in two populations of *B. hookeri* near Taupo supports an herbarium record that a population of *B. talbotii* once existed in this locality.

Two **rare alleles** were found in each of the taxa *B. hookeri* [Gpi-1(c) and Mdh-3(d)], *B. gibbsii* var. *balanifera* [Gpi-1(c) and Mdh-2(b)] and *B. angustifolia* [Gpi-1(e) and Mdh-1(c)]. Four rare alleles were found in *B. modesta* [Mdh-2(d), 6Pgd-1(c), 6Pgd-2(b) and Pgm-1(c)]. These rare alleles were either detected only in the named taxon or detected as well in occasional populations of other taxa. Alleles which were rare in one taxon but common in another are considered under introgression rather than here. That the allele Icd-1(c) was present in all four populations of *B. modesta* studied and absent from all populations of other taxa, may suggest gene flow among the populations, and is supporting evidence for *B. modesta* remaining a discrete entity.

The inbreeding coefficient, $\mathbf{F_{IS}}$, was the **F-statistic** of interest **within taxa** (table 7.7), while $\mathbf{F_{ST}}$ gave the level of differentiation **between taxa** (tables 7.8 and 7.9). **Within Moore's (1964) taxa**, the lowest level of inbreeding was found in *B. modesta* (0.162) and the highest in *B. gibbsii* var. *balanifera* (0.617), closely followed by *B. rossii* (0.613). The F_{IS} for the other taxa described by Moore (1964) ranged from 0.425 to 0.498 (table 7.7). The F_{IS} values **within** the **taxonomic groupings** inferred from the **allozyme data** (table 7.7) ranged from 0.425 to 0.617. A high F_{IS} suggested that the taxon was separated into small breeding groups (isolated populations) with little gene flow occurring between them. A low F_{IS} may

Table 7.7. The F_{IS} values (inbreeding coefficients) within the taxa identified by Moore (1964) and the taxonomic groupings (clusters) inferred from the dendrogram (fig. 7.28). The F_{IS} could not be calculated for *B. gibbsii* var. *gibbsii* because the one plant examined was monomorphic at all 14 loci. Legend: B.h. = *B. hookeri*, B.gvb. = *B. gibbsii* var. *balanifera*, B.r. = *B. rossii*, B.m. = *B. modesta*, B.t. = *B. talbotii* and B.a. = *B. angustifolia*. All populations examined of each taxon are included. B.h.^{0} includes populations 1-16 and 29-34. B.h.^{1} includes populations 1, 4, 5, 6, 7, 8, 10, 13 & 14. B.h.^{2} includes populations 2, 3, 9, 11, 12, 15, 16, & 30-34. B.h.^{3} includes the same populations as B.h.^{2} except population 30. B.h.^{4} includes populations 2, 3, 9, 11, 12, 15 & 16. B.a.^{1} includes populations 37 - 55 and B.a.^{2} includes populations 56 - 61. Six of the seven taxomonic groups identified by Moore (1964) were B.h., B.gvb., B.r., B.m., B.t. and B.a. The four taxonomic groupings (clusters) inferred from fig. 7.28 were B.h.^{0}, B.gvb., B.t. and B.a.

Taxon	**F_{IS}**
B.h.	0.498
B.gvb.	0.617
B.r.	0.613
B.m.	0.162
B.t.	0.425
B.a.	0.474
B.h.^{0}	0.455
B.h.^{1} (sub-cluster 1)	0.480
B.h.^{2} (sub-cluster 2, B.h. + B.m. + B.r.)	0.271
B.h.^{3} (sub-cluster 2, B.h. + B.m.)	0.256
B.h.^{4} (sub-cluster 2, B.h. only)	0.312
B.a.^{1} (northern)	0.334
B.a.^{2} (southern)	0.191

Table 7.8. Pairwise F_{ST} values between *Bulbinella* taxa in New Zealand (Moore 1964). Legend: B.h. = *B. hookeri*, B.gvb. = *B. gibbsii* var. *balanifera*, B.gvg. = *B. gibbsii* var. *gibbsii*, B.r. = *B. rossii*, B.m. = *B. modesta*, B.t. = *B. talbotii* and B.a. = *B. angustifolia*. All populations examined of each taxon are included.

Taxon	F_{ST}
B.h. x	
B.gvb.	0.230
B.gvg.	0.225
B.r.	0.181
B.m.	0.099
B.t.	0.339
B.a.	0.472
B.gvb. x	
B.gvg	0.411
B.r.	0.360
B.m.	0.218
B.t.	0.430
B.a.	0.542
B.gvg. x	
B.r.	0.319
B.m.	0.099
B.t.	0.494
B.a.	0.721
B.r. x	
B.m.	0.125
B.t.	0.414
B.a.	0.524
B.m. x	
B.t.	0.293
B.a.	0.482
B.t. x	
B.a.	0.623

Table 7.9. Pairwise F_{ST} values between taxonomic groupings (clusters) inferred from the dendrogram (fig. 7.28). Legend: B.h. = *B. hookeri*, B.gvb. = *B. gibbsii* var. *balanifera*, B.t. = *B. talbotii* and B.a. = *B. angustifolia* B.h.^{0} includes populations 1 - 16 and 29-34. The taxonomic groupings inferred from allozyme data (above dotted line) were B.h.^{0}, B.gvb., B.t. and B.a. Sub-groupings inferred from the allozyme data (below dotted line) were B.h.^{1}, B.h.^{2}, B.h.^{4}, B.a.^{1} and B.a.^{2}. B.h.^{1} includes populations 1, 4, 5, 6, 7, 8, 10, 13 & 14. B.h.^{2} includes populations 2, 3, 9, 11, 12, 15, 16, & 29-34. B.h.^{4} includes populations 2, 3, 9, 11, 12, 15 & 16 only. B.a.^{1} includes populations 37 - 55 and B.a.^{2} includes populations 56 - 61.

Taxon or cluster	F_{ST}
B.h.^{0} x	
B.gvb.	0.206
B.t.	0.298
B.a.	0.451
B.gvb. x	
B.t.	0.430
B.a.	0.542
B.t. x	
B.a.	0.623
B.a.^{1} x	
B.a.^{2}	0.325
B.h.^{1} x	
B.m.	0.170
B.r.	0.241
B.h.^{4} x	
B.m.	0.058
B.r.	0.184
B.h.^{1} x	
B.h.^{2}	0.142
B.gvb.	0.246
B.t.	0.374
B.a.	0.469
B.h.^{2} x	
B.gvb.	0.231
B.t.	0.309
B.a.	0.498

indicate that gene exchange among the populations was more common in the taxon. For example the F_{IS} value within *B. modesta* was 0.162.

Moore's (1964) taxa which could not be separated by allozymes alone (*B. hookeri, B. gibbsii* var. *gibbsii, B. rossii* and *B. modesta*) showed the lowest pairwise $\mathbf{F_{ST}}$ (differentiation) values (table 7.8). Other pairwise F_{ST} values between Moore's (1964) taxa ranged between 0.181 and 0.721 (table 7.8), with the highest values found between taxa having one or more fixed differences, for example *B. talbotii* x *B. angustifolia* (F_{ST} = 0.623).

Using the **taxonomic groupings** inferred from the **allozyme data** (table 7.9) the lowest level of differentiation (F_{ST} = 0.206) was found between *B. gibbsii* var. *balanifera* and the *B. hookeri* complex (designated B.h.^{0}). Other values between taxonomic groups inferred from the allozyme data ranged from 0.298 to 0.623.

7.3.6 Correlation of taxonomic level with genetic distance

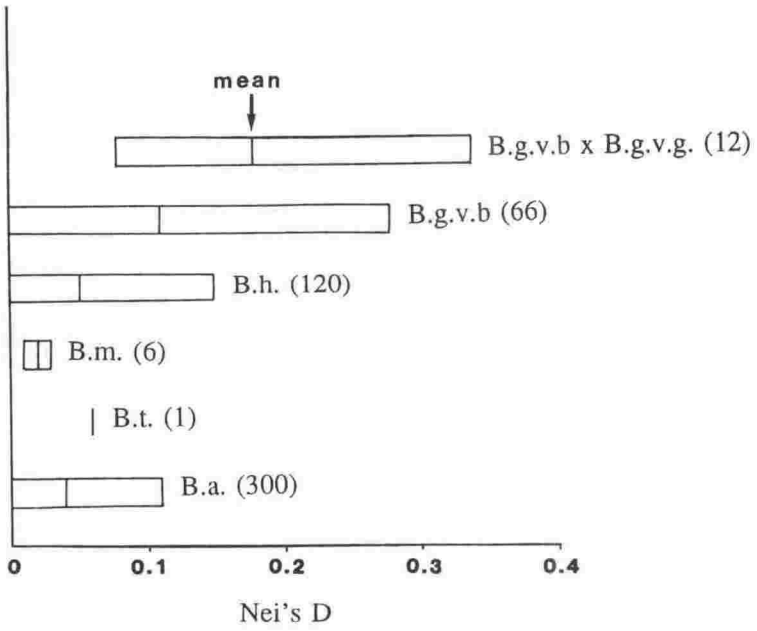
The genetic distance, D, a pairwise measure, is secondarily useful for assessing patterns of divergence. The values of D obtained between populations showed only a moderate correlation with taxonomic levels in this study (table 7.10).

While the mean D values **within a single species** (fig. 7.22) for *B. hookeri* and *B. angustifolia* were the same (0.05), the range for *B. hookeri* (0 - 0.15) was wider than that found in *B. angustifolia* (0 - 0.11). Within species the D between populations was lowest (0.02) for *B. modesta*, but only six comparisons were available. The highest within species values for mean distance (0.11) and range (0 - 0.28) were obtained for *B. gibbsii* var. *balanifera*. It is worth noting that these highest values were obtained **within** the same **variety** under current taxonomy. Because the mean D value and range **between** the two **varieties** of *B. gibbsii*

Table 7.10. Mean pairwise unbiased genetic distances (Nei 1978) between and within *Bulbinella* taxa in New Zealand. The full matrix of Nei's I and Nei's D for the 61 populations examined is not given in this study. * indicates the inclusion of *B. gibbsii* var. *balanifera* X *B. gibbsii* var. *gibbsii* data.

Taxon	mean D	Range
Within variety		
B. gibbsii var. *balanifera*	0.113	0-0.276
Between varieties		
B. gibbsii var. *balanifera* X var. *gibbsii*	0.183	0.075-0.336
Within species		
B. hookeri	0.051	0-0.146
B. modesta	0.020	0.007-0.031
B. talbotii	0.062	-
B. angustifolia	0.037	0-0.105
Between species		
B. hookeri X		
B. gibbsii var. *balanifera*	0.180	0.072-0.369
B. gibbsii var. *gibbsii*	0.064	0-0.146
B. modesta	0.070	0.005-0.196
B. talbotii	0.189	0.088-0.314
B. angustifolia	0.258	0.147-0.381
B. rossii	0.073	0.023-0.181
B. gibbsii var. *balanifera* X		
B. gibbsii var. *gibbsii*	0.183	0.075-0.336
B. modesta	0.155	0.063-0.328
B. talbotii	0.328	0.179-0.562
B. angustifolia	0.433	0.236-0.730
B. rossii	0.199	0.089-0.336
B. gibbsii var. *gibbsii* X		
B. modesta	0.021	0.007-0.034
B. talbotii	0.120	0.090-0.149
B. angustifolia	0.227	0.158-0.272
B. rossii	0.027	-
B. modesta X		
B. talbotii	0.139	0.083-0.202
B. angustifolia	0.232	0.137-0.287
B. rossii	0.036	0.016-0.062
B. talbotii X		
B. angustifolia	0.383	0.269-0.474
B. rossii	0.149	0.110-0.187
B. angustifolia X		
B. rossii	0.186	0.174-0.211
Overall:		
Within species	0.050	0-0.276
Within species*	0.054	0-0.336
Between species*	0.262	0-0.730

Figure 7.22. Nei's D means and ranges within individual species. Legend: The number in parentheses is the number of comparisons. B.h. = *B. hookeri*, B.g.v.b. = *B. gibbsii* var. *balanifera*, B.g.v.g. = *B. gibbsii* var. *gibbsii*, B.a. = *B. angustifolia*, B.m. = *B. modesta* and B.t. = *B. talbotii*.



examined (table 7.10) exceeded the values obtained between some pairs of species (table 7.10), it was considered useful to regard these two varieties as separate "taxa" for the purpose of the between species pairwise comparisons.

Comparisons of mean D values **between** the following pairs of **species**: *B. hookeri, B. gibbsii* var. *balanifera, B. angustifolia, B. modesta* and *B. talbotii* (table 7.10 and fig. 7.23) revealed that the highest mean D (0.43) and the greatest range in D values (0.24 - 0.73) were obtained between *B. gibbsii* var. *balanifera* and *B. angustifolia*. Excluding pairwise comparisons where only one population of one of the species involved was examined, the lowest mean D (0.07) was found between *B. hookeri* and *B. modesta* (range 0.01 - 0.2). The other pairs of species ranged in mean D values between these two extremes (fig. 7.23).

The overall mean D **within species** (including all taxa where more than one population was examined) was 0.050 (range 0 - 0.276). For comparison, the overall mean D when data from *B. gibbsii* var. *balanifera* was omitted was lower, being 0.040 (range 0 - 0.146) [fig. 7.24]. The overall mean D **between species** including all available comparisons was 0.262 (range 0 - 0.730, fig. 7.24).

That the pairwise mean genetic distance between the two varieties of *B. gibbsii* exceeds that within any other single species of *Bulbinella* (table 7.10), casts serious doubt on the validity of the placement of these two varieties within the same species. Furthermore, that the mean distance within the variety *B. gibbsii* var. *balanifera* exceeds that within any other single species (table 7.10) strongly implies that *B. gibbsii* var. *balanifera* may be composed of several taxa. The low mean D between *B. hookeri* and *B. modesta* casts doubt on existing taxonomy or suggests that these two species have recently diverged.

Figure 7.23. Nei's D means and ranges between species, pairwise comparisons. Legend: The number in parentheses is the number of comparisons. B.h. = *B. hookeri*, B.g.v.b. = *B. gibbsii* var. *balanifera*, B.g.v.g. = *B. gibbsii* var. *gibbsii*, B.a. = *B. angustifolia*, B.m. = *B. modesta*, B.t. = *B. talbotii* and B.r. = *B. rossii*.

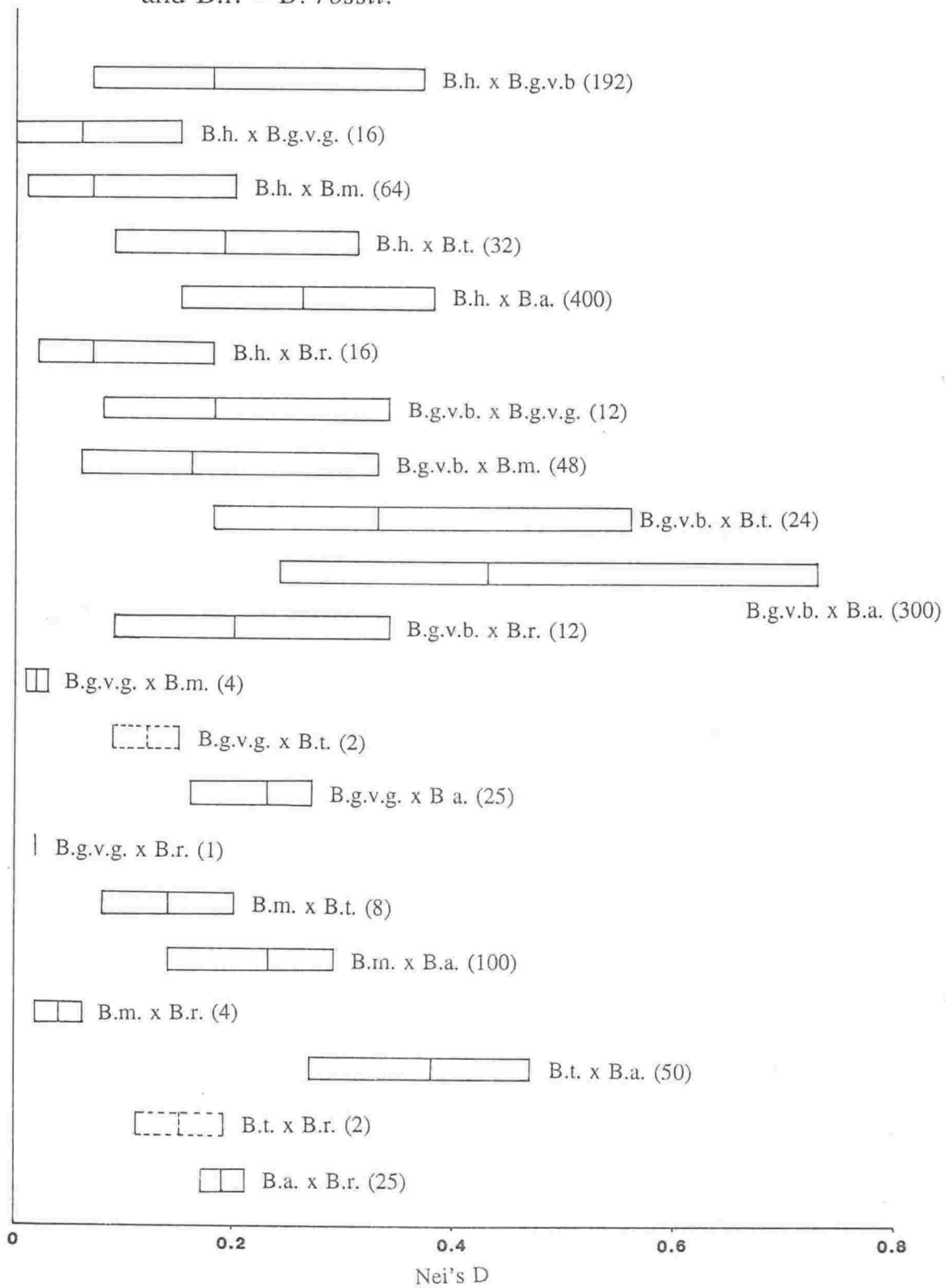
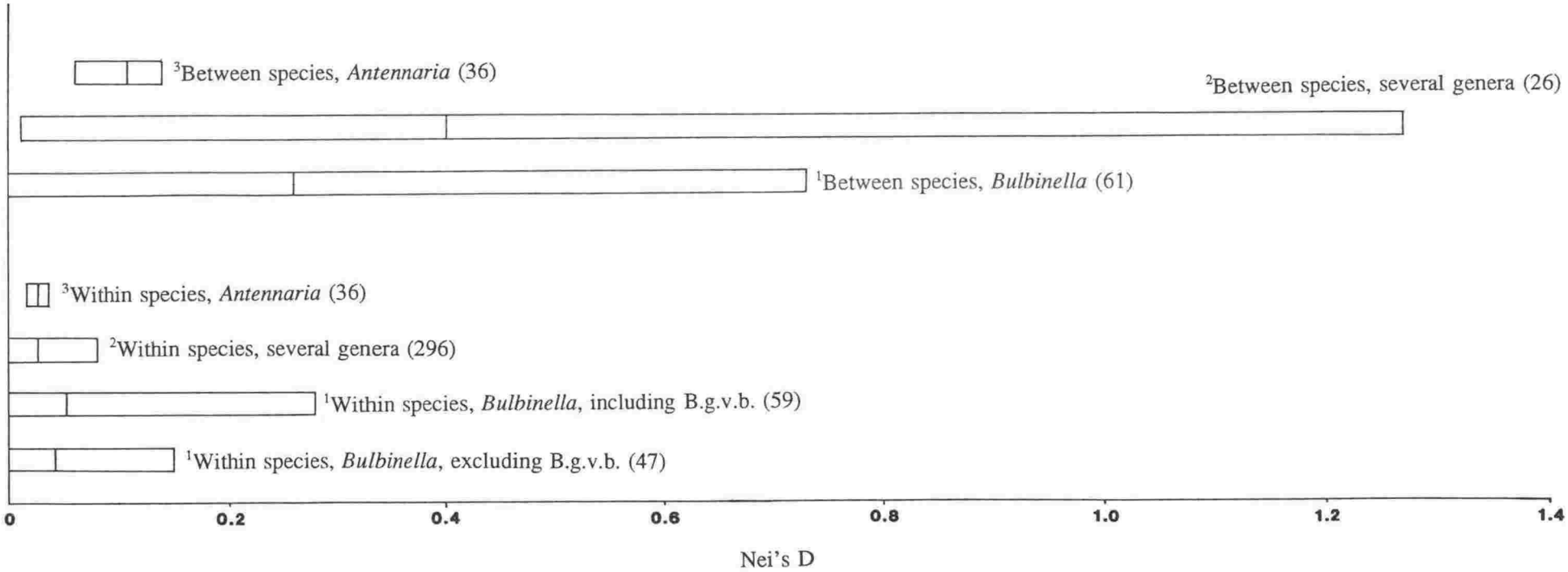


Figure 7.24. Nei's D: means and ranges within species and between congeneric species. Legend: B.g.v.b. = *B. gibbsii* var. *balanifera*. The number in parentheses is the number of populations examined. References are: [1] = this study, [2] = Gottlieb (1977) and [3] = Bayer and Crawford (1986).



In this study, although the mean D within a single species was lower than that between congeneric species, the ranges in the two sets of D values did overlap (fig. 7.24). Comparisons with data from other studies (fig. 7.24) reveal that generally genetic distances found within a single species are smaller than those found between congeneric species (as in this study). In the study of *Antennaria* (Bayer and Crawford 1986), these two classes of data (within a species and between congeneric species) did not overlap. In reviewed data (Gottlieb 1977), mean distances found within a species and between congeneric species did not overlap in most cases. Exceptions were data for *Oenothera* and *Gaura*. The mean genetic distance between 21 pairs of congeneric plant species surveyed by Gottlieb (1981) was 0.40, suggesting that about 40 detectable changes per 100 loci had occurred during the separate evolution of an average pair of congeneric plant species. Such a genetic distance represented more than a ten-fold increase over the average distance between conspecific populations (Gottlieb 1981).

7.3.6.1 Genetic distance distribution relating to taxonomic levels

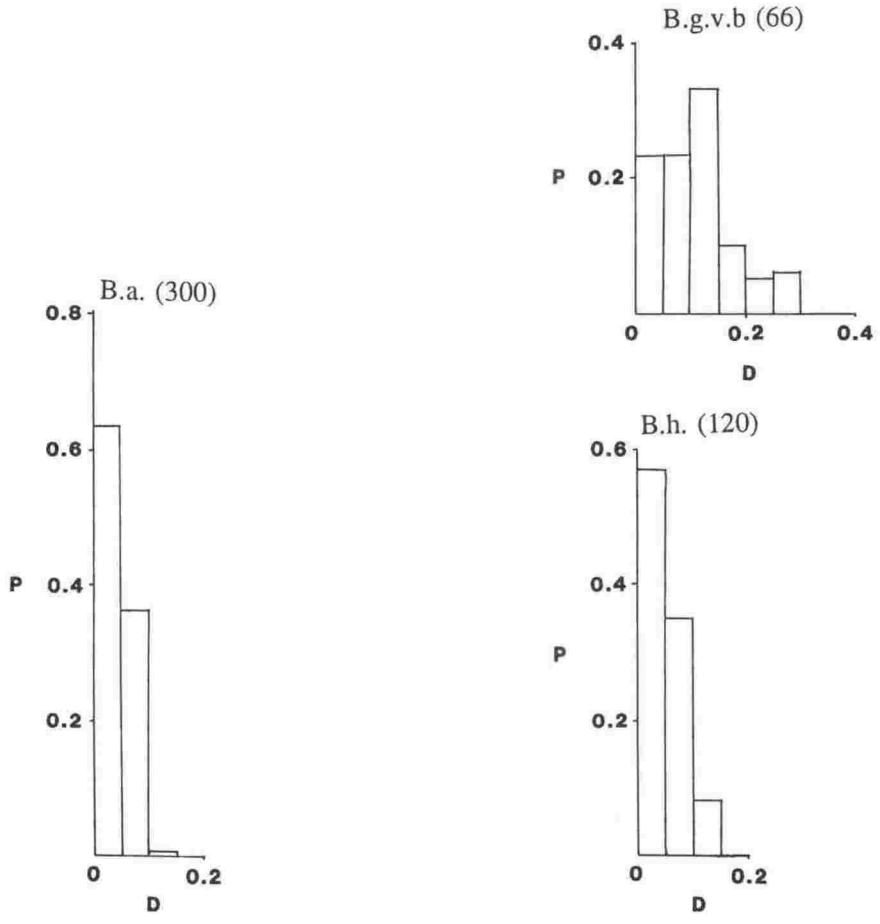
B. hookeri and *B. angustifolia* showed the expected distribution pattern (most values <0.1), for D values **within a single species** [Avise 1974, table 7.11 and fig. 7.25]. *B. gibbsii* var. *balanifera* did not conform to this pattern. There were insufficient observations for the patterns to be determined for *B. modesta* and *B. talbotii*.

Most of the **between species** pairwise comparisons (*B. hookeri* x *B. gibbsii* var. *balanifera*, *B. hookeri* x *B. angustifolia*, *B. hookeri* x *B. talbotii*, *B. gibbsii* var. *balanifera* x *B. angustifolia*, *B. modesta* x *B. angustifolia* and *B. talbotii* x *B. angustifolia*) showed the common between species patterns illustrated (Avise 1974),

Table 7.11. The distribution of D values within individual species. Legend: The number in parentheses is the number of measurements in each class. The figures in the body of the table represent the proportion of values which were in that range. The D values were summed at intervals of 0.05. This gave ranges of 0 - 0.05; 0.06 - 0.10; 0.11 - 0.15 and so on. The highest D value in the interval is given in the table (for example 0.05 represents the interval 0 - 0.05) under "D". B.h. = *B. hookeri,* B.gvb. = *B. gibbsii* var. *balanifera*, B.a. = *B. angustifolia,* B.m. = *B modesta* and B.t. = *B. talbotii*.

D	**B.h.** (120)	**B.gvb.** (66)	**B.a.** (300)	**B.m.** (6)	**B.t.** (1)
0.05	0.57	0.23	0.63	1.0	-
0.10	0.35	0.23	0.36	-	1.0
0.15	0.08	0.33	0.01	-	-
0.20	-	0.10	-	-	-
0.25	-	0.05	-	-	-
0.30	-	0.06	-	-	-

Figure 7.25. The distribution of Nei's D values within individual species. Legend: P = proportion of values, D = Nei's genetic distance and the number in parentheses is the number of observations. B.h. = *B. hookeri*, B.g.v.b. = *B. gibbsii* var. *balanifera* and B.a. = *B. angustifolia*.



although the position of the majority of values and the range in values obtained varied (table 7.12 and fig. 7.26). The pattern obtained between *B. hookeri* and *B. modesta* was more like that expected within a species. That 76% of the D values obtained between these two taxa were in the range 0 - 0.1 challenges existing taxonomy or suggests later divergence of this species from *B. hookeri.*

7.3.6.2 Summary (table 7.13 and fig. 7.27)

Generally, the majority of values **within species** fell between 0 - 0.1. Few were found above this value. *B. gibbsii* was an exception in that neither the pattern obtained within *B. gibbsii* var. *balanifera* nor that found between *B. gibbsii* var. *balanifera* and *B. gibbsii* var. *gibbsii* conformed to the one expected for within species data. **Between congeneric species** the distance values showed a common distribution pattern with most observations falling between 0.10 and 0.45, few lower (<0.10) and few higher (>0.45) values being recorded.

7.3.7 Patterns of relationships

Clusters are defined by the presence of at least one fixed allozymic difference between them and distances within a cluster being less than those between clusters. The phenetic cluster analysis (WPGMA) of unbiased genetic distance (D) reveals **four discrete clusters** (fig. 7.28). The pattern of clustering does not entirely conform to existing taxonomy.

One cluster contains all populations of *B. hookeri*, plus *B. rossii, B. gibbsii* var. *gibbsii* and all four populations of *B. modesta.* This cluster contains the taxa in which no distinguishing alleles were found. The taxa *B. talbotii, B. gibbsii* var. *balanifera* and *B. angustifolia* each correspond to **one cluster**. All **three** of these

Table 7.12. The distribution of D values between pairs of species. Legend: The number in parentheses is the number of measurements in each class. The figures in the body of the table represent the proportion of values which were in that range. The D values were summed at intervals of 0.05. This gave ranges of 0 - 0.05; 0.06 - 0.10; 0.11 - 0.15 and so on. The highest D value in the interval is given in the table (for example 0.05 represents the interval 0 - 0.05) under "D". B.h. = *B. hookeri,* B.gvb. = *B. gibbsii* var. *balanifera*, B.a. = *B. angustifolia,* B.m. = *B modesta* and B.t. = *B. talbotii*.

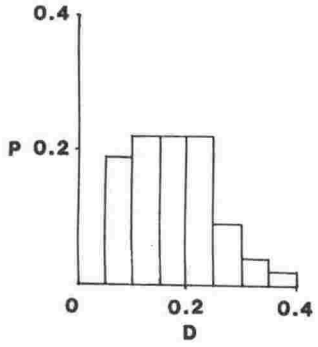
D	**B.h. x B.gvb.** (192)	**B.h. x B.a.** (400)	**B.h. x B.m.** (64)	**B.h. x B.t.** (32)	**B.gvb. x B.a.** (300)
0.05	-	-	0.45	-	-
0.10	0.19	-	0.31	0.06	-
0.15	0.22	<0.01	0.16	0.25	-
0.20	0.22	0.11	0.08	0.32	-
0.25	0.22	0.45	-	0.25	0.01
0.30	0.09	0.26	-	0.06	0.08
0.35	0.04	0.17	-	0.06	0.21
0.40	0.02	0.01	-	-	0.09
0.45	-	-	-	-	0.27
0.50	-	-	-	-	0.09
0.55	-	-	-	-	0.07
0.60	-	-	-	-	0.12
0.65	-	-	-	-	0.02
0.70	-	-	-	-	0.04
0.75	-	-	-	-	0.01

Table 7.12 continued

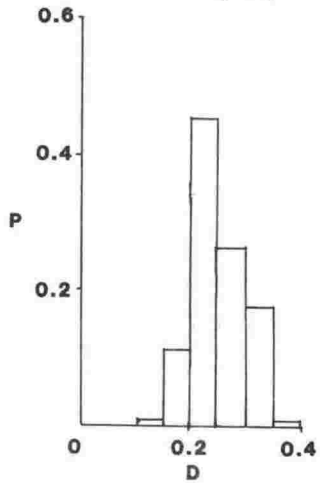
D	**B.gvb. x B.m.** (48)	**B.gvb. x B.t.** (24)	**B.m. x B.a.** (100)	**B.t. x B.a.** (50)	**B.m. x B.t.** (8)
0.05	-	-	-	-	-
0.10	0.29	-	-	-	0.38
0.15	0.27	-	0.03	-	0.25
0.20	0.17	0.12	0.19	-	0.37
0.25	0.19	0.17	0.51	-	-
0.30	0.06	0.17	0.27	0.12	-
0.35	0.02	0.17	-	0.10	-
0.40	-	0.12	-	0.42	-
0.45	-	0.17	-	0.34	-
0.50	-	0.04	-	0.02	-
0.55	-	-	-	-	-
0.60	-	0.04	-	-	-

Figure 7.26. The distribution of Nei's D values between pairs of species. Legend: P = proportion of values, D = Nei's genetic distance and the number in parentheses is the number of observations. B.h. = *B. hookeri*, B.g.v.b. = *B. gibbsii* var. *balanifera*, B.a. = *B. angustifolia*, B.m. = *B. modesta* and B.t. = *B. talbotii*.

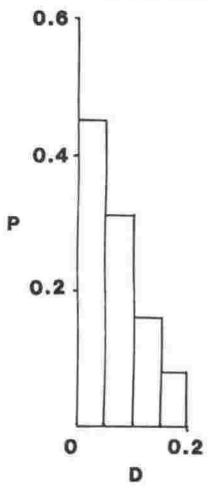
B.h. x B.g.v.b (192)



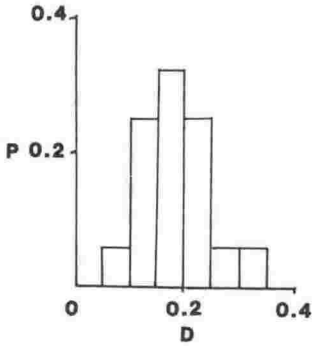
B.h. x B.a. (400)



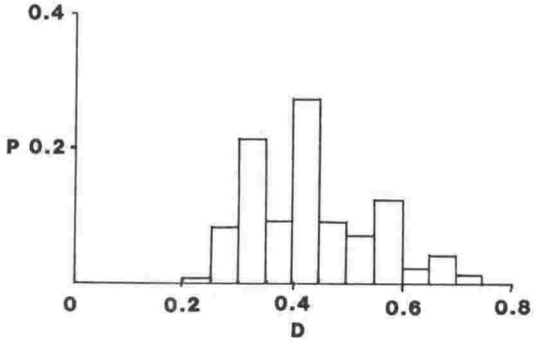
B.h. x B.m. (64)



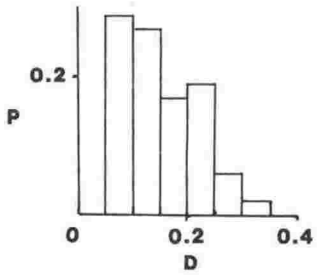
B.h. x B.t. (32)



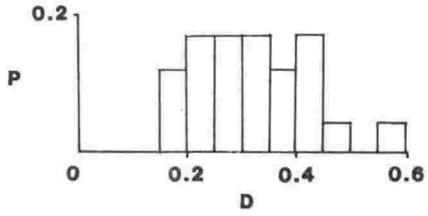
B.g.v.b. x B.a. (300)



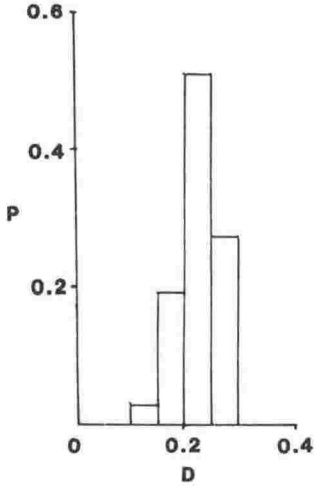
B.g.v.b. x B.m. (48)



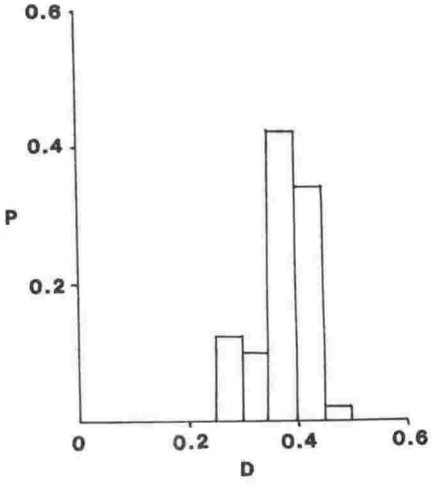
B.g.v.b. x B.t. (24)



B.m. x B.a. (100)



B.t. x B.a. (50)



B.m. x B.t. (8)

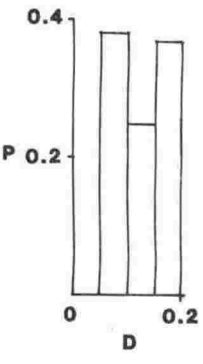


Table 7.13. The distribution of D values over all the taxonomic levels studied. Legend: The number in parentheses is the number of measurements in each class. The figures in the body of the table represent the proportion of values which were in that range. The D values were summed at intervals of 0.05. This gave ranges of 0 - 0.05; 0.06 - 0.10; 0.11 - 0.15 and so on. The highest D value in the interval is given in the table (for example 0.05 represents the interval 0 - 0.05) under "D". B.gvb. = *B. gibbsii* var. *balanifera* and B. gvg. = *B. gibbsii* var. *gibbsii*. B.gvb. x B.gvg. gave the between varieties data.

D	**Within variety B.gvb.** (66)	**Between varieties** (12)	**Within species** (492)	**Between species** (1218)
0.05	0.23	-	0.57	0.02
0.10	0.23	0.25	0.34	0.06
0.15	0.33	0.25	0.07	0.07
0.20	0.10	0.08	0.01	0.11
0.25	0.05	0.25	<0.01	0.24
0.30	0.06	0.08	0.01	0.15
0.35	-	0.08	-	0.12
0.40	-	-	-	0.05
0.45	-	-	-	0.08
0.50	-	-	-	0.02
0.55	-	-	-	0.02
0.60	-	-	-	0.03
0.65	-	-	-	0.01
0.70	-	-	-	0.01
0.75	-	-	-	<0.01

Figure 7.27. The distribution of Nei's D values relating to taxonomic levels. Legend: P = proportion of values, D = Nei's genetic distance and the number in parentheses is the number of observations.

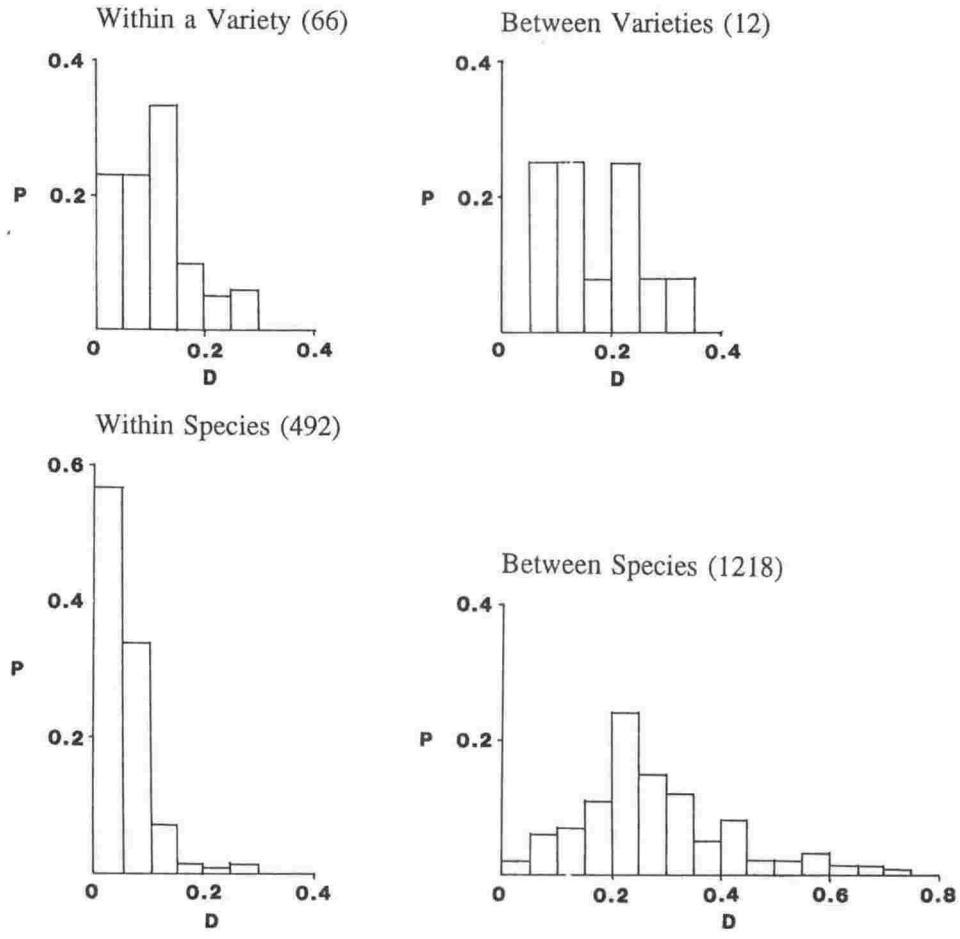
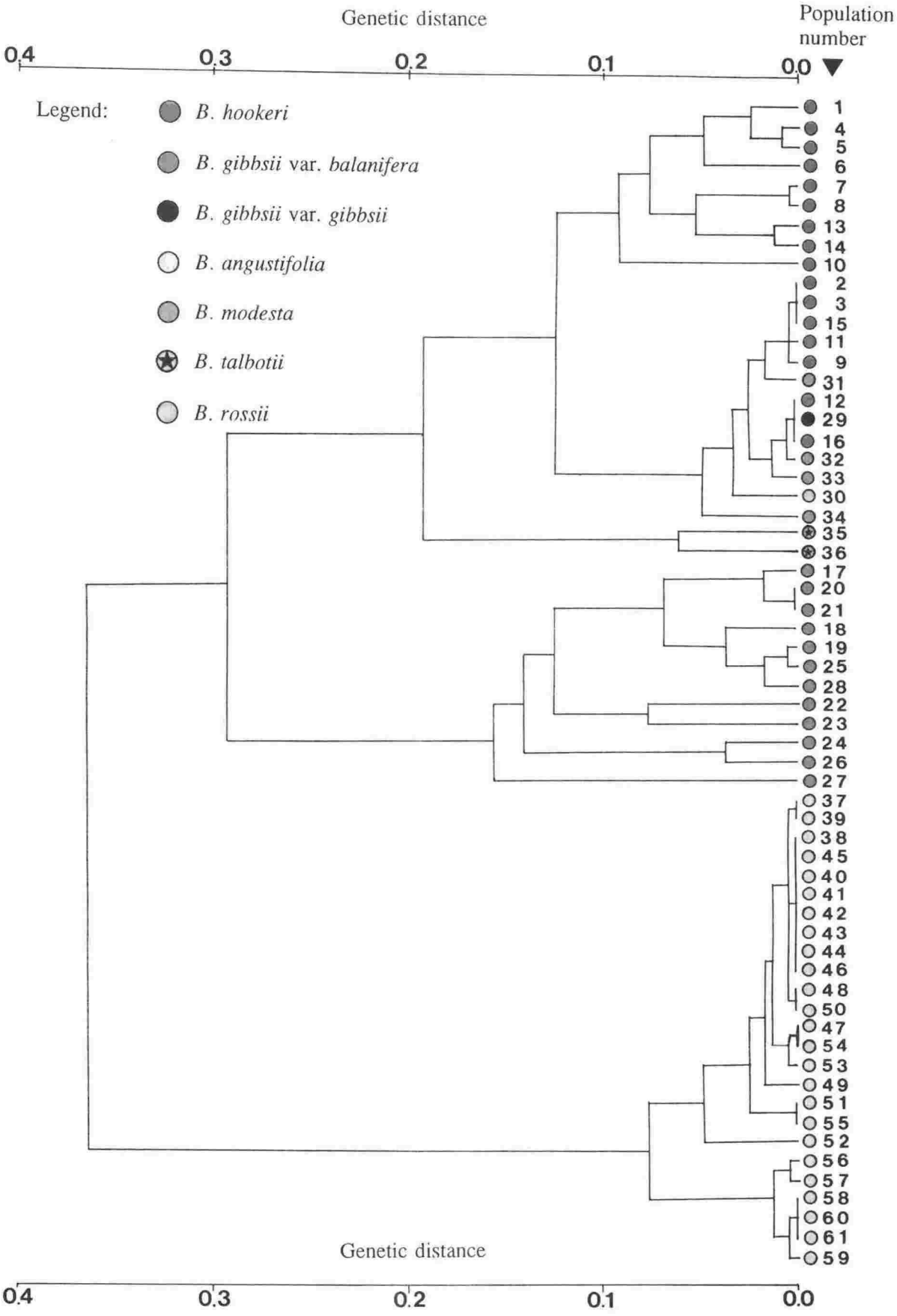


Figure 7.28. Dendrogram resulting from cluster analysis using Nei's unbiased genetic distances. The population numbers correspond to those in table 7.1.



taxa contain at least one fixed (but not diagnostic) allele, and *B. angustifolia* is characterised by a combination of two unique alleles as noted earlier (section 7.3.5).

The taxon *B. hookeri* is divided between two sub-clusters at a lower value of Nei's D in the dendrogram (fig. 7.28). While one of these groupings contains 9/16 populations of *B. hookeri*, the other is composed of the remaining 7/16 populations of *B. hookeri* plus *B. rossii, B. gibbsii* var. *gibbsii* and all four populations of *B. modesta.* The taxa which cluster with *B. hookeri* cannot be separated from any populations of this species using allozyme data. In *B. gibbsii* var. *gibbsii* and *B. rossii* the small sample sizes may be a contributing factor to the failure to distinguish the taxa allozymically.

The D within *B. talbotii* is entirely attributable to an allele frequency difference at one locus (Mdh-1, fig. 7.16) between the two populations.

The dendrogram (fig. 7.28) reveals several sub-clusters of populations at a lower D value and thus reflects the higher genetic distances within the taxon *B. gibbsii* var. *balanifera* compared to those within any of the other discrete clusters. Substantial differences (0 - 1.0) in allele frequency at three loci [Gpi-1 (fig. 7.13), Mdh-3 (fig. 7.18) and Pgm-1 (fig. 7.21)] within this taxon account for the greater distances on the dendrogram. Such differences are perhaps accentuated by the small number of loci examined.

While the present taxonomy of *B. angustifolia* is supported genetically, geographic groupings exist within this species in the form of populations which are characterised by the possession of alleles which are rare in or absent from other populations. The main groupings are northern (populations 37 - 50) and in the far south of the South Island (populations 56 - 61). The remaining populations (51 - 55) in the south-east of the South Island do not form a discrete sub-cluster on the

dendrogram (fig. 7.28) because they share some alleles with each of the two other geographic areas.

The clustering indicates that genetically *B. gibbsii* var. *gibbsii* is more similar to *B. hookeri, B. rossii* and *B. modesta* than to the alternative variety, *B. gibbsii* var. *balanifera*. That these four taxa cluster together, and are inseparable by allozyme analysis alone, questions existing taxonomy.

CHAPTER 8: APPLICATION TO TAXONOMY: CONCLUSIONS

8.1 Taxonomy

8.1.1 Introduction

The following **data** are now available for ***Bulbinella* in New Zealand**:

(1) **Morphological data** collected by Moore prior to her review of the taxonomy of the genus published in 1964. Most of these morphological data were obtained from the detailed study of herbarium specimens. A small number of live plants were also examined. In Moore's work, no mention was made of the variable nature of some populations and taxa reported in the present study, probably because the specimens she examined did not reveal this information. Much data of this nature have been collected for various plant groups. Analysis was almost invariably subjective and usually the evolutionary history of taxa was not evaluated.

(2) **Distribution data**, demonstrating the complete allopatry of taxa, compiled by Moore from her extensive study of herbarium specimens. These are still applicable although the ranges of some species have changed since 1964 (for example, the range of *B. modesta* has extended south of Okarito).

(3) **Morphological data** compiled by the present author. These data are based mainly on the study of natural populations of live plants as well as herbarium specimens.

(4) **Allelic frequency data** of the present study. These were obtained for 61 natural populations for each of 14 loci detected, of which 11 were variable.

(5) **Phenetic cluster analysis**. This was used to further define genetic and taxonomic affinities of the 61 study populations.

The **taxonomic implications** of the allozyme data (Chapter 7) are:

(a) The taxa *B. talbotii, B. gibbsii* var. *balanifera* and *B. angustifolia* are clearly separate species (section 7.3.7).

(b) Because the taxa *B. hookeri, B. modesta, B. gibbsii* var. *gibbsii* and *B. rossii* cannot be distinguished by allozyme analysis they are closely related (section 7.3.7).

(c) The high mean D and range in D values obtained between populations within *B. gibbsii* var. *balanifera* suggest that this group may contain several taxa (section 7.3.6).

(d) *B. gibbsii* var. *gibbsii* is more similar genetically to *B. hookeri, B. rossii* and *B. modesta* than to the alternative variety, var. *balanifera*, suggesting that these two taxa should not remain as varieties of the same species (section 7.3.7).

The **purposes** of a **taxonomy** are:

(i) to **classify** organisms.

(ii) to **summarise variation** within and between groups of related organisms.

(iii) to represent the **evolutionary history** of groups of organisms.

Although **species** are the basic units of **taxonomy**, taxonomists hold differing opinions of what these units should be, and by what criteria they should be delimited (Davis and Manos 1991). Until well into this century species delimitation typically involved the characterisation of distinct entities, groups of comparable individuals that could be distinguished by unique combinations of characters (usually morphological). The absence of a reproductive barrier between two systems of populations which are morphologically distinct is considered by some workers to be a criterion for withholding species status. However, other workers do consider

morphological differences in the absence of reproductive isolation sufficient criteria to place two groups of populations in two different species (Davis and Manos 1991).

Although **species** play a central role in ecology, systematics and evolutionary biology, **species concepts** are a controversial issue. Great confusion still exists as to what species are and how they can be recognised (Baum 1992). Other **species concepts** are presented for comparison to that given initially (Chapter 1, p.3), which was:

(a) "**Species** are the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means" [Conquist (1978) cited by Grant (1981)].

(b) **Biological** species are groups of interbreeding natural populations that are reproductively isolated from other such groups (Mayr 1970, p.12). The biological species concept does not account for hybridization. A number of (especially plant) taxa are not reproductively isolated, evidenced by the occurrence of natural hybrids. Because the parent taxa of these F_1 hybrids are often markedly different in appearance, with the hybrid being intermediate, all three taxa are usually named; with the parent taxa often being separated at least at the specific level. The biological species concept is more useful when assessing animal taxa where interfertility is relatively rare compared to the level found in some plant groups.

(c) An **evolutionary** species is a single lineage of ancestor - descendant populations which maintains its identity from other such lineages, and which has its own evolutionary tendencies and historical fate (Wiley 1978, cited in Wiley 1981). "Maintaining identity" from other lineages does not account for hybridization but can be assessed reasonably easily. Assessing the evolutionary tendency and historical fate of some groups may result in several taxa being merged if they are interfertile.

For this reason the evolutionary species concept is most usefully applied to reproductively isolated or endemic taxa.

(d) **Phylogenetic** species concepts focus on the historical relatedness of organisms and the distribution of characters. A **phylogenetic** species is an irreducible cluster of organisms possessing at least one diagnostic character. Diagnostic characters for a phylogenetic species must be discrete, fixed in the species and absent from close relatives. These characters may be morphological, behavioral or chemical provided they can be inferred to have a genetic basis (Baum 1992). Assessment of taxonomic status in light of the phylogenetic species concept is reasonably simple. It is relatively easy to decide whether characters are fixed and/or discrete. The phylogenetic species concept differs from the initial definition, (a), in the objectivity of the methods used to analyse the data compared to the subjective nature of previous methods of assessment. Although data of many types are applicable, the use of the phylogenetic species concept can result in a proliferation of taxa, separated only by minor differences (Frost and Hillis 1990).

8.1.2 Evaluation

When using the **initial species definition** [(a), p. 98], the size of the detectable difference classed as "distinguishable" depends on the nature of the taxon in question and is determined by worker(s) involved. Such decisions are invariably subjective and may lead to different workers making different taxonomic decisions concerning the same group of organisms. Therefore this definition was rejected.

Because some taxa of *Bulbinella* are not reproductively isolated (evidenced by introgression, Chapter 7), although all are allopatric, the use of the **biological** species concept as a species definition supports placing all the seven currently recognised

taxa into one species, and viewing the discrete groups as ecotypes. This species concept was rejected because it gives no indication of the distribution of variation or evolutionary history.

Although the evaluation of the evolutionary tendency and historical fate of a taxon may be difficult, the distribution of the endemic taxon *B. rossii* (Campbell Island and Auckland Island Group) suggests that this taxon has its own evolutionary tendency and historical fate and therefore can be regarded as a separate **evolutionary** species. One could argue that plant populations on the three main islands of New Zealand (North Island, South Island and Stewart Island) all have the same evolutionary tendency and historical fate, evidenced by hybridization, and therefore should all be placed in one species. Although the evolutionary species concept supports retaining *B. rossii* as a separate entity, this concept was rejected because like the biological species definition, this taxonomic option does not summarise variation (taxa, most of which are different allozymically, are combined) or represent the evolutionary history of the genus.

The use of the **phylogenetic** species concept relies on discrete, fixed characters. This concept can be applied to various data types. Few characters (morphological or allozyme) recorded for *Bulbinella* are completely fixed in any single taxon. A character typical of one species is often found (although usually in low frequency) in at least one other taxonomic group. The only completely fixed characters are the dioecious reproduction and cylindrical inflorescences (as opposed to gynodioecious or hermaphroditic reproduction and conical inflorescences) in *B. rossii* and the long bracts (compared to short) in *B. gibbsii* var. *gibbsii*. Nearly fixed allozymic characters are Icd-2(b) in *B. talbotii*, Icd-1(b) in *B. gibbsii* var. *balanifera* and Gdh-1(b) and Mdh-2(c) in *B. angustifolia*. Fixed and almost fixed characters can be used

to separate *B. rossii, B. gibbsii* var. *gibbsii, B. talbotii* and *B. angustifolia* from the *B. hookeri* / *B. modesta* complex. Use of the **phylogenetic** species concept results in the following taxa being recorded as separate species: *B. rossii, B. gibbsii* var. *gibbsii, B. hookeri* and *B. modesta* together, *B. talbotii, B. gibbsii* var. *balanifera* and *B. angustifolia*. That *B. hookeri* and *B. modesta* can be distinguished by discrete morphological characters (the lax flower racemes and prostrate growth habit of *B. modesta*) indicate that *B. modesta* should be assigned to a sub-species of *B. hookeri*, not completely merged with it.

Taxa recognised by Moore (1964)	**Taxa supported by this study♦**
B. rossii	*B. rossii*
B. gibbsii var. *gibbsii*	*B. gibbsii**
B. hookeri	*B. hookeri* ssp. *hookeri*
B. modesta	*B. hookeri* ssp. *modesta*
B. talbotii	*B. talbotii*
B. gibbsii var. *balanifera*	*B. balanifera*
B. angustifolia	*B. angustifolia*

* more samples required

♦ using phylogenetic species concept

Using the **phylogenetic** species concept, both morphological and allozyme data can be readily evaluated. Because application of the **phylogenetic species concept** to *Bulbinella* results not only in **classifying** the **taxa**, but **summarising variation** and **evaluating** the **evolutionary history** as well, this species concept was accepted as the most suitable species definition for this study. The use of the phylogenetic

species concept in relation to *Bulbinella* did not lead to a proliferation of taxa as often occurs (Frost and Hillis 1990).

8.1.2.1 Justification for taxonomic decisions

Retaining ***B. rossii*** as a separate species is supported by its endemic distribution, fixed morphological differences and reluctance to base taxonomic decisions on few samples (only six plants were analysed).

Retaining ***B. gibbsii* var. *gibbsii*** as a separate taxon is supported by morphological differences (one of which is fixed) and conservatism (only one sample was available for analysis). Although Sarich (1977) demonstrated that the information provided by the analysis of **two** individuals from one population was sufficient to assess species level relationships, he employed a large number of loci. The number of loci used in this study may have been insufficient to make a certain decision using only two individuals. The raising of the taxonomic status of *B. gibbsii* var. *gibbsii* to to that of a species is supported by allozyme data. This taxon is genetically more closely related to *B. hookeri, B. rossii* and *B. modesta* than to the alternative variety, var. *balanifera*. Although plant populations belonging to the same taxon (at species level) are very similar enzymatically, and one population may be nearly representative of the species as a whole (Crawford 1989), this should not be used as an argument for limited populational sampling. I am therefore reluctant to formally alter the taxonomic status of *B. gibbsii* var. *gibbsii* in the absence of further samples from more individuals and from more populations of this current variety. In *B. gibbsii* var. *gibbsii* and *B. rossii*, the small sample sizes may have been a contributing factor to the failure to distinguish these two taxa from each other

or from either *B. hookeri* or *B. modesta.* Small sample sizes were not a contributing factor in the taxa *B. modesta* or *B. hookeri.*

B. modesta has not diverged completely from ***B. hookeri*** (although it may do so in future) and should be renamed as a **sub-species** of this taxon. Large sample sizes for both *B. modesta* and *B. hookeri* and a low level of differentiation between these two taxa (F_{ST} = 0.099, table 7.8) support this decision. The presence of discrete morphological differences between *B. hookeri* and *B. modesta* supports the subspecific differentiation of these two taxa.

Fixed morphological differences and an almost fixed allozymic difference support ***B. gibbsii* var. *balanifera*** remaining a separate taxon and being **raised to specific rank**. Because the differences in allelic frequencies at several loci within *B. gibbsii* var. *balanifera* were not detected between the same populations at each locus when they were found, subdivision of this taxon into cryptic species is inappropriate.

Fixed morphological differences and one almost fixed allozymic difference support ***B. talbotii*** remaining a **separate species**.

The presence of two unique alleles supports ***B. angustifolia*** also remaining a **separate species**. The two groups of populations within *B. angustifolia* (B.a.[1] and B.a.[2], table 7.9) should be recorded as different because they are differentiated from each other (F_{ST} = 0.325, table 7.9), but the species should not be split taxonomically since morphological differences between the two groups (narrow leaves and lax flower racemes) are present only in the most southern populations of B.a.[2] and the allozymic differences [for example the presence of allele 6Pgd-1(a)] are not fixed in B.a.[2]. The frequency of the allele 6Pgd-1(a) forms a 'cline' with increasing frequency of 6Pgd-1(a) compared to 6Pgd-1(b) being detected on moving south from Otago (fig. 7.19). Sub-division of this species is therefore inappropriate.

The **non-concordance** of different types of data, reported by Crawford (1985), was also encountered in this study. There are several possible **explanations**.

(1) Genetic differences are present but were not detected. (For example, proteins were different in structure but showed the same mobilities on a gel).

(2) Only a minute fraction of the genome was sampled. If more loci had been resolved, fixed or nearly fixed differences may have been found between taxa which presently cannot be separated using allozymes.

(3) The small numbers of individuals and populations sampled from *B. gibbsii* var. *gibbsii* (1) and *B. rossii* (6) lead to the erroneous conclusion of their being allozymically indistinguishable from each other and from *B. hookeri* and *B. modesta*. An inherent difficulty with the technique of starch gel electrophoresis is that small differences in the mobilities of alleles cannot always be clearly resolved. Differences are more obvious/convincing when a larger number of samples is analysed.

(4) Differences in allozyme data between taxa can be interpreted as real, but no detectable difference does not neccessarily infer a common evolutionary history.

(5) Taxa which presently cannot be separated by allozymes (*B. hookeri, B. modesta, B. gibbsii* var. *gibbsii* and *B. rossii*) have retained the ancestral (original) character state for most loci, while those which can be distinguished have derived character states for some loci. Ancestral character states may be those found most commonly in the study taxon (*Bulbinella*) and which are also present in close relatives (other members of Liliaceae, such as *Allium*) [Brooks et al. 1984 p. 4]. In time, the currently inseparable taxa may diverge. The allele Icd-1(c), unique to *B. modesta*, may indicate that this taxon is in the process of diverging from the remainder of the group. It may be that Icd-1(c) is an autapomorphy (a feature unique to one group of individuals and thought to originate in that group) that

justifies continued recognition of *B. modesta* as a separate entity and is also evidence of gene flow. A purely phenetic analysis was used previously (Chapter 7). Cladistic methods might give a different result and would be more appropriate for understanding evolutionary history.

(6) There is no neccessary or causal relationship between speciation and allozyme divergence (or between morphology and speciation).

Non-concordance between **electrophoretic data** and **other data** sets (morphological, ecological and interfertility) has been found in other plant groups (Crawford 1985). Allozyme data are most useful for testing hypothesised modes of speciation when evaluated in the context of all other data available (Crawford 1985). Divergence at genes specifying soluble enzymes is often uncorrelated with plant speciation. That is, speciation may occur but the divergence between species is no greater than that normally found between populations of a single taxon (Crawford 1989). In this study, the four taxa of *Bulbinella* between which no fixed differences in allozymes could be demonstrated *(B. hookeri, B. gibbsii* var. *gibbsii, B. rossii* and *B. modesta*), may illustrate this phenomenon. Conversely, allozyme divergence may occur between populations in the absence of speciation (Crawford 1989). The fixation of the allele Mdh-3(b) [as opposed to Mdh-3(a)] in some populations of *B. gibbsii* var. *balanifera* (fig. 7.18) illustrates this latter point.

8.2 Phylogenetic analyses

8.2.1 Introduction

Phenetic analysis of data leads to a taxonomic arrangement reflecting overall similarity and is based on a large set of characters, usually without emphasising any

particular character or character set. **Phenetic** relationships do not neccessarily reflect phylogenetic (evolutionary) relationships, although genetic similarity can be expected to reflect the amount of time since the divergence of the groups (Richardson et al. 1986).

Phylogenetic analysis or **cladistics** is the only way to estimate evolutionary history. Allozyme data seldom stand alone. Interpretation must take account of other data such as morphology and karyology (Richardson et al. 1986).

8.2.2 Materials and Methods

Swofford's 1985, 1990 version 3.0 of PAUP (Phylogenetic Analysis Using Parsimony) was used to construct phylogenies. PAUP was applied to selected data sets (table 8.1) which included morphological and allozyme data, both together and separately. The morphological data were the character states discussed previously (Chapter 4, table 4.5). The allozyme data were entered in the form of alleles which were regarded as characters and their states were recorded as presence or absence of that allele. The use of loci as characters did not provide a large enough data set to be useful, especially when allozyme data alone were used.

Allium triquetrum, a member of Liliaceae (the same family to which *Bulbinella* belongs), was defined as an outgroup and used to root the trees which were constructed by the branch and bound method. The consistency index (C.I.) is the number of **unique** character state changes divided by the **total** number of changes. Changes which occur more than once, and reverse changes lower the C.I. from 1, the maximum possible value. The C.I. was calculated by PAUP (1) for each character used in the data set and (2) the mean value was recorded over all characters for each tree or set of equally parsimonious trees. One inadequacy of PAUP is that reticulate

Table 8.1. The data sets which were used to construct phylogenies using PAUP (Swofford 1985, 1990 version 3.0). The taxa are those supported by this study: *B.r.* = *B. rossii, B.g.* = *B. gibbsii, B.h.* = *B. hookeri, B.t* = *B. talbotii, B.b.* = *B. balanifera, B.a.* = *B. angustifolia* and *Allium* = *Allium triquetrum*. The tree number is that assigned to the output from PAUP.

Taxa	Data	Tree number
B.r. *B.g.* *B.h.* *B.t.* *B.b.* *B.a.* *[Allium]*	allozyme only	1
as above	morphology only	2
as above	allozyme & morphology	3

Table 8.2. Summary of the phylogeny results. Legend: a = allozyme data, m = morphological data, c = concensus (tree).

Tree number	Data	Number of shortest trees	Number of characters	Number of character state changes	Consistency index (C.I.)
1	a	7	30	37	0.811
1c	a	1	30	45	0.667
2	m	1	21	33	0.848
3	a & m	1	51	74	0.784

evolution is not taken into account, which is clearly inappropriate for use with taxa which are not reproductively isolated.

8.2.3 Results and discussion

PAUP was applied to three data sets (table 8.2). Seven equally parsimonious trees were constructed using allozyme data alone (fig. 8.1 illustrates one example). Because of the occurrence of reticulate evolution in this group (evidenced by hybridization), a concensus tree derived from these seven was not useful as an indicator of evolutionary history. Application of PAUP to the morphological data alone (fig. 8.2) and to both morphological and allozyme data combined (fig. 8.3), produced only one shortest tree on each occasion.

In none of the trees 1-3 were *B. balanifera* and *B. gibbsii* represented as sister groups. The combination of the morphology and allozyme data produced a similar phylogeny (fig. 8.3) to that obtained using morphological data alone (fig. 8.2). The only difference between the trees generated using these two data sets was the presentation of *B. rossii* and *B. balanifera* as sister groups using morphological data alone and *B. rossii* and *B. gibbsii* as sister groups using both allozyme and morphological data. The difference between the two trees reflect that *B. rossii* and *B. balanifera* were the most similar of the three morphologically, and *B. rossii* and *B. gibbsii* were the most similar genetically (both have retained ancestral character states for many loci and were indistinguishable by allozyme analysis).

That the two current varieties of *B. gibbsii* are not depicted as sister groups using any of the data sets is no surprise, and this supports their being placed in separate species.

Figures 8.1. to 8.3. Phylogenetic trees constructed using PAUP (Swofford 1985, 1990 version 3.0). Legend: The tree numbers correspond to those in tables 8.1 and 8.2. C.I. = consistency index. Vertical lines represent changes in character state. The taxa are: *B.r.* = *B. rossii*, *B.g.* = *B. gibbsii*, *B.h.* = *B. hookeri*, *B.t.* = *B. talbotii*, *B.b.* = *B. balanifera*, *B.a.* = *B. angustifolia* and *Allium* = *Allium triquetrum*.

Figure 8.1.

Tree number	1 (of 7)
Data	allozyme only
C.I.	0.811

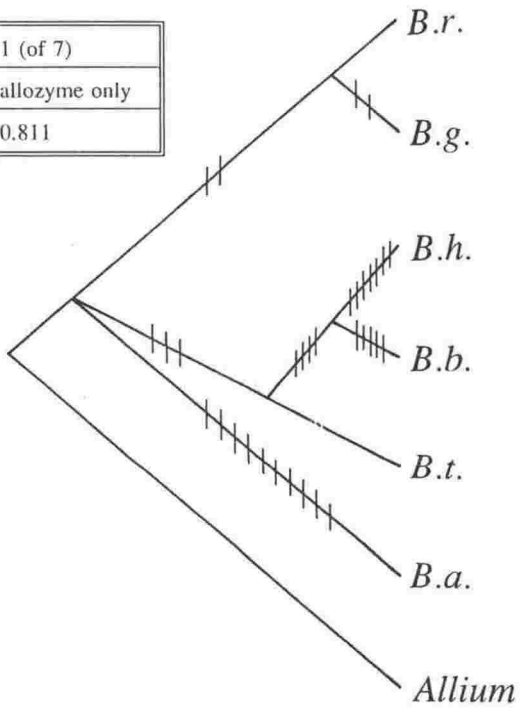


Figure 8.2.

Tree number	2
Data	morphology only
C.I.	0.848

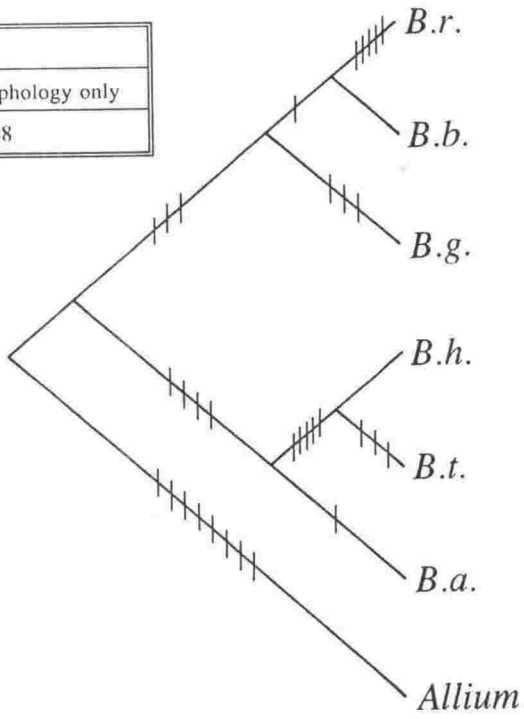
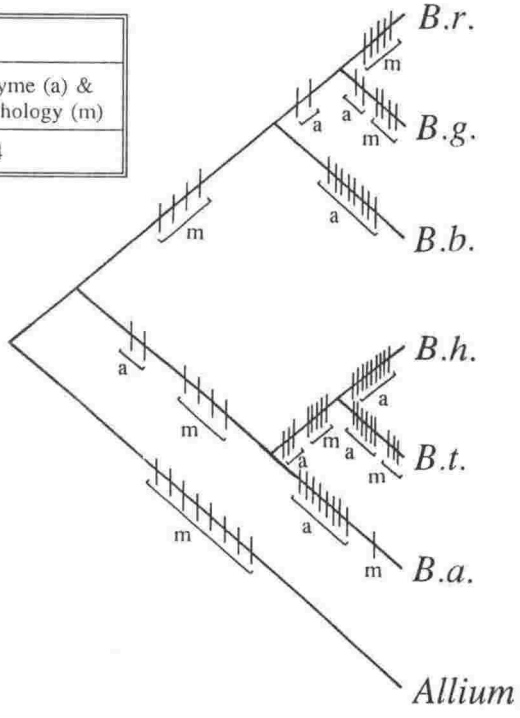


Figure 8.3.

Tree number	3
Data	allozyme (a) & morphology (m)
C.I.	0.784



8.3 Implications for conservation

8.3.1 Introduction

The **aspects** of **conservation** identified or confirmed by this study are:

(1) Some taxa are recorded as "**local**" (*B. talbotii*) or "**endemic**" (*B. rossii*) because of their restricted geographic distribution.

(2) **Habitat destruction** was most evident in reference to the taxa *B. modesta* and *B. angustifolia*. It is likely that extensive farming, in the south east of the South Island particularly, has eliminated some previously recorded populations of *Bulbinella* by destroying their habitat.

(3) This study identified certain **populations** and **groups of populations** as genetically and in some cases morphologically different from the remainder of their taxon.

The decision as to what should be conserved preferentially, that is, which populations/localities should be given priority, is a difficult one.

One of the three main objectives of the living resource conservation of the World Conservation Strategy is to preserve genetic diversity (IUCN 1980). Vane-Wright et al. (1991) confirm that urgent action is required to preserve biological diversity, and discuss various methods of measuring biodiversity. They employed cladistic procedures to develop taxic diversity measures and applied these to the assessment of conservation priorities.

The conservation of nature is also affected by distribution and biogeography. A taxon restricted to one locality or region must be preserved there, or samples obtained from there for relocation (Vane-Wright et al. 1991).

Avise et al. (1987) addressed the concept of **intraspecific phylogeny** and geographic population structure. Their five categories have been summarised (table 8.3).

8.3.2 Appropriate conservation measures

Both local (*B. talbotii*) and endemic (*B. rossii*) taxa probably do not require further conservation measures to those already in place.

The preservation of genetic diversity (IUCN 1980 and Vane-Wright et al. 1991) in *Bulbinella* results in retaining representatives of the most different (morphological and allozymic) types of plant.

The identification of some populations and groups of populations as different from the majority of their taxon has not resulted in subdivision of the taxa in which they occur because the unusual/unique features form a cline within the taxon rather than being clear cut. These clines were typical of *B. angustifolia* and *B. gibbsii* var. *balanifera*. Such populations or groups of populations may be regarded as separate phylogeographic units and were noted as different, but have not been distinguished taxonomically. The unique groups within *B. angustifolia* are of special interest because a number of the populations composing them contain rare alleles and are small remnants of once larger populations (such as the ones at Backline Road and Alton Burn).

The categories of phylogeographic pattern (Avise et al. 1987) which applied to this study, particularly to *B. angustifolia* and to a lesser extent to *B. gibbsii* var. *balanifera* were:

I (discontinuous with spatial separation) in relation to the locus 6Pgd-1 in *B. angustifolia* where most intermediate genotypes have been eliminated between the

Table 8.3. General phylogeographic patterns (relationships between phylogeny and geography) discussed by Avise et al. (1987) and applied to this study.

Category	Genetic divergence pattern	Geographic distribution	Likely evolutionary circumstances	Applications to *Bulbinella*
I	discontinuous	spatial separation	(A) long-term extrinsic barriers to gene flow; and/or (B) extinctions of intermediate genotypes in species with limited gene flow.	(A) may apply to some populations of *B. gibbsii* var. *balanifera*. (B) particularly applicable to *B. angustifolia*.
II	discontinuous	lack of spatial separation	(A) recent secondary admixture zones; or (B) intrinsic barriers among sympatric sibling species.	not applicable to *Bulbinella*.
III	continuous	spatial separation	limited gene flow in a species not sub-divided by long-term extrinsic barriers.	may apply to groups of populations of *B. angustifolia* which share otherwise rare alleles in certain geographic regions (for example the south-east of the South Island).
IV	continuous	lack of spatial separation	very extensive gene flow in a species not sub-divided by long-term extrinsic barriers.	not applicable to *Bulbinella*.
V	continuous	partial spatial separation	intermediate gene flow in a species not sub-divided by long term extrinsic barriers.	may also apply in the situation mentioned with respect to pattern III, and may be difficult to distinguish from this category.

areas where either allele (b) or allele (a) was found in higher frequency (fig. 7.19). Phylogeographic category I may also be applied to the loci Mdh-3, 6Pgd-1 and Pgm-1 in *B. gibbsii* var. *balanifera* (figs 7.18, 7.19 and 7.21 respectively), with geography providing a long term extrinsic barrier to gene flow between some populations of this species.

III (continuous with spatial separation) concerning gene flow within certain geographic areas containing *B. angustifolia*, such as those in the south-east and the far south of the South Island. Over the entire range of this species, this information could also be an example of category I, with the presence of long-term barriers to gene flow between these 'sub-sets'.

Phylogeographic category **V** may be anticipated in species or sub-sets of species with historically intermediate levels of gene flow between geographic populations. This intermediate situation may be difficult to distinguish clearly from categories III or IV (Avis et al. 1987). This was certainly the case in this study, especially in relation to 'sub-sets' of populations of *B. angustifolia* in the south-east and the far south of the South Island.

Avise et al. (1987) point out that conspecific populations can occupy easily recognisable branches on an intraspecific evolutionary tree. Differences can exist within one species and it is therefore important not to restrict oneself to any taxonomy when making decisions relating to conservation. Conserving phylogeographic units of a group of related organisms results in retaining representatives of the most different types of that group.

8.4 Conclusions

Critical evaluation of some species concepts (biological, evolutionary and phylogenetic) resulted in the selection of the phylogenetic species concept for application to the data obtained in this study. Use of the phylogenetic species definition classified the taxa, summarised variation and represented evolutionary history. Results of this study supported changing the taxonomy of *Bulbinella* as follows:

B. rossii, B. talbotii and *B. angustifolia* remain separate taxa at the specific level. *B. gibbsii* var. *balanifera* is raised to specific rank (*B. balanifera*), as is *B. gibbsii* var. *gibbsii* (*B. gibbsii*, subject to analysis of further samples). *B. modesta* is recognised as a sub-species of *B. hookeri*.

Phylogenetic analysis, which was not entirely appropriate because of the interfertility among taxa, showed clearly that *B. balanifera* and *B. gibbsii* are not sister groups and thus supported their being placed in separate species.

CHAPTER 9: SUMMARY

Chapter 2: Natural history

All seven taxa of *Bulbinella* in New Zealand have the same basic features: swollen, fleshy roots, strap-like leaves and a raceme of yellow star-like flowers. Plants of all taxa favour a wet habitat and are entirely allopatric.

Plants of the genus *Bulbinella* in South Africa also produce a raceme of stellate flowers, but they are more variable than plants of the New Zealand genus regarding features such as plant size, leaf shape, root appearance and flower colour.

Chapter 3: Conservation aspects

While the habitats of most of the 57 populations of *Bulbinella* examined by the author were considered stable, there was evidence for some habitats being diminished by human enterprises such as farming, road-making and mining. The two species most affected by these activities were *B. modesta* and *B. angustifolia.* Diminished habitat of the latter species was particularly noticeable in the form of small remnants of once larger populations. The results of this study suggest that many previously recorded populations of *B. angustifolia* in the southern South Island have been eliminated by farming practices. The few which remain are considered vulnerable.

The only taxon considered to be at risk was *B. talbotii*, recorded as local (not an IUCN category) because its distribution is now restricted to one area, the Gouland Downs.

Chapter 4: Growth and Morphology

Eighteen morphological characters were recorded for each of the seven taxa. Most of these characters were observed in growing plants in natural populations, while a few (for example reproduction) were obtained or confirmed from the

literature. Although generally the seven taxa were morphologically distinguishable, occasional plants differed from the remainder of their population and occasional populations differed from most others in their taxon.

The greatest percentage of germination was achieved following a period of moist chilling applied to seeds sown as soon as possible after ripening.

Chapter 5: Cytogenetics

Karyotyping was achieved for at least one plant from each of 13 populations. The diploid chromosome number was 14 (seven pairs) except in the one plant of *B. rossii* examined where the presence of a supernumerary 'B' chromosome was suspected. Giemsa-banding, demonstrating one band on each of four chromosomes, was achieved in one slide of material from one plant.

Chapter 6: Allozyme techniques

After screening six tissues of *Bulbinella* with a total of 52 protein stains, a maximum of 14 loci was consistently resolved from flower tissue, using eight enzyme stains.

Chapter 7: Geographic variation in allozymes

Floral tissue from 61 natural populations of *Bulbinella* was examined. Eleven of the 14 loci resolved were polymorphic. A maximum of five alleles was detected in any one locus over the seven taxa. No single allele was both fixed (frequency = 1.0) and diagnostic for any one taxon. Some alleles were fixed in one species but not diagnostic as a result of introgression.

Four discrete clusters were identified in the dendrogram (fig. 7.28), which was constructed using Nei's D values derived from allelic frequency data. Three discrete clusters corresponded to three of Moore's (1964) taxa, while the remaining cluster

contained the other four currently recognised taxa, which could not be separated allozymically.

Chapter 8: Taxonomy and conclusions

The application of the phylogenetic species concept to the results of this study of *Bulbinella* in New Zealand supports the following decisions concerning taxonomy:

(1) *B. rossii, B. talbotii, B. gibbsii* var. *balanifera* and *B. angustifolia* should remain as separate taxa.

(2) *B. gibbsii* var. *balanifera* and *B. gibbsii* var. *gibbsii* should both be raised to specific status subject to the analysis of more individuals of the latter variety.

(3) *B. modesta* should be named as a sub-species of *B. hookeri* to which it is genetically closely related.

Appendix I. Site visits to assess the habitat stability of *Bulbinella* in New Zealand. The localities followed were those given in personal communications, literature or recorded herbarium collections. Where several herbarium specimens from the same locality were examined a range of dates has been given. Legend: sources of localities: [p] = pers. comm., [h] = herbarium record; literature: [1] = Salmon 1985, [2] = Robins 1968 and [3] = Moore 1964.

Locality	Latitude/ longitude	Dates of visits	*Bulbinella* plants found yes / no	Date(s) of previous sitings
B. hookeri				
Mt. Manuoha	37° 50′S 177° 05′E	12/87	n	1984[h], 1988[p]
Desert Road	39° 15′S 175° 44′E	12/90	y	1933[h] - 1964[h]
Umukarikari Range	39° 13′S 175° 53′E	12/90	y	1989[p]
Waipakihi River	39° 14′S 175° 48′E	12/86 03/87 12/90	y y y	
Tukino Ski Road	39° 14′S 175° 38′E	05/86 12/86	n n	1986[p]
Poled Track	30° 18′S 175° 47′E	12/86 03/87	y y	1986[p]
Mangaio Stream	39° 19′S 175° 44′E	12/90	y	1965[h]
Mangaohane	39° 35′S 176° 08′E	05/86 05/86 12/86 03/87 12/90	n y y y y	1948[h] - 1986[p]
Pouakai Plateau	39° 14′S 174° 02′E	03/86 12/87 12/90	n y y	1885[h], 1962[h], 1986[p]
Ahukawakawa Swamp	39° 15′S 174° 02′E	03/86 12/87	y y	1933[h] - 1960[h], 1986[p]
Purity Hut	39° 50′S 176° 03′E	12/88 12/90	y y	1988[p]
Cobb Valley	41° 08′S 172° 37′E	02/86 02/87 12/90	y y y	1952[h] - 1986[p]
Red Hills	41° 44′S 172° 59′E	11/90	y	1980[h], 1986[p]

Appendix I continued

Locality	Latitude/ longitude	Dates of visits	*Bulbinella* plants found yes / no	Date(s) of previous sitings
Puhi Peaks	42° 11′S 173° 46′E	02/88	y	1964[h] - 1988[p]
near Oaro	42° 32′S 173° 29′E	01/88 01/89	n n	1988[p]
Molesworth	42° 06′S 173° 10′E	01/88	y	
Acheron Valley	42° 21′S 173° 00′E	01/88	y	January, no year given[1]
Jacks Pass	42° 31′S 172° 50′E	01/87 01/88 11/90	y y y	1963[h] - 1986[p]
Mt. Isobel	42° 30′S 172° 51′E	01/87 11/90	y y	1919[h] - 1986[p]
B. gibbsii* var. *balanifera				
Whanahuia Range	39° 53′S 176° 02′E	12/88 12/90	y y	1952[h] - 1975[h]
Holdsworth	40° 53′S 175° 26′E	01/87	y	1908[h] - 1986[p]
Bridge Peak	40° 56′S 175° 17′E	12/86 12/90	y y	1922[h] - 1986[p]
Carroll Hut	42° 48′S 171° 33′E	02/89 12/90	y y	1966[h]
Tuhua	42° 49′S 171° 11′E	02/89 11/89 11/90	y y y	1963[h]
Mt. Fox	43° 30′S 170° 01′E	02/89	y	1967[h]
Red Tarns	43° 45′S 170° 06′E	02/87 02/88 11/89	y y y	1947[h] - 1972[h], 1987[p]
Hooker Valley	43° 42′S 170° 05′E	02/87 02/88	n y	1911[h], 1987[p]
Lake Marian	44° 44′S 168° 07′E	02/88 12/89	y y	1962[h]
Lake Harris	44° 45′S 168° 10′E	02/87 02/88 12/89	y y y	1963[h], 1968[h], 1987[p]

Appendix I continued

Locality	Latitude/ longitude	Dates of visits	*Bulbinella* plants found yes / no	Date(s) of previous sitings
Homer	44° 44′S 168° 03′E	02/88 12/89	y y	1943[h] - 1967[h]
Monkey Creek	44° 44′S 168° 03′E	02/88 12/89	y y	1972[h]
Wilmot Pass	45° 26′S 167° 07′E	02/88 01/90	y y	1943[h] - 1962[h]
B. modesta				
Charming Creek	41° 37′S 171° 56′E	01/87 11/87 11/89 11/90	n y y y	1970[h], 1986[p]
Stockton / Millerton	41° 38′S 171° 53′E	01/87 11/87	n n	1964[h], 1986[p]
Caroline Terrace, near Westport	41° 50′S 171° 35'E	11/87	n	1965[l2]
south of Charleston	41° 55'S 171° 26'E	11/87	n	1967[h]
Atarau	42° 20′S 171° 30′E	01/87 11/87 11/89 11/90	y y y y	1963[h], 1986[p]
Blackball - Roa Road	42° 21′S 171° 23′E	01/87 11/87	n n	1963[h]
Blackball rubbish dump	42° 21′S 171° 24′E	01/87 11/87	n n	1965[h]
Croesus Track	42° 13′S 171° 25′E	01/87 11/87	n n	1963[h]
Kumara, Stafford, Dillmanstown area	42° 40′S 171° 12′E	11/87	n	1970[h]
Okarito Swamp	43° 15′S 170° 13′E	11/87 02/89 11/89 11/90	y y y y	1950[h] - 1983[h]
Poerua State Forest, South Wanganui River	43° 15′S 170° 17′E	11/87	n	1948[h]

Appendix I continued

Locality	Latitude/ longitude	Dates of visits	*Bulbinella* plants found yes / no	Date(s) of previous sitings
Lake Ellery Track	44° 03′S 168° 42′E	11/87 02/89 11/89 11/90	y y y y	1965[h]
B. talbotii				
Gouland Downs	40° 53′S 172° 22′E	02/87 12/90	y y	1953[h] - 1973[h]
Saxon Hut	40° 53′S 172° 18′E	12/90	y	
B. angustifolia				
Lake Taylor	42° 47′S 172° 19′E	01/89 11/90	y n	1964[h] - 1971[h]
Virginia Road	42° 55′S 172° 27′E	11/90	y	
Okuku Pass	43° 07′S 172° 27′E	11/90	y	1976[h]
Ashley Gorge	43° 14′S 172° 13′E	01/89 11/89	y y	1975[h], 1989[p]
Lake Lyndon	43° 18′S 171° 43′E	01/87 12/89	y y	1963[h]
Porters Pass	43° 18′S 171° 45′E	01/87 12/89	y y	1964[h] - 1967[h], 1986[p]
Mt. Sinclair	43° 43′S 172° 52′E	01/88	n	1974[h]
Le Bons Bay	43° 47′S 173° 01′E	01/88 12/89	y y	1964[3]
Lakes Emma & Clearwater	43° 37′S 171° 03′E	02/87 11/89	y y	1962[h] - 1966[h]
Fairlie - Geraldine Road	44° 03′S 170° 53′E	02/89 11/89	n y	1965[h]
Clayton Wetlands	43° 46′S 170° 52′E	01/89 11/89	y y	1989[p]
Backline Road	44° 28′S 170° 53′E	02/89 11/89	y y	1989[p]

Appendix I continued

Locality	Latitude/ longitude	Dates of visits	*Bulbinella* plants found yes / no	Date(s) of previous sitings
Lake Ohau	44° 10′S 169° 49′E	02/89 11/89 11/90	y y y	1969[h], 1976[h], 1989[p]
Lindis Pass	44° 33′S 169° 42′E	02/87 11/89 11/90	y y y	1948[h] - 1965[h]
Crown Range	44° 59′S 168° 56′E	02/87 11/87 12/89	y y y	1962[h]
Coronet Peak	44° 55′S 168° 45′E	02/87	n	1968[h]
Danseys Pass	44° 58′S 170° 29′E	11/89 11/90	y y	1989[p]
Palmerston - Ranfurly Road	45° 18′S 170° 33′E	02/89 11/89 11/90	n y y	1950[h]
Macraes Flat	45° 24′S 170° 20′E	02/89 11/89 11/90	y y y	1988[p]
Rock & Pillar Range	45° 33′S 170° 03′E	02/89 11/89	y y	1969[h]
Dunedin - Middlemarch Road	45° 44′S 170° 03′E	11/89	y	
Flagstaff Hill, Dunedin	45° 51′S 170° 27′E	02/87 02/88 11/89	y y y	1906[h] - 1950[h]
Signal Hill, Dunedin	45° 51′S 170° 33′E	02/87	n	1910[h]
Lake Waipori	45° 59′S 170° 06′E	02/87	n	1963[h]
Waitahuna, west of Milton	45° 59′S 169° 46′E	02/87	n	1967[h]
Tapanui, west of Balclutha	45° 57′S 169° 16′E	02/89 11/89	n n	1915[h]
Clinton - Mataura Road	46° 13′S 169° 13′E	02/89	n	1952[h]

Appendix I continued

Locality	**Latitude/ longitude**	**Dates of visits**	***Bulbinella* plants found** yes / no	**Date(s) of previous sitings**
Otapiri Gorge	46° 02′S 168° 27′E	11/90	n	1966[3]
Raes Junction	45° 48′S 169° 18′E	11/90	y	
Te Anau - Mossburn Road	45° 33′S 168° 01′E	02/89 12/89	n y	1962[h]
Manapouri - Blackmount Road	45° 39′S 167° 37′E	02/89 12/89	n y	
Blackmount	45° 47′S 167° 42′E	02/88 02/89 12/89	y y y	1972[h], 1988[p]
Lilburn Valley	46° 00′S 167° 28′E	02/89 12/89	y y	1989[p]
Alton Burn	46° 05′S 167° 38′E	02/89 12/89	y y	1989[p]

Appendix II. Plant deaths in cultivation.

Plant species	Proportion of deaths	% of plants collected	% of total deaths
B. hookeri	14/100	14%	26%
B. gibbsii var. *balanifera*	12/68	18%	22%
B. gibbsii var. *gibbsii*	2/10	20%	4%
B. gibbsii (both varieties)	14/78	18%	26%
B. rossii	8/16	50%	15%
B. modesta	0/26	-	-
B. talbotii	1/15	5%	2%
B. angustifolia	17/128	13%	31%

Summary:
Total deaths = 54 (15%)
Total plants collected = 364
Plants remaining alive at September 1991 = 310 (85%)

Appendix III. Solutions for cytogenetics stains

Propionic Orcein

2g orcein was added to 100ml 40% propionic acid and boiled gently in a flask with a reflux condenser for several hours. The resulting solution was cooled and filtered into stock bottles. Stock was diluted 1:1 with 45% propionic acid and further filtered before use.

Feulgen's Stain

400ml distilled water was boiled and the bunsen turned off. 2g basic fuchsin was added slowly while stirring. When the temperature had dropped to 50°C, 4g potassium metabisulphite was added. When the temperature had further dropped to room temperature (about 20°C) 4ml HCl was added. The solution was left in a dark cupboard overnight to bleach. The following day 5g of activated charcoal was added and the mixture left for 1 hour. If the solution was not glass clear more charcoal was added until it was. After filtering, the stain was stored in a dark bottle in the refrigerator.

Barium hydroxide solution

0.064M barium hydroxide ($Ba(OH)_2$)
2.02g barium hydroxide was dissolved in 100ml distilled water.

SSC (Saline Sodium Citrate)

Make 17.532g NaCl and 8.823g trisodium citrate to 1L with distilled water. Adjust pH to 7.0 with 0.1M HCl.

McIlvaine's Buffer

0.2M disodium hydrogen phosphate (Na_2HPO_4) adjusted to pH 7.0 with 0.1M citric acid.

Sörenson's Phosphate Buffer

Make 4.623g potassium dihydrogen phosphate (KH_2PO_4) and 4.674g disodium hydrogen phosphate (Na_2HPO_4) to 1L with distilled water. Check pH is 6.8.

Giemsa Stain

Stock Giemsa stain (BDH No. 35014) was diluted to give a final concentration of 2% with Sörenson's phosphate buffer.

Appendix IV. Grinding buffer recipes

1. TRIS/DTT (pers. comm. Dr G.C. Lindsay)

1.211g TRIS + 0.0772g DTT per 100ml
pH 7.4

TRIS = Tris[hydroxymethyl]-aminomethane MW 121.1 (T1378)
DTT = Dithiothreitol (Cleland's Reagent) MW 154.3 (D0632)
Both chemicals from Sigma

2. Phosphate (Conkle et al. 1982)

0.384g Monosodium dihydrogen phosphate (NaH_2PO_4) +
2.386g Disodium hydrogen phosphate (Na_2HPO_4) +
1 drop 2-mercaptoethanol per 100ml.
pH 7.5

Both buffers were made in distilled water and frozen in aliquots of about 20ml which were thawed when required.

Appendix V. Recipes for the electrophoretic buffers used in both the pilot study (examining tissue from Liliaceae) and the secondary study (examining *Bulbinella* tissue). Legend: TRIS = Tris[hydroxymethyl]-aminomethane. References: AC, TC-1, and RW; Allendorf et al. 1977, PH, TC-2, TC-3, and PK; Selander et al. 1971.

System	Gel	Electrode
AC	pH 6.0 0.841g citric acid / 2L adjust pH with N-(3-aminopropyl)-morpholine	pH 6.1 16.8g citric acid / 2L adjust pH with N-(3-aminopropyl)-morpholine
PH	pH 6.7 12.5ml electrode buffer + 17.5mg NADP for 250ml	pH 6.7 KH_2PO_4 37.56g + NaOH 4.96g / 2L adjust pH with conc. HCl
TC-1	pH 6.7 TRIS 1.94g + citric acid 1.26g / 2L adjust pH with NaOH	pH 6.3 TRIS 54g + citric acid 36.14g / 2L adjust pH with NaOH
TC-2	pH 8.0 TRIS 5.54g + citric acid 2.20g / 2L	pH 8.0 TRIS 166.4g + citric acid 60.0g / 2L
TC-3	pH 7.0 TRIS 2.18g + citric acid 1.206g / 2L	pH 7.0 TRIS 32.7g + citric acid 18.08g / 2L
PK	pH 8.7 TRIS 18.40g + citric acid 2.10g / 2L	pH 8.2 Boric acid 37.10g + NaOH 4.80g / 2L
RW	pH 8.5 TRIS 7.27g + citric acid 2.10g / 2L add 10ml electrode buffer per liter	pH 8.1 Boric acid 37.1g + LiOH 5.04g / 2L

Appendix VI. Method for preparing starch gels (Allendorf et al. 1977).

1. Moulds were assembled and labelled, one for each gel, using glass plates, plastic sides and rubber bands.

2. 30gm Sigma starch was placed in a 1L evacuating flask.

3. Gel buffer was poured into a 250ml volumetric flask to the mark. Approximately 1/3 of this was used to emulsify the starch.

4. The remaining buffer was heated to boiling in the volumetric flask.

5. The starch was thoroughly emulsified in the evacuating flask and then the boiling buffer was added whilst stirring continuously.

6. The entire mixture was heated to boiling, with frequent swirling to mix.

7. Prolonged boiling was unneccessary and inadvisable since the preparation had a greater tendency to stick to the bottom of the flask, and took longer to degas.

8. When the mixture was of an even consistency and lightly bubbling, it was removed from the heat.

9. The gel was then degassed with the aid of a vaccuum pump. The mixture was swirled gently while degassing. The appearance of large bubbles in the gel and a change in the tone of the pump indicated that the gel had been successfully degassed.

10. The gel was poured to fill the mould evenly.

11. Any small bubbles remaining were removed using forceps before the gel began to set.

12. After allowing to cool (at least 1 hour at room temperature) the gel was enclosed in plastic wrap and placed in the refrigerator until required.

Appendix VII. Stain recipes. Legend: * Denotes that these staining methods were taken from Allendorf et al. (1977). Abbreviations used were: NAD = β-nicotinamide adenine dinucleotide, NADP = β-nicotinamide adenine dinucleotide phosphate, NBT = nitro blue tetrazolium, MTT = methyl thiazolyl blue (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), PMS = phenazine methosulphate, HCl = hydrochloric acid, TRIS = Tris[hydroxymethyl]-aminomethane and $MgCl_2$ = magnesium chloride. The stain buffers (S.B.) were: S.B. I = 0.2M TRIS (24.2g TRIS/L) pH 8.0 with HCl; S.B. II = 0.04M TRIS (100ml S.B. I + 400ml distilled water) and S.B. III = RW gel buffer (appendix V).

ADH alcohol dehydrogenase (modified Allendorf et al. 1977)

50ml S.B. II
0.5ml absolute alcohol
20mg NAD
10mg MTT
5mg PMS

GDH glutamate dehydrogenase *

50ml S.B. III
2g L-glutamic acid
5mg NAD
10mg NBT
5mg PMS

G6PDH glucose-6-phosphate dehydrogenase *

50ml S.B. II
200mg glucose-6-phosphate
5mg NADP
10mg NBT
5mg PMS
10mg $MgCl_2$

GPI glucose phosphate isomerase *

50ml S.B. III
25mg fructose-6-phosphate
10u G6PDH
5mg NADP
10mg NBT
5mg PMS
10mg $MgCl_2$

ICD isocitrate dehydrogenase *

50ml S.B. II
200mg DL-isocitric acid
5mg NADP
10mg NBT
5mg PMS
10mg $MgCl_2$

MDH malate dehdrogenase *

50ml S.B. III
25ml 0.5M DL-malic acid, pH 7.0 (malic acid solution)
5mg NAD
10mg NBT
5mg PMS

malic acid solution:
8.9g DL-malic acid / 100ml distilled water (0.5M)
pH was adjusted to 7.0 with 1N HCl.

6PGDH 6-phosphogluconate dehydrogenase *

50ml S.B. II
100mg 6-phosphogluconic acid
5mg NADP
10mg NBT
5mg PMS
10mg $MgCl_2$

PGM phosphoglucomutase (modified from Stuber and Goodman 1983)
25ml S.B. I + 25ml distilled water
200mg glucose-1-phosphate
25mg EDTA
25u G6PDH
5mg NADP
7.5mg MTT
1mg PMS
100mg $MgCl_2$ (weighed out and added as crystals)

For ease and speed of staining:

(1) The substrate was added to a measured volume of the stain buffer on the day of the laboratory work and left at room temperature until required.

(2) G6PDH was received as a powder in vials containing 1000 Units(u). Ten ml distilled water was added to reconstitute the powder. This solution was then

dispensed into aliquots in AA tubes, each containing 100u in 1ml of liquid, frozen at -20°C and thawed when required.

(3) The following ingredients were dissolved in distilled water to form a solution of the concentration indicated. They were made freshly on the day of the laboratory work. Because these chemicals deteriorated in solution when exposed to light (except $MgCl_2$), they were stored in the refrigerator in small conical flasks wrapped in silver foil. Adding 1ml of solution was easier and more economical than weighing out the small quantity of powder required for each indiviual stain.

Chemical	**Concentration**
NAD	5mg/ml
NADP	5mg/ml
NBT	10mg/ml
MTT	5mg/ml
PMS	5mg/ml
$MgCl_2$	10mg/ml

All the above chemicals were added to the remainder of the stain mixture immediately prior to staining. A further modification was: when either NBT or MTT was required, an equal quantity (0.5ml) of each was added.

(4) When bands were optimally stained (largely achieved by trial and error initially) the excess staining solution was carefully tipped out and the gels washed with tap water. The gels were then covered with destaining solution (methanol, glacial acetic acid, distilled water; 5:1:5). After 20-30 minutes, half the volume of destain was tipped out and replaced by tap water, to prevent rapid fading of the bands and dye markers.

Appendix VIII. Photographs of some of the enzymes detected in floral tissue of *Bulbinella* using starch gel electrophoresis.

In each figure the origin is at the bottom of the photograph. Backslices have been omitted because no enzymes were detected on these. Bold numerals in the text refer to numbers on the photographs.

ADH [fig. VIII (a)]: **1** indicates an example of the genotype Adh-1(aa) and **2** shows genotype Adh-1(ab).

GDH [fig. VIII (b)]: **1** indicates an example of the genotype Gdh-1(aa), **2** shows Gdh-1(bb) and **3**, Gdh-1(ac).

GPI [fig. VIII (c)]: All samples have genotype Gpi-2(aa). Most samples are genotype Gpi-1(aa) shown by **1**. **2** indicates Gpi-1(bb) and **3**, Gpi-1(ad).

ICD [fig. VIII (d)]: Most samples have genotype Icd-1(aa) and **1** indicates such an example. **2** shows genotype Icd-1(bb), **3**, Icd-1(ac) and **4**, Icd-1(cc). **5** shows a sample of genotype Icd-2(aa), while adjacent samples have the genotype Icd-2(bb).

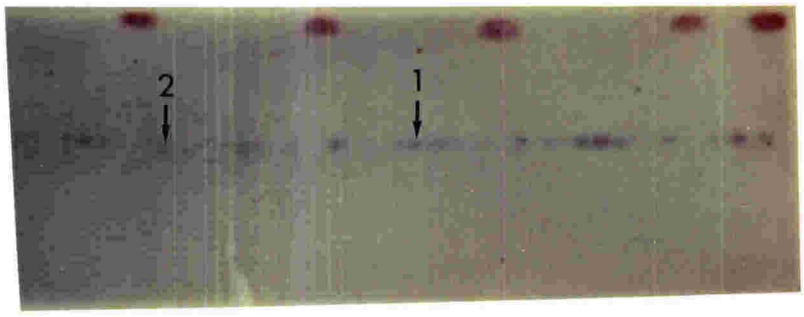
MDH [figs VIII (e) and VIII (f)]: The bands of stain close to the origin were not scored routinely because they were not detected consistently in all samples. In fig. VIII (e), **1** shows Mdh-1(aa), **2** shows Mdh-1(ab) and **3**, Mdh-1(bb). **4** indicates a sample of genotype Mdh-2(ad). All samples in this figure are of genotype Mdh-3(aa). In fig. VIII (f), all samples are of genotype Mdh-1(aa) and Mdh-2(aa). **5** shows a sample of genotype Mdh-3(aa), **6**, genotype Mdh-3(bb) and **7**, Mdh-3(ab).

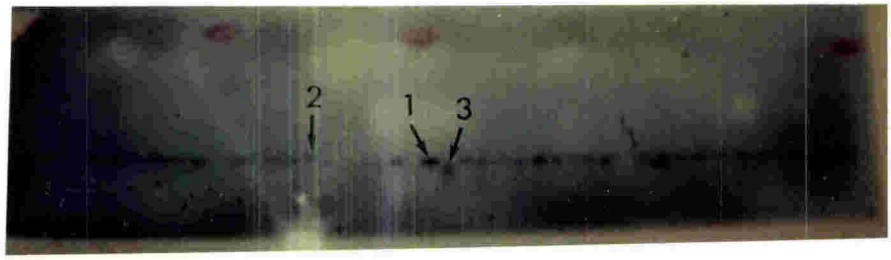
Figure VIII (a). ADH on RW gel.

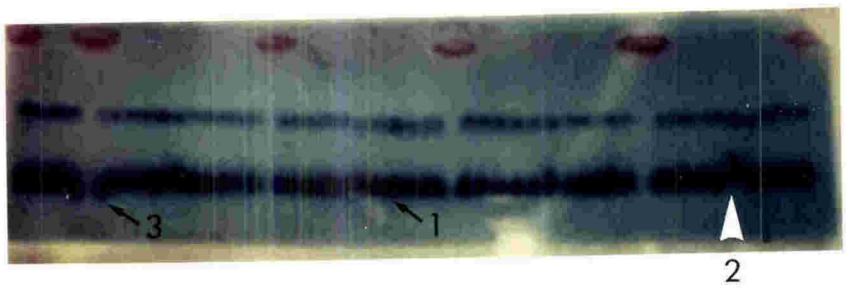
Figure VIII (b). GDH on RW gel.

Figure VIII (c). GPI on RW gel.

Figure VIII (d). ICD on AC gel.


2
1


2
1
3


3
1
2

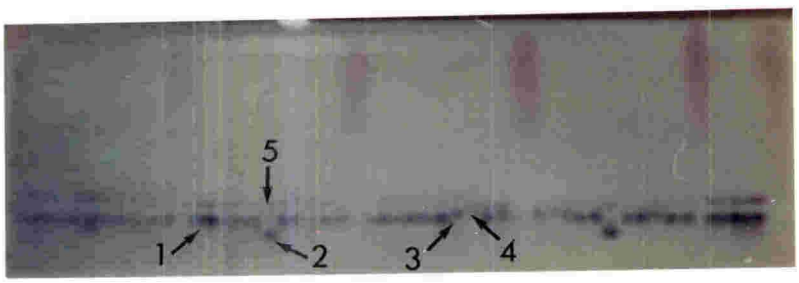
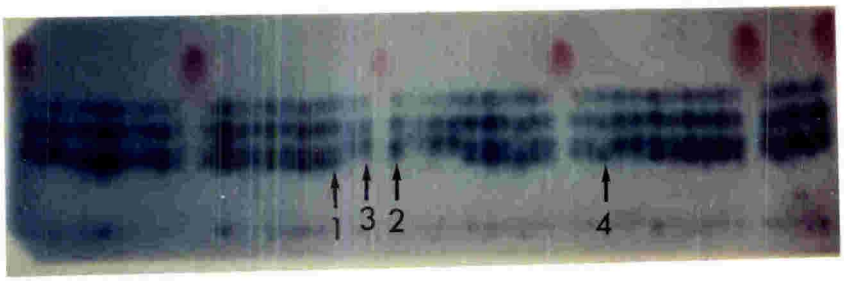

5
1
2
3
4

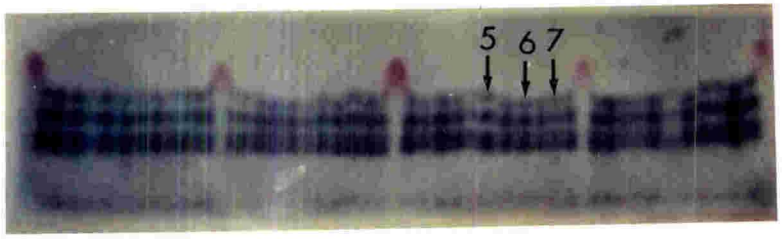
Figure VIII (e). MDH on TC_2 gel.

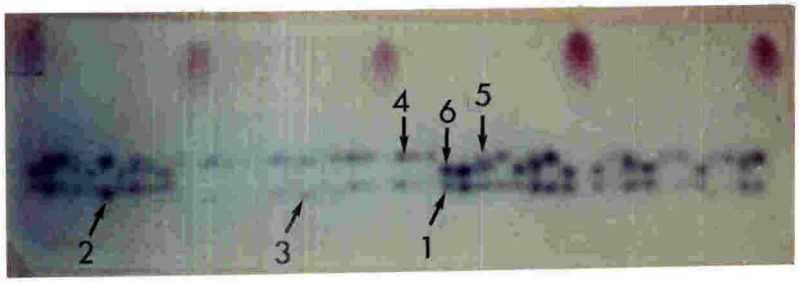
Figure VIII (f). MDH on TC_3 gel.

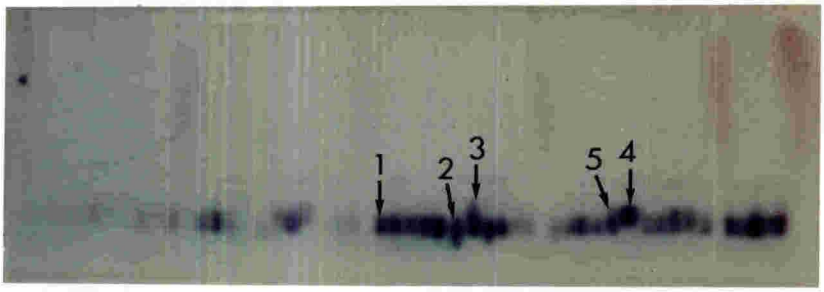
Figure VIII (g). 6PGD on TC_2 gel.

Figure VIII (h). PGM on AC gel.


1
3
2
4


5
6
7


4
6
5
2
3
1


1
2
3
5
4

6PGD [fig. VIII (g)]: **1** shows a sample of genotype 6Pgd-1(aa), **2** shows 6Pgd-1(ac) and **3**, 6Pgd-1(cc). **4** indicates a sample of genotype 6Pgd-2(aa), **5** shows 6Pgd-2(ac) and **6**, 6Pgd-2(cc).

PGM [fig. VIII (h)]: All samples are of genotype Pgm-2(aa), although this locus is faint in the photograph. **1** indicates a sample of genotype Pgm-1(aa), **2** shows Pgm-1(ad), **3**, Pgm-1(ac), **4**, Pgm-1(bb) and **5**, Pgm-1(ab).

REFERENCES

Allendorf, F.W.; Mitchell, N.; Ryan, N.; Stahl, G. 1977: Isozyme loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data. *Hereditas 86*: 179-190.

Avise, J.C. 1974: Systematic value of electrophoretic data. *Systematic zoology 23*: 465-481.

Avise, J.C.; Arnold, J.; Ball, R.M.; Bermingham, E.; Lamb, T.; Neigel, J.E.; Reeb, C.A.; Saunders, N.C. 1987: Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual review of ecology and systematics 18*: 489-522.

Avise, J.C. 1989: A role for molecular genetics in the recognition and conservation of endangered species. *Trends in ecology and evolution 4(9):* 279-281.

Babbel, G.R.; Selander, R.K. 1974: Genetic variability in edaphically restricted and widespread plant species. *Evolution 28*: 619-630.

Baker, J.; Maynard Smith, J.; Strobeck, C. 1975: Genetic polymorphism in the bladder campion *Silene maritima. Biochemical genetics 13(7/8)*: 393-410.

Bayer, R.J.; Crawford, D.J. 1986: Allozyme divergence among five diploid species of *Antennaria* (Asteraceae: inuleae) and their allopolyploid derivatives. *American journal of botany 73(2)*: 287-296.

Baum, D. 1992: Phylogenetic species concepts. *Trends in ecology and evolution 7(1):* 1-2

Berrie, A.M.M. 1984: Germination and dormancy. *In:* Wilkins, M.B. *ed.* Advanced plant physiology. Pitman Publishing Co. Ltd. London. Pp. 440-468.

Brewer, G.J. 1970: An introduction to isozyme techniques. Academic Press, London. Pp. 1-175.

Brooks, D.R.; Caira, J.N.; Platt T.R.; Pritchard M.R. 1984: Principles and methods of phylogenetic systematics: a cladistics workbook. University of Kanzas publications, museum of natural history.

Brown, A.H.D. 1978: Isozymes, plant population genetic structure and genetic conservation. *Theoretical and applied genetics 52*: 145-157.

Brunsfeld, S.J.; Soltis, D.E.; Soltis, P.S. 1991: Patterns of genetic variation in *Salix* section *Longifoliae* (Salicaceae). *American journal of botany 78(6)*: 855-869.

Buchanan, J. 1868: Sketch of the botany of Otago. *Transactions and proceedings of the New Zealand Institute 1*: Pp 23, 24, 36, 50. (essay)

Cartman, J. 1985: Growing New Zealand alpine plants. Reed Methuen Publishers Ltd. Auckland. Pp. 39, 40.

Cheeseman, T.F. 1906: Manual of the New Zealand flora. Government Printer, Wellington. Pp. 717-718.

Cheliak, W.M.; Pitel, J.A. 1984: Techniques for starch gel electrophoresis of enzymes from forest tree species. *In*: Information report. Petawawa National Forestry Institute. Pp. 20-43.

Cherry, J.P.; Katterman, F.R.H.; Endrizzi, J.E. 1972: Seed esterases, leucine aminopeptidases and catalases of species of the genus *Gossypium. Theoretical and applied genetics 42*: 218-226.

Cockayne, L.; Laing, R.M. 1910: The Mount Arrowsmith district: a study in physiography and plant ecology. Part 2. Plant ecology. *Transactions of the New Zealand Institute 43:* 342-378.

Conkle, M.T.; Hodgskiss, P.D.; Nunnally L.B.; Hunter, S.C. 1982: Starch gel electrophoresis of conifer seeds: a laboratory manual. United States Department of Agriculture.

Crawford, D.J.; Julian, E.A. 1976: Seed protein profiles in the narrow-leaved species of *Chenopodium* of the western United States: taxonomic value and comparison with distribution of flavonoid compounds. *American journal of botany 63(3)*: 302-308.

Crawford, D.J.; Wilson, H.D. 1979: Allozyme variation in several closely related diploid species of *Chenopodium* of the western United States. *American journal of botany 66(3)*: 237-244.

Crawford, D.J.; Smith, E.B. 1984: Allozyme divergence and intraspecific variation in *Coreopsis grandiflora* (Compositae). *Systematic botany 9(2)*: 219-225.

Crawford, D.J. 1985: Electrophoretic data and plant speciation. *Systematic botany 10 (4):* 405-416.

Crawford, D.J. 1989: Enzyme electrophoresis and plant systematics. *In:* Soltis, D.E.; Soltis, P.S. *ed.* Isozymes in plant biology. Discorides press, Portland, Oregon.

Dahlgren, R.M.T.; Clifford, H.T.; Yeo, P.T. 1985: The families of the Monocotyledons. Structure, evolution and taxonomy. Springer-Verlag, Berlin, Germany.

Darlington, C.D.; Wylie, A.P. 1955: Chromosome atlas of flowering plants. George Allen and Unwin, London. p. 364.

Davis, J.I.; Manos, P.S. 1991: Isozyme variation and species delimitation in the *Puccinellia nuttalliana* complex (Poaceae): an application of the phylogenetic species concept. *Systematic botany 16(3):* 431-445.

Evans, A. 1987: New Zealand in flower. An illustrated guide to native flowering plants. Reed Methuen publishers Ltd., Birkenhead, Auckland.

Filion, W.G. 1974: Differential giemsa staining in plants I. Banding patterns in three cultivars of *Tulipa*. *Chromosoma 49:* 51-60.

Fisher, M.E.; Satchell, E.; Watkins, J.M. 1970: Gardening with New Zealand plants, shrubs and trees. Collins Bros., Auckland. p. 163.

Frankel, O.H. and Soulé, M.E. 1981: Conservation and evolution. Cambridge University Press, Cambridge. Pp. 31-77.

Frost, D.R.; Hillis, D.M. 1990: Species in concept and practice: herpetological applications. *Herpetologica 46(1):* 87-104.

Fuller, David. 1978: Maori food and cookery. A.H. and A.W. Reed, Wellington. p. 9.

Given, D.R. 1981: Rare and endangered plants of N.Z. A.H. and A.W. Reed.

Gottlieb, L.D. 1973: Genetic control of glutamate oxaloacetate transaminase isozymes in the diploid plant *Stephanomeria exigua* and its allotetraploid derivative. *Biochemical genetics 9(1)*: 97-107.

Gottlieb, L.D. 1977: Electrophoretic evidence and plant systematics. *Annals of the Missouri botanical garden 64:* 161-180.

Gottlieb, L.D. 1981: Electrophoretic evidence and plant populations. *Progress in phytochemistry 7*: 1-46.

Goulding, J.H. 1971: Identification of archaeological and ethnological specimens of fibre-plant material used by the Maori. *Records. Auckland institute and museum 8*: 57-101.

Grant, V. 1981: Plant speciation. Columbia University Press, New York.

Hair, J.B.; Beuzenberg E.J. 1966: Contributions to a chromosome atlas of the New Zealand flora - 7 miscellaneous families. *New Zealand journal of botany 4*: 255-266.

Hamrick, J.L.; Linhart, Y.B.; Mitton, J.B. 1979: Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual review of ecology and systematics 10*: 173-200.

Hamrick, J.L. and Godt, M.J.W. 1990: Allozyme diversity in plant species. *In:* Brown, A.H.D.; Clegg, M.T.; Kohler, A.L.; Weir, B.S. *Ed.* Plant population genetics, breeding and genetic resources. Sinauer associates Inc. publishers. Sunderland, Massachusetts.

Harris, H.; Hopkinson, D.A. 1976: Handbook of enzyme electrophoresis in human genetics. North-Holland publishing Co., Amsterdam, the Netherlands.

Harrison, R.E. 1977: Bulbs and perennials. A southern hemisphere gardening book. A.H. and A.W. Reed Ltd., Wellington.

Hawkins, B.J.; Sweet, G.B. 1989: Genetic variation in Rimu - an investigation using isozyme analysis. *New Zealand journal of botany 27*: 83-90.

Heywood, J.S.; Levin, D.A. 1984: Allozyme variation in *Gaillardia pulchella* and *G. amblyodon* (Compositae): relation to morphological and chromosomal variation and to geographical isolation. *Systematic botany 9(4)*: 448-457.

Hurka, H. 1980: Enzymes as a taxonomic tool: a botanist's view. *In:* Bisby, F.A.; Vaughan, J.G.; Wright, C.A. *ed.* Systematics association special volume number 16. Chemosystematics: principles and practice. Academic press, London. Pp. 103-121.

IUCN 1980: World conservation strategy. Living resource conservation for sustainable development. Prepared by the International Union for Conservation of Nature and Natural Resources (IUCN) with the advice, cooperation and financial assistance of the United Nations Environment Programme (UNEP) and the World Wildlife Fund (WWF) and in collaboration with the Food and Agriculture Organisation of the United Nations (FAO) and the United Nations Educational, Scientific and Cultural Organisation (Unesco).

Johnson, P.N.; Campbell, D.J. 1975: Vascular plants of the Auckland Islands. *New Zealand journal of botany 13(4):* 665-720.

Lewontin, R.C. 1974: The genetic basis of evolutionary change. Columbia University Press, New York.

Littler, M.M.; Littler, D.S. *ed.* 1985: Handbook of phycological methods. Ecological field methods: macroalgae. Cambridge University Press.

Lowrey, T.K.; Crawford, D.J. 1985: Allozyme divergence and evolution in *Tetramolopium* (Compositae: Astereae) on the Hawaiian islands. *Systematic botany 10(1)*: 64-72.

Mark, A.F.; Adams, N.M. 1979: New Zealand alpine plants. A.H. and A.W. Reed, Wellington.

Marks, G.E. 1975: The giemsa-staining centromeres of *Nigella damascena*. *Journal of cell science 18*: 19-25.

Marks, G.E. 1983: Feulgen banding of heterochromatin in plant chromosomes. *Journal of cell science 62*: 171-176.

Martinez-Zapater, J.M.; Oliver, J.L. 1984: Genetic analysis of isozyme loci in tetraploid potatoes (*Solanum tuberosum* L.). *Genetics 108*: 669-679.

Matthews, B. 1979: Growing native plants. A.H. and A.W. Reed Ltd., Wellington.

Matthews, J.W.; Matthews, B. 1968: The New Zealand garden dictionary; a practical guide to the growing of flowers, fruits and vegetables under New Zealand conditions. A.H. and A.W. Reed, Wellington.

Mayr, E. 1970: Populations, species and evolution. An abridgement of animal species and evolution. The Belknap Press of Harvard University Press, Cambridge, Massachusetts.

Merril, C.R.; Goldman, D.; Van Keuren, M.L. 1982: Simplified silver protein detection and image enhancement methods in polyacrylamide gels. *Electrophoresis 3*: 17-23.

Moore, L.B. 1964: The New Zealand species of *Bulbinella* (Liliaceae). *New Zealand journal of botany 2*: 286-304.

Moore, L.B.; Edgar, E. 1970: Flora of New Zealand volume II. A.R. Shearer Govt. Printer, Wellington. Pp. 21-25.

Moore, L.B.; Irwin, J.B. 1978: The Oxford book of New Zealand plants. Oxford University Press.

Morikawa, T.; Leggett, J.M. 1990: Isozyme polymorphism in natural populations of *Avena canariensis* from the Canary Islands. *Heredity 64*: 403-411.

Murphy, R.W.; Crabtree, C.B. 1985: Evolutionary aspects of isozyme patterns, number of loci and tissue-specific gene expression in the prairie rattlesnake, *Crotalus viridis viridis*. *Herpetologica 41*: 451-470.

Nei, M. 1977: F-statistics and analysis of gene diversity in subdivided populations. *Annals of human genetics 41*: 225-233.

Nei, M. 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics 89*: 583-590.

Nordenstam, B. 1982: Chromosome numbers of Southern African plants: 2. *Journal of South African botany 48(2)*: 273-275.

Panda, R.C.; Aniel Kumar, O.; Raja Rao, K.G. 1986: The use of seed protein electrophoresis in the study of phylogenetic relationships in Chili pepper (*Capsicum* L.). *Theoretical and applied genetics 72*: 665-670.

Parks, C.R.; Wendel, J.F.; Sevell, M.M.; Qui, Y.L. 1990: Genetic control of isozyme variation in the genus *Liriodendron* L. (Magnoliaceae). *Journal of heredity 81(4)*: 317-323.

Partridge, T.R.; Wilson, M.D. 1990: A germination inhibitor in the seeds of Mahoe (*Melicytus ramiflorus*). *New Zealand journal of botany 28*: 475-478.

Peat, N. 1990: A walk on the wild side. *Southern skies (the inflight magazine of Ansett New Zealand) June 1990*: 44-49.

Perry, P.L. 1987: A synoptic review of the genus *Bulbinella* (Asphodelaceae) in South Africa. *South African journal of botany 53(6)*: 431-444.

Philipson, W.R.; Hearn, D. 1962: Rock garden plants of the Southern Alps. Caxton Press, Christchurch. Pp. 92-93.

Rajora, O.P. 1990: Genetics of allozymes in *Populus deltoides* Marsh., *P. nigra* L., and *P. maximowiczii* Henry. *Journal of heredity 81(4)*: 301-308.

Richardson, B.J.; Baverstock, P.R.; Adams, M. 1986: Allozyme electrophoresis. A handbook for animal systematics and population studies. Academic Press, (Harcourt Brace Jovanovich, publishers) Sydney, Australia.

Rigg, H.H. 1962: The pakihi bogs of Westport, New Zealand. *Transactions of the Royal Society of New Zealand Botany 1(7)*: 91-108.

Robins, I. 1968: The *modesta* story. *Wellington botanical society bulletin 35*: 34-36.

Salmon, J.T. 1985: Collins guide to the alpine plants of New Zealand. William Collins Publishers Ltd., Auckland.

Salmon, J.T. 1986: New Zealand flowers and plants in colour. Mural books, Auckland.

Samuel, R.; Pinsker, W.; Ehrendorfer, F. 1990: Allozyme polymorphism in diploid and polyploid populations of *Galium*. *Heredity 65*: 369-378.

Sarich, V.M. 1977: Rates, sample sizes and the neutrality hypothesis for electrophoresis in evolutionary studies. *Nature 265:* 24-28.

Schaal, B.A. 1975: Population structure and local differentiation in *Liatris cylindracea*. *The American naturalist 109(969)*: 511-528.

Schwaegerle, K.E.; Schaal, B.A. 1979: Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea*. *Evolution 33(4)*: 1210-1218.

Schweizer, D. 1973: Differential staining of plant chromosomes with giemsa. *Chromosoma 40*: 307-320.

Selander, R.K.; Smith, M.H.; Yang, S.Y.; Johnson, W.E.: Gentry, J.B. 1971: Biochemical polymorphisms and systematics in the genus *Peromyscus*. I Variation in the old-field mouse, *Peromyscus polionotus*. Studies in genetics, University Texas publication 7103. Pp. 49-90.

Shaw, C.R.; Prasad, R. 1970: Starch gel electrophoresis of enzymes - a compilation of recipes. *Biochemical genetics 4*: 297-320.

Shields, C.R.; Orton, T.J.; Stuber, C.W. 1983: An outline of general resource needs and procedures for electrophoretic separation of active enzymes from plant tissue. *In*: Tanksley, S.D.; Orton, T.J. *Ed.* Isozymes in plant genetics and

breeding part A: 443-458. Elsevier Science Publishers B.V., Amsterdam, the Netherlands.

Siciliano, M.J.; Shaw, C.R. 1976: Separation and visualisation of enzymes on gels. *In*: Smith, I. *ed.* Chromatographic and electrophoretic techniques, volume 2, zone electrophoresis. Heinmann, London.

Simberloff, D. 1988: The contribution of population and community biology to conservation science. *Annual review of ecology and systematics 19*: 473-511.

Skinner, G. 1981: Simply living. A gatherers' guide to New Zealand's fields, forests and shores. A.H. and A.W. Reed Ltd., Wellington.

Sneath, P.H.A.; Sokal, R.R. 1973: Numerical taxonomy. W.H. Freeman, San Francisco.

Soltis, D.E.; Haufler, C.H.; Darrow, D.C.; Gastony, G.J. 1983: Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American fern journal 73(1)*: 9-27.

Spiess, E.B. 1977: Genes in populations. John Wiley and sons, New York.

Stuber, C.W.; Goodman, M.M. 1983: Inheritance, intracellular localization, and genetic variation of phosphoglucomutase isozymes in maize (*Zea mays* L.). *Biochemical genetics 21(7/8)*: 667-689.

Swofford, D.L.; Selander, R.B. 1981: BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of heredity 72*: 281-283.

Swofford, D.L. 1985, 1990: PAUP - Phylogenetic analysis using parsimony version 3.0. Illinois Natural History Survey, Illinois.

Templeton, A.R.; Shaw, K.; Routman, E.; Davis, S.K. 1990: The genetic consequences of habitat fragmentation. *Annals of the Missouri botanical garden 77*: 13-27.

Torres, A.M.; Soost, R.K.; Mau-Lastovicka, T. 1982: Citrus isozymes genetics and distinguishing nucellar from zygotic seedlings. *Journal of heredity 73*: 335-339.

Tsumura, Y.; Uchida, K.; Ohba, K. 1989: Genetic control of isozyme variation in needle tissues of *Cryptomeria japonica*. *Journal of heredity 80*: 291-297.

Turner, A.P. 1985: Giemsa banding of chromosomes reviewed and applied to hybrid rye. B.Sc(Hons) project (unpublished) VUW.

Upritchard, E.A. (*compiler*) 1986: A guide to the identification of New Zealand common weeds in colour. New Zealand weed and pest control society, Palmerston North.

Vane-Wright, R.I.; Humphries, C.J.; Williams, P.H. 1991: What to protect? - systematics and the agony of choice. *Biological conservation 55:* 235-254.

Webb, C.J.; Johnson, P.N.; Sykes, W.R. 1990: Flowering plants of New Zealand DSIR Botany, Christchurch.

Weeden, N.F.; Gottlieb, L.D. 1979: Distinguishing allozymes and isozymes of phosphoglucoisomerases by electrophoretic comparisons of pollen and somatic tissues. *Biochemical genetics 17(3/4)*: 287-296.

Wendel, J.F.; Parks, C.R. 1982: Genetic control of isozyme variation in *Camellia japonica* L. *Journal of heredity 73*: 197-204.

Wendel, J.F.; Weeden, N.F. 1989: Visualisation and interpretation of plant isozymes. *In:* Soltis, D.E.; Soltis, P.S. *ed.* Isozymes in plant biology. Discorides Press, Portland, Oregon.

Wiley, E.O. 1981: Phylogenetics. The theory and practice of phylogenetic systematics. John Wiley and Sons, New York.

Wilson, H.D. 1982: Stewart Island plants. Field guide publications, Christchurch. p. 296.

Wright, A.E. 1984: New Zealand herbarium resources. *New Zealand journal of botany 22:* 323-335.