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**An investigation into the evolutionary relationships
of the North Island alpine *Ranunculus***

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

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Abstract

Ranunculus nivicola is a member of the alpine *Ranunculus* (Section *Pseudadonis*), a group of 18 species that occupy distinct ecological habitats within mountainous environments of New Zealand and Australia. There are currently three species of alpine *Ranunculus* found in the North Island of New Zealand. Of those three species *R. nivicola* is the only species endemic to the North Island. With a ploidy of $2n = 96$, *R. nivicola* is the exception to the hexaploid state ($2n = 48$) of all other members of the alpine *Ranunculus*. It has been hypothesised that the elevated chromosome number in *R. nivicola* could be explained by a hybridisation event between the other two North Island species, *R. insignis* and *R. verticillatus*, with subsequent polyploidisation.

The aim of the present study was to investigate the evolutionary history and habitat differences of the North Island species of *Ranunculus* through phylogenetic analyses of sequence data and the use of LENZ (Land Environments of New Zealand) data layers with geographic information systems. Nuclear ITS (ITS), chloroplast J_{SA} and *trnL* - *trnF* sequences were determined and analysed using median networks and maximum likelihood tree building. Positional data (from GPS grid points and herbarium records) for populations of each of the three species was used to query the LENZ database and extract information on environmental envelopes.

The phylogenetic analyses indicate that *R. verticillatus* and *R. insignis* have different population histories, with range expansion occurring at different periods from the South to the North Island. Multiple events of dispersal between the South and the North Island could be inferred for *R. verticillatus*. However, only a single event of range expansion has occurred for *R. insignis*. A paucity of sequence variation among widely sampled accessions of *R. nivicola* may point to a recent (late Pleistocene-Holocene) origin for this allopolyploid. Interestingly, while *R. nivicola* nuclear ITS sequence is very similar to an extant genotype of *R. verticillatus*, its chloroplast sequence is intermediate between haplotypes shared by *R. insignis* and *R. ensyii*. There is no evidence for recurrent allopolyploid formation.

Analyses of environmental data revealed that there were significant differences between the three species. *R. insignis* occupied the widest range of environments, with the

environmental envelopes of *R. verticillatus* and *R. nivicola* occurring as subsets of the *R. insignis* envelope. It was also found that *R. insignis* could potentially occur in areas currently occupied exclusively by *R. nivicola*.

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List of Abbreviations

cpDNA	Chloroplast DNA
DNA	Deoxyribonucleic Acid
dNTP	Dinucleotidetriphosphate
GIS	Geographic Information System
GPS	Geographic Positioning System
Indel	Insertion or Deletion
J _{SA}	Junction of the chloroplast short single copy region and inverted repeat A
LENZ	Land Environments of New Zealand
ML	Maximum Likelihood
mtDNA	Mitochondrial DNA
Mya	Million years ago
ITS	Internal Transcribed Spacer
NJ	Neighbor Joining
nDNA	Nuclear DNA
nrDNA	Nuclear ribosomal DNA
PCR	Polymerase Chain Reaction
rDNA	Ribosomal DNA
rRNA	Ribosomal RNA
RNA	Ribonucleic Acid
Subsp.	Subspecies
trnL – trnF	trn L (UAA) 5'exon – trn F (GAA) intergenic spacer region

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Chapter 1 General Introduction

1.1. Introduction to the Themes of the Thesis

1.1.1 Overview

This thesis investigates the role of allopolyploidy in angiosperm evolution through molecular systematics and Geographic Information Systems (GIS) studies on the New Zealand alpine *Ranunculus* (Fisher 1965). Range expansion of taxa into North Island habitats of New Zealand has been inferred based on phylogenetic analyses of chloroplast and nuclear DNA sequences. Habitat differences for diploid and allopolyploid species were characterised using the LENZ (Land Environments of New Zealand) database and ArcView GIS software. Hypotheses are proposed to explain range expansion and extant species distributions.

1.1.2 Role of Polyploidy in Angiosperm Evolution

Polyploidy is regarded as a fundamentally important phenomenon in the evolution of angiosperms (Stebbins, 1971; Grant, 1981; Baack and Staton, 2005). Many studies suggest a significant number of angiosperm species have undergone a polyploidisation event in either their recent evolutionary past (neopolyploids) or in their deep evolutionary past (paleopolyploids) (Soltis and Soltis, 1993; Masterson, 1994; Leitch and Bennett, 1997; Ramsey and Schemske, 1998; Widmer and Baltisberger, 1999; Rieseberg, 2001; Schranz and Osborn, 2004; Soltis *et al.*, 2004b; Adams and Wendel, 2005; Soltis, 2005). It is estimated that 30-80% of angiosperms are either descended from, or are themselves polyploid (Muntzing, 1936; Stebbins, 1950; Grant, 1981; Masterson, 1994). Furthermore, recent data suggest that even species with relatively small genomes, such as *Arabidopsis* ($n = 5$), have been involved in at least one polyploid event (Simillion *et al.*, 2002; Blanc *et al.*, 2003; Adams and Wendel, 2005). It appears that polyploidy is ongoing and new polyploid taxa have been observed in the last hundred years in several genera (*e.g.* *Tragopogon* (Ownbey, 1950); *Spartina* (Thompson, 1991), *Senecio* (Lowe and Abbott, 1996) and *Cardamine* (Urbanska *et al.*, 1997)).

Polyploidy is defined as the possession of two or more genomes per cell (Grant, 1981; Ramsey and Schemske, 1998; Soltis and Soltis, 2000). A polyploid species can arise as the result of genome doubling within one species (autopolyploidy) or following interspecific hybridisation (allopolyploidy) (Soltis and Soltis, 2000; Ramsey and Schemske, 2002). Allopolyploids are considered to be more prevalent in nature than autopolyploids, although Soltis and Soltis (2000) suggest that further examination will find that the numbers of autopolyploids are underestimated.

Chromosome doubling has been considered to be maladaptive by some (Levin, 1983) due to factors such as minority cytotype exclusion (Levin, 1975; Rausch and Morgan, 2005), epigenetic instability and the disruption of mitosis and meiosis (Whitton, 2004; Comai, 2005). These features and others have prompted some to downplay the role of polyploidy as a progressive evolutionary state (Dobzhansky, 1950; Wagner, 1970; Mable, 2004). An alternative view is that doubling of chromosome number in allopolyploids is not maladaptive, as it prevents the problems associated with the bringing together of divergent genomes *i.e.* inviability and sterility (Wendel, 2000). Doubling of chromosomes creates a homologue which restores pairing and fertility in chromosomally divergent species (Dobzhansky, 1933). It is thought that genome inflation via polyploidy is temporary and that over time there are rounds of removal of redundant genetic material. Thus over time a species undergoes cycles of expansion via polyploid doubling and contraction via differential gene loss (Adams and Wendel, 2005).

Polyploidy is in effect an instantaneous speciation event with the resulting polyploid usually resistant to introgression with its parental species due to differences in ploidy level (Ramsey and Schemske, 1998; Gross *et al.*, 2003). However, if a newly formed polyploid is to be successful in establishing itself, it needs to be sufficiently different in character from its parental species so as not to be in direct competition with them (Welch and Rieseberg, 2002). Polyploids are often reported to have broader ecological tolerances than their parental species, and this property can facilitate their expansion into habitats not occupied by either parent (Levin, 1983; Schwarzbach *et al.*, 2001). Furthermore, polyploids are purported to be better colonizers than diploids (Soltis and Soltis, 2000). Polyploidisation can also have the effect of changing a plant's morphology into more robust forms (Pal and Khoshoo, 1973) as well as influencing its phenology

(Petit *et al.*, 1997) and physiology (Chen and Tang, 1945). The rearrangement of genes that results from genome doubling can bring about changes that result in the formation of novel phenotypes or the expression of transgressive traits (Adams and Wendel, 2005).

There is significant evidence for the natural occurrence of polyploidy; a question that then arises is whether polyploid formation between two species occurs once or is recurrent. Increasingly, the evidence points towards polyploidy being recurrent in nature (Soltis and Soltis, 1999). Soltis and Soltis (1999) go as far to say that recurrent origins of polyploidy are the rule rather than the exception, and in a study by the same authors (Soltis and Soltis, 1993) they cite 30 examples of recurrent formation from angiosperms, ferns and bryophytes. The mixing of polyploids formed from different populations creates the potential for abundant genetic diversity. The massive potential for genetic variability not only at the individual level but at the population level could be a reason why polyploids are so successful at colonising sites compared to their diploid progenitors.

1.1.3 Polyploidy and the Evolution of the New Zealand Alpine Flora

The high morphological diversity of the New Zealand alpine flora appears to be anomalous given the recent development of the New Zealand mountain ranges (Raven, 1973; Winkworth, 2000; Winkworth *et al.*, 2005). It has been proposed that this diversity is the product of rapid species radiations that occurred in relative isolation (Winkworth *et al.*, 2002b). The role of polyploidy in these radiations is uncertain. Polyploidy has been reported for 63% of New Zealand plant species (Hair, 1966), including species from most of the major angiosperm families present in New Zealand (Table 1.1). However, many plant groups in the New Zealand angiosperm flora have also radiated without forming polyploidy series. The species of New Zealand alpine *Ranunculus* are perhaps one of the best examples of an alpine polyploid group.

One of the first proposed allopolyploid species in the New Zealand flora was *Ranunculus nivicola* (Fisher, 1965). It was found that *R. nivicola* has $2n = 96$ chromosomes, whilst the nearest known relatives have $2n = 48$ chromosomes (Fisher, 1965). It was hypothesised by Fisher (1965) that *R. nivicola* is an allopolyploid of *R. insignis* and *R. verticillatus*. There

are molecular data supporting this hypothesis (Lockhart *et al.*, 2001); however, it remains to be thoroughly tested.

Family	Example genus
Asteliaceae	<i>Astelia</i> (Wheeler, 1966)
Asteraceae	<i>Anaphalioides</i> (Glenny, 1997)
Cyperaceae	<i>Uncinia</i> (Hair, 1966)
Ericaceae	<i>Epacris</i> (Hair, 1966)
Fabaceae	<i>Carmichaelia</i> (Wagstaff <i>et al.</i> , 1999)
Iridaceae	<i>Libertia</i> (Blanchon <i>et al.</i> , 2000)
Lobeliaceae	<i>Pratia</i> (Murray <i>et al.</i> , 1992)
Onagraceae	<i>Fuchsia</i> (Raven, 1979)
Plantaginaceae	<i>Hebe</i> (Hair, 1966)
Poaceae	<i>Poa</i> (Hair, 1966)
Ranunculaceae	<i>Ranunculus</i> (Fisher, 1965)
Rhamnaceae	<i>Pomaderris</i> (Hair, 1963)
Rubiaceae	<i>Coprosma</i> (Hair, 1966)
Verbenaceae	<i>Vitex</i> (Barrell <i>et al.</i> , 1997)
Violaceae	<i>Melicytus</i> (Molloy and Clarkson, 1996)

Table 1.1: Incidence of polyploidy in the angiosperm flora of New Zealand.

1.1.4 Taxonomy and Systematics of Family Ranunculaceae Juss.

The Ranunculaceae is a large family with a worldwide distribution described as containing between 50-66 genera and approximately 2000 species (Webb *et al.*, 1988; Ziman and Keener, 1989; Hoot, 1991; Johansson and Jansen, 1993). Members of this family, which includes the genus *Ranunculus*, are found predominantly in temperate to arctic/sub-Antarctic climates (Johansson and Jansen, 1993). Outside of montane regions, they are uncommon in tropical and subtropical zones (Ziman and Keener, 1989).

The Ranunculaceae is believed to have originated in the montane temperate floras of the Northern Hemisphere during the Cretaceous (Ziman and Keener, 1989). The family is often considered a “basal” herbaceous eudicot family as members exhibit a number of proposed ancestral traits (Stebbins, 1938; Jury, 1993; APG, 2003), such as numerous and spirally arranged floral parts, apocarpy, imperfect carpel closure and follicles (Hoot, 1991). Yet, it is very successful in the contemporary setting and demonstrates characters considered to be derived, such as finely dissected leaves, achenes, racemose inflorescences and zygomorphic flowers (Hoot, 1991).

On a worldwide scale the distribution of Ranunculaceae can be divided into six floristic kingdoms, with eight subkingdoms, 34 regions and 150 provinces (Ziman and Keener, 1989). Of relevance to this study is the Holoantarctic kingdom which covers South America from latitude 30° south and includes the Neozeylandic region that encompasses New Zealand and its outlying islands.

1.1.5 Genus *Ranunculus* L.

Ranunculus is the largest genus of Ranunculaceae and was described by Linnaeus in the 18th century under the name ‘buttercup’, which is now the common name of this genus (Rendel, 1987). The genus contains approximately 600 species (Tamara, 1995) and is characteristically morphologically diverse with leaf forms ranging from large peltate leaves to small, highly dissected ones. Parallel evolution appears to be prevalent, with distantly related species often showing convergent leaf morphology (Fisher, 1965).

Ployploidy and hybridisation appear to have been important phenomena in the evolution of this genus (Gregory, 1941; Fisher, 1965; Hörandl *et al.*, 2005).

There is a high degree of ecological diversity within the genus *Ranunculus*, with members found in a variety of terrestrial and aquatic environments, from lowland to alpine zones (Johansson, 1998; Hörandl *et al.*, 2005). *Ranunculus* species have a variety of sexual reproductive strategies such as protogyny and agamospermy, and asexual vegetative reproductive structures such as stolons and bulbs (Fisher, 1965; Hörandl *et al.*, 2005). A high degree of out-crossing is maintained via pollination from generalist insects (Fisher, 1965; Jury, 1993). Species are considered taxonomically difficult to categorise due to high levels of intraspecific morphological diversity, and frequent hybridisation (Rendel and Murray, 1989).

1.1.6 Infrageneric Taxonomy of the New Zealand *Ranunculus*

In the Neozeylandic region there are 38 species of *Ranunculus* divided into three sections. Section *Chrysanthe* (Spach) Benson has 16 species endemic to New Zealand. Section *Epiroteles* (Prantel) Benson has 6 species endemic to New Zealand and section *Pseudadonis* F. Muell. has 16 species endemic to New Zealand and 2 species in Australia (Fisher, 1965; Lockhart *et al.*, 2001).

Section *Chrysanthe* contains species found in a range of habitats from sub-alpine to lowland (Rendel and Murray, 1989). Previously, species of section *Epiroteles* were divided into two ecologically and genetically distinct groups: those that occupied high-altitude or alpine environments, and lowland plants of wet or damp environments (Rendel, 1987). Tamura (1995) transferred the high altitude and alpine species into section *Pseudadonis*, which is the focus of the current study (Table 1.2).

Ranunculus subgenus *Ranunculus* Peterm.

Members of section *Pseudadonis* F. Muell.

R. anemoneus F. Muell.
R. buchananii Hook. f.
R. crithmifolius subsp. *crithmifolius* Fisher
R. crithmifolius subsp. *paucifolius* (T. Kirk) Fisher.
R. enysii T. Kirk.
R. godleyanus Hook. f.
R. gracilipes Hook. f.
R. grahamii Petrie
R. gunnianus Hook.
R. haastii subsp. *haastii* Fisher
R. haastii subsp. *pilferus* Fisher
R. insignis Hook. f.
R. lyallii Hook. f.
R. nivicola Hook.
R. pachyrrhizus Hook. f.
R. pinguis Hook. f.
R. scirthalis P.J Garnock Jones
R. sericophyllus Hook. f.
R. verticillatus T. Kirk
R. viridis H.D. Wilson & P.J Garnock Jones

Table 1.2: *Ranunculus* species from section *Pseudadonis* included in this study.

1.1.7 Origin of the New Zealand Alpine *Ranunculus*

The most widely held hypothesis regarding the origin of the New Zealand alpine *Ranunculus* (section *Pseudadonis*) is that they are a derivative of a South American ancestor (Fisher, 1965; Wardle, 1978; Santisuk, 1979; Ziman and Keener, 1989). Alternatively, an ancestor of the New Zealand species may have dispersed via the New Guinea highlands down through the Australian Alps and then to New Zealand (Raven, 1973). It is also

possible that they arose from a lowland relative (Fisher, 1965). The likely candidate for this is *R. acaulis* Banks & Sol. as this species has a wide distribution (Fisher, 1965). However, that hypothesis is not supported by analysis of nuclear and chloroplast DNA sequences (Lockhart *et al.*, 2001).

The prevalence of species in the South Island led Fisher (1965) to conclude that the group originated there, and subsequently dispersed to the mountainous areas of the North, Stewart and sub-Antarctic islands, and into the Australian Alps. There is some molecular support (Lockhart *et al.*, 2001) for dispersal from New Zealand to Australia, with the Australian species *R. gunnianus* and *R. anemoneus* grouping closely with New Zealand species (Lockhart *et al.*, 2001). Such transoceanic dispersal events would have had to occur against the prevailing westerly circumpolar winds. These winds, which still occur today, are thought to have existed since the Tertiary period (Stewart and Neall, 1984), and are a major discussion topic in studies of south-western Pacific biogeography (Raven, 1973; Wagstaff *et al.*, 1999; von Hagen and Kadereit, 2001; Winkworth *et al.*, 2002a; Winkworth *et al.*, 2002b; Munoz *et al.*, 2004).

1.1.8 Habitat Origins: A Result of Tectonic and Climatic Upheaval

New Zealand arrived at its current location *c* 60 million years ago (mya), after splitting off from the super-continent of Gondwana approximately 80 mya and rafting across the Tasman basin (Cooper and Millener, 1993; Cooper and Cooper, 1995). New Zealand is an area of particularly active geological processes, largely due to the fact that it straddles the margins of the Pacific and Indo-Australian continental plates (Fleming, 1979). This marginal position led to an intense period of mountain building in the Southern Alps of the South Island during the last 5-6 million years (Fleming, 1979; Batt *et al.*, 2000; Chamberlain and Poage, 2000). New Zealand has undergone a series of climatic fluctuations; most notably, *c* 18 glacial-interglacial cycles that occurred during the Pleistocene (Cooper and Millener, 1993). The lowering of the sea level with glacial maxima increased the amount of coastal land and joined the three main islands (Te Punga, 1953; Fleming, 1962).

1.1.9 Environmental Specialisation in the New Zealand Alpine *Ranunculus*

Distinctive alpine habitats have been recognised for the alpine species of *Ranunculus* section *Pseudadonis* (Table 1.3). Fisher (1965) identified five of these general environments, which range from rock ledges and cervices among outcrops in the zone of permanent snow to slowly draining seepage zones alongside streams (Table 1.3). These habitats reflect the environmental specialisation of some species. An exception concerns *R. insignis* which is one more environmentally variable species of alpine *Ranunculus*. Fisher (1965) described this species as being restricted to sheltered situations, *i.e.*, protected from direct sunlight or wind. However, it can be observed that there is a gradient in ecological preferences from the North Island where it occurs in damp shady sheltered areas such as stream sides and gorges, to the South Island where *R. insignis* is found in more open tussock grassland or shrub land. While there is going to be variation from these general descriptions it provides a convenient starting point to distinguish between the habitats of the alpine *Ranunculus*.

Habitat	Species
High altitude snowfield	<i>R. buchananii</i>
	<i>R. grahamii</i>
Snowline fringe	<i>R. godleyanus</i>
	<i>R. sericophyllus</i>
	<i>R. pachyrrhizus</i>
Stony debris	<i>R. crithmifolius</i> subsp. <i>crithmifolius</i>
	<i>R. baastii</i>
Sheltered situations	<i>R. lyallii</i>
	<i>R. pinguis</i>
	<i>R. insignis</i>
	<i>R. enysii</i>
	<i>R. nivicola</i>
	<i>R. pinguis</i>
Slowly draining	<i>R. gracilipes</i>
	<i>R. verticillatus</i>

Table 1.3: Characteristic habitats of the alpine *Ranunculus* (section *Pseudadonis*) as identified by Fisher (1965).

1.1.10 Morphological Variation of the New Zealand Alpine *Ranunculus*

Morphologically, the New Zealand species follow the familial trend of varying widely in form and leaf shape. For example, consider *R. lyallii* and *R. gracilipes*: *R. lyallii* has large, entire peltate leaves up to 40cm in diameter and scapes up to 1.0m in height, whereas the leaves of *R. gracilipes* may be only 3cm long with bipinnasect divisions, and the entire plant rarely exceeding 10cm in height.

It was proposed by Fisher (1965) that the morphological variation within section *Pseudadonis* fell into eight categories ranging from uniform morphology to variation

recognised at the level of subspecies. At the simplest level are species such as *R. lyallii* which maintain a uniform morphology across their entire range. More complex is the morphological variation observed in *R. verticillatus* and *R. insignis*.

The vegetative morphology of *R. verticillatus* varies widely across its range and within any one population it is possible to observe the entire range of forms. This species is considered to represent a multiform species under Fisher's (1965) system. *R. insignis* also varies significantly in morphology across its range, grading from broadly reniform leaves (10 – 23 cm wide) to lanceolate forms (2.5 to 10cm wide) (Fisher, 1965). However, unlike *R. verticillatus* where the variation has no geographic structure, in *R. insignis* the variation is clinal and regional forms grade continuously through the species' entire population.

1.1.11 Breeding Groups in the New Zealand Alpine *Ranunculus*

In his study of the New Zealand alpine *Ranunculus* (section *Pseudadonis*), Fisher (1965) found that species could be divided into two breeding groups. These groups could be distinguished on the basis of petal number and hair type (Table 1.4). It was hypothesised that these two groups (many-petalled *i.e.* 10-16 petals with silky hair and few-petalled *i.e.* 5-6, petals with coarse hair) represented an ancestral split in the lineage. In the many-petalled, silky-haired group a further division could be made based on flower colour (Table 1.4). The predominant flower colour in the section *Pseudadonis* is yellow, and it is thought that the two white-flowered species (*R. lyallii* and *R. buchananii*) represent a more recent split in the lineage (Fisher, 1965).

Group	Species	Flower colour
Many-petalled, silky hair	<i>R. lyallii</i>	White
	<i>R. buchananii</i>	White
	<i>R. haastii</i>	Yellow
	<i>R. grahamii</i>	Yellow
	<i>R. sericophyllus</i>	Yellow
	<i>R. pachyrrhizus</i>	Yellow
	<i>R. verticillatus</i>	Yellow
	<i>R. pinguis</i>	Yellow
	<i>R. nivicola</i>	Yellow
Few-petalled, coarse hair	<i>R. insignis</i>	Yellow
	<i>R. godleyanus</i>	Yellow
	<i>R. crithmifolius</i> subsp. <i>crithmifolius</i>	Yellow
	<i>R. enysii</i>	Yellow
	<i>R. gracilipes</i>	Yellow

Table 1.4: Morphological division of the New Zealand alpine *Ranunculus* (section *Pseudadonis*), as identified by Fisher (1965).

A recent study by Lockhart *et al.* (2001) of the alpine *Ranunculus* of New Zealand and Australia, using DNA marker sequencing of the nuclear Internal Transcribed Spacer (ITS) region, found that section *Pseudadonis* can be divided into 4 genetically distinct groups (Table 1.5). Group I is comprised of alpine species from New Zealand and Australia. The species of group II are found in alpine and sub-Antarctic environments of New Zealand and Australia. The group III species are all found in alpine areas of New Zealand. *R. sciribalis*, the solitary group IV species, is a scree specialist and is both ecologically and genetically distinct from the other members of section *Pseudadonis* (Lockhart *et al.*, 2001).

Group	Species	Distribution
I	<i>R. lyallii</i>	South Island, New Zealand
	<i>R. baastii</i>	South Island, New Zealand
	<i>R. buchananii</i>	South Island, New Zealand
	<i>R. nivicola</i> (ITS)*	North Island, New Zealand
	<i>R. verticillatus</i>	North and South Islands, New Zealand
	<i>R. grahamii</i>	South Island, New Zealand
	<i>R. anemoneus</i>	Mt Kosciusko, Australia
II	<i>R. sericophyllus</i>	South Island, New Zealand
	<i>R. pachyrrhizus</i>	South Island, New Zealand
	<i>R. viridis</i>	Stewart Island, New Zealand
	<i>R. pinguis</i>	Campbell and Auckland Islands, New Zealand
	<i>R. gunnianus</i>	Australian Alps, Australia
III	<i>R. insignis</i>	North and South Islands, New Zealand
	<i>R. nivicola</i> (J _{SA})*	North Island, New Zealand
	<i>R. godleyanus</i>	South Island, New Zealand
	<i>R. crithmifolius</i> subsp. <i>crithmifolius</i>	South Island, New Zealand
	<i>R. crithmifolius</i> subsp. <i>paucifolius</i>	South Island, New Zealand
	<i>R. enysii</i>	South Island, New Zealand
	<i>R. gracilipes</i>	South Island, New Zealand
IV	<i>R. scirithalis</i>	South Island, New Zealand

Table 1.5: Phylogenetic divisions identified by J_{SA} and ITS, and geographic locations of the members of section *Pseudadonis*. *It is noted that there is conflict in the placement of *R. nivicola*.

These findings of Lockhart *et al.* (2001) were largely congruent with the findings of Fisher (1965). Fisher's many-petalled line is contained in groups I and II and the few-petalled lineage corresponds to group III. The group IV species of *R. scirithalis* was described by Garnock-Jones (1987) and thus was not discussed in Fisher's 1965 monograph. Lockhart *et al.*, (2001) estimated that the primary divergence between group III and groups I and II occurred approximately 5 mya, which coincides with the onset of Tertiary mountain building in New Zealand (Batt *et al.*, 2000).

Lockhart *et al.* (2001) also characterised the same taxa for the chloroplast encoded marker J_{SA} , which is found at the junction of the inverted repeat A (IR_A) and small single-copy region (SSC) of the chloroplast genome. This region was identified as a fast-evolving marker via Amplified Fragment Length Polymorphism (AFLP) studies of the alpine *Ranunculus* (Lockhart and McLenachan, 1997). Phylogenetic analysis of J_{SA} also suggested the major groupings identified with ITS, with the notable exceptions of *R. verticillatus* and *R. nivicola*. In the J_{SA} phylogeny, *R. verticillatus* was sister to the group I species, rather than being a distinct member of the clade as observed in the ITS phylogeny. In the chloroplast phylogeny, *R. nivicola* was associated with group III rather than group I, as was the case in the ITS phylogeny (Table 1.5). The shift of *R. nivicola* from group I, where it is associated with *R. verticillatus*, to group III (in association with *R. insignis*) is consistent with the hypothesis that this species is the progeny of *R. insignis* and *R. verticillatus*.

1.2 Dispersal and Polyploidy in the North Island *Ranunculus*

1.2.1 Introduction to the Study

Currently there are three species of alpine *Ranunculus* in the North Island, namely *R. insignis*, *R. verticillatus* and *R. nivicola* (Fig. 1.1). It has been hypothesised that during the Pleistocene *R. insignis* and *R. verticillatus*, which are thought to have originated in the South Island, expanded their range across Cook Strait and into the North Island (Fisher, 1965; Lockhart *et al.*, 2001). This most likely occurred during glacial periods of the Pleistocene when lower sea levels meant the islands were connected (Te Punga, 1953; Fleming, 1962; Fisher, 1965; Lockhart *et al.*, 2001). Fisher (1965) hypothesised that *R. nivicola* formed in the North Island as an allopolyploid between *R. verticillatus* and *R. insignis*. In support of this inference Fisher (1965) noted that *R. nivicola* exhibits phenotypic traits intermediate between that of its putative parents (Fig. 1.2).

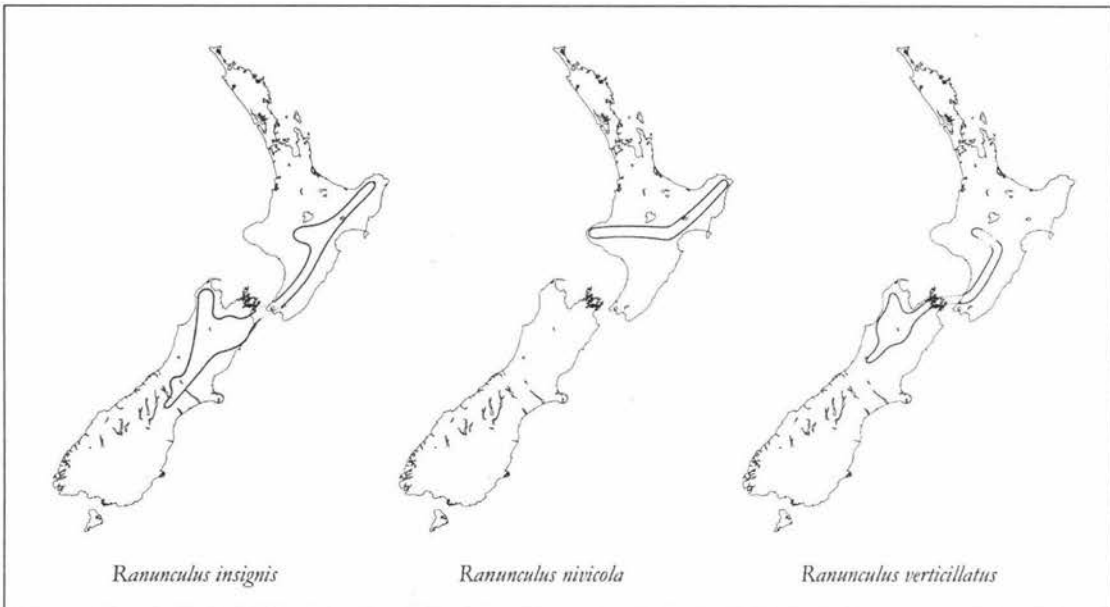


Figure 1.1: The current distributions of *R. insignis*, *R. nivicola* and *R. verticillatus* from Fisher (1965).

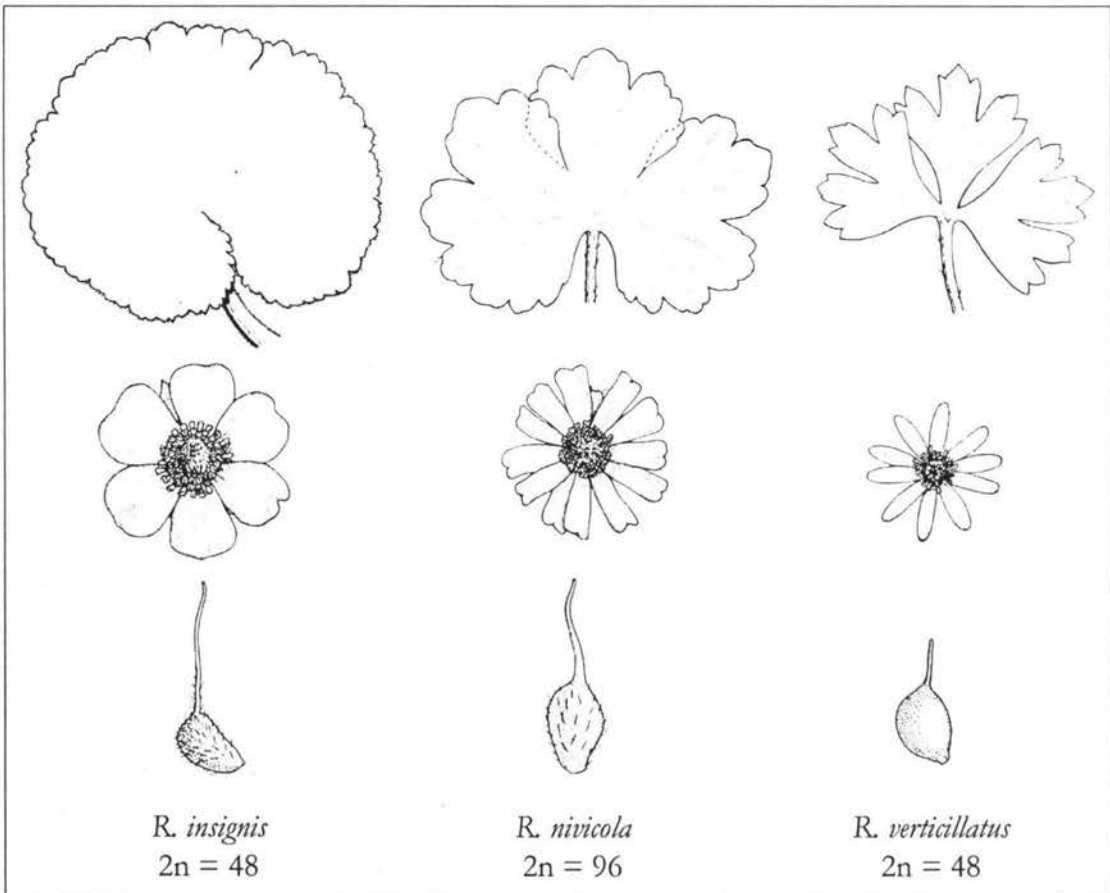


Figure 1.2: Leaf and floral morphology of *R. insignis*, *R. nivicola* and *R. verticillatus*. (Modified from Fisher (1965)).

The principal question that has motivated the present study is whether the formation of the allopolyploid *R. nivicola* might have facilitated range expansion of New Zealand alpine *Ranunculus* into habitats unsuitable for either of the parent species. The present study makes a contribution towards answering this general question by describing phylogenetic relationships of all three species and by analysing ecological diversity with ArcGIS.

1.2.2 Hypotheses and Aims

Hypothesis 1:

Range expansion of *R. insignis* and *R. verticillatus* into the North Island involved rare dispersal events in the evolutionary history of New Zealand alpine *Ranunculus*.

Aim:

Using phylogenetic analyses of J_{SA} and ITS sequences, and more extensive taxon sampling than that used by Lockhart *et al.* (2001), reconstruct the evolutionary relationships among individuals from North and South Island populations of *R. insignis* and *R. verticillatus* and infer the frequency of seed dispersal events between the two islands.

Hypothesis 2:

In the North Island, a single allopolyploidisation event between *R. insignis* and *R. verticillatus* gave rise to *R. nivicola*.

Aim:

With improved taxon sampling compared to that used by Lockhart *et al.*, (2001), perform phylogenetic analyses of J_{SA} and ITS sequences to investigate (a) whether the origin of *R. nivicola* can be identified with respect to North Island populations of *R. insignis* and *R. verticillatus*, and (b) whether or not *R. nivicola* has formed recurrently.

Hypothesis 3:

The polyploid *Ranunculus nivicola* extends the geographic range of alpine *Ranunculus* in the North Island into areas that would not be otherwise occupied by this genus.

Aim:

Use LENZ and ArcGIS techniques to describe the habitat differences of the three North Island species, and infer whether *R. nivicola* occupies habitat that could not be occupied by *R. insignis* or *R. verticillatus*.