Breeding Systems and Rarity in New Zealand *Myosotis*

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Andrea Mary Brandon
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Abstract

Rarity in New Zealand *Myosotis* was explored at several different levels for this thesis. Within the genus, there are many rare species, encompassing several different types of rarity, and many of these are considered threatened in some way. In addition, the genus contains species that exhibit a wide array of floral forms, each of which can be associated with a distinct form of rarity.

The reproductive biology of selected Nelson taxa was investigated in several species covering a range of floral morphologies, including the previously unstudied brush blossom floral syndrome. Species were found to fall into one of two mating types based on the degree of herkogamy[1] exhibited by the flowers. Species with flowers that are herkogamous throughout their life, require pollinators to set seed while those that are not herkogamous at some stage during anther dehiscence, are self-fertile and able to self-pollinate autonomously. All species studied were self-compatible. Considerable variation in seed production was observed under natural conditions for those species that required pollinators to set seed. For these pollinator-requiring species, local population density had strong effects on seed set, while population size had no effect. These plants were always pollen limited in low-density patches. For a self-fertile species, seed set was always high and unaffected by either local density or population size and pollen was never limiting. These results indicate reproductive success in pollinator-requiring species of *Myosotis* is subject to Allee effects and these effects occur at a very local scale. These results have implications for the assessment and management of threatened *Myosotis* species in particular and rare plants generally. It is essential to know the pollination requirements and levels of density dependence for reproduction both for the assessment of threat and for determining management strategies for threatened plants.

Self-compatible, but pollinator-requiring species are prone to autogamy and geitonogamy and therefore inbreeding depression. One strategy to avoid this is to increase dichogamy and synchronise inflorescence development. Pollination was studied in the geographically restricted, ultramafic endemic *Myosotis monroi,* a species that has a relatively large floral display and whose flowers often bear precocious styles prior to

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[1] A glossary of technical terms follows the appendices

buds opening. In general, precocious styles are assumed to be receptive and indicate protogyny. Stigmas were receptive at this stage and some pollination occurs during this precocious phase. Stylar precocity effectively lengthens the female-only phase in this species. This is the first time that precocious styles have been proved to be receptive at this stage. *M. monroi* also shows far greater phenological synchrony of within plant flowering than five other species of New Zealand *Myosotis*. It is thought that the impact of a large floral display on levels of geitonogamy in *M. monroi* is alleviated to some degree by the relatively long, initial female-only phase and phenological synchrony of flowering stages.

Rarity is generally considered "the precursor to extinction" (Darwin, 1872). However, there are several different forms of rarity and not all rare species are threatened. I examined the different rarity patterns observed within New Zealand *Myosotis*. New Zealand *Myosotis* species are never common, all are rare in different ways and some species are threatened. Some species are known to occur at one locality where they may be locally common, others may occur in two or more widely disjunct geographic areas, while others may be widespread but never common where they occur. A comparative analysis was carried out to determine whether there are morphological correlates of the rarity patterns seen within the genus. Local abundance, population disjunctions and distribution patterns of 33 *Myosotis* taxa were compared to aspects of their morphologies, including traits related to mating systems, dispersal ability and life-histories. Taxa requiring pollinators to set seed had smaller range sizes and higher local population densities than those that were able to self-pollinate. Apparent adaptations for dispersal and life-history traits were not correlated with range size. The disjunct distributions exhibited by some taxa within the genus were not associated with any of the morphological traits. My results can assist threat assessment and conservation management for New Zealand *Myosotis*. Locally dense, geographically restricted taxa are pollinator requiring while sparse, widespread taxa are selfing. Cases that break this general rule may be used to identify taxa at risk.

In the most recent taxonomic treatment, New Zealand *Myosotis* is initially split into two major clades depending the degree of anther exertion exhibited by the flowers. This has led to the recognition of species that cannot be distinguished in any other way. Species limits were examined in one such species complex; the vegetatively indistinguishable *M.*

forsteri and *M. venosa*. Several floral and vegetative characters were measured and compared. Filament length was the only significant character that could be used to distinguish the species, and even this was not 100% reliable. Filament length determines the degree of self-pollination that can occur and whether or not reproduction is assured. The continuous variation observed in filament, style and anther lengths and corolla sizes shows that there is a lot of phenotypic variation within each taxon. Geographic clines are observed in vegetative traits that are independent of mating system. This, in addition to the continuous variation observed in floral characters, lends some strength to the proposition that this species pair may be switching between the two breeding systems.

The increased knowledge provided by the studies contained in this thesis on New Zealand *Myosotis* has provided a much-needed boost to our understanding of the population dynamics of these rare species, which ultimately can be used to guide conservation management for those taxa considered at risk of extinction. It can assist in identifying those populations that are not threatened and it can direct efforts towards the more pressing problem situations. It has also highlighted the necessity for a taxonomic revision for the southern hemisphere section of the genus.

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CHAPTER ONE

The biology of rarity and New Zealand *Myosotis*

This chapter introduces the thesis by providing a literature review on the biology of rarity with particular emphasis on rare plant reproductive biology. This chapter also provides an overview of the systematic position of New Zealand *Myosotis* and summarizes work carried out to date on the New Zealand species in the genus. Several taxa are considered threatened in some way in lists compiled for conservation purposes, and there are many rare species. This chapter will also provide an outline of the contents of the thesis.

The biology of rarity

'Rarity, as geology tells us, is the precursor to extinction'

C. Darwin (1872)

An integral part of attempting to preserve biological diversity is the conservation of threatened species. The premise that rare species face increased extinction risk has driven the assumption that rare species *per se* are threatened. This is certainly true in some cases. However, we now know from work carried out in the last couple of decades that there are different types of rarity, and that not all rare species face the same risk of extinction. The more knowledge we have about the biology of rarity the better we will be able to understand our rare species and make informed decisions regarding population management.

Darwin wrote about rarity and its relationship to extinction, commenting that until the reasons for rarity were understood extinctions could not be explained (Darwin, 1872). Previously Lyell (1830-33) had proposed a geological explanation for rarity, in which rare species were old and about to go extinct. Although Darwin's concept of rarity also encompassed evolution, ecology, geography and genetics, it was the geological theme that was carried forward to explain rarity well into the 20th century. This was the basis of Willis's (1922) 'Age and Area' hypothesis; he thought that rare species were newly diverged (incipient). Some still thought that they were old (relictual) (e.g. Fernald, 1918), and others (e.g. Gleason, 1924) argued that they could be either (Fiedler and Ahouse, 1992). In this respect rare species have been found to be old, young or of intermediate age (Stebbins, 1980). However it is still a useful tool to use when considering possibilities as to why a species is rare. When looking at natural forms of rarity within genera, the age of the taxon has been used to explain distributions. A recent study of eucalypts examined rarity from the perspective of newly diverged species and those of old or intermediate age (Prober, *et al.* 1990). In their study Prober *et al.* (1990) used phylogeny to infer taxon age. Another method for determining age from cytological data has been proposed in which ploidy level was used to categorise endemic plants (Fiedler, 1986). Fiedler (1986) lists age of taxon as a factor that may be important in determining rarity.

Poor competitive ability has been put forward as an explanation for rarity and could possibly explain the restriction of some taxa to ultramafic areas (Kruckeberg, 1951). The rare plant, dwarf pipewort *Eriocaulon kornickianum*, a declining species with a disjunct distribution, was found to have poor competitive abilities (Watson *et al.* 1994). However, in a study of the differences between rare and common prairie grasses, sparse species have been found to have good competitive abilities that may offset low density and increase local persistence (Rabinowitz and Rapp, 1984). Again, there are examples that confirm the theory but there are also exceptions.

Another theory was that rare species are colonisers of temporary habitats (Stebbins, 1980). However, while some rare species are confined to pioneer habitats, there are also a large number that are confined to climax forest habitats (Stebbins, 1980).

Many rare species occur on ‘ecological islands’ which have different environmental factor/s, often soils of an unusual type (e.g. serpentine areas), which isolate them from the surrounding ‘ocean’ of unsuitable habitat (Stebbins, 1980). Again, there certainly are examples of rare plants confined to these areas, but it is not a general pattern of all rare plants.

Stebbins (1980) called for a synthetic approach to understanding rarity, having found good examples for many of the hypotheses proposed. In short, there is no single factor that can be identified as the cause of rarity.

The meaning of rarity

Descriptive work carried out in the first half of this century on abundances established the well known fact that within a community there are many rare species and a few common ones (Fiedler and Ahouse, 1992). However, most of these rare species will be common elsewhere within their ranges.

The word rare has different meanings and is used to describe many different patterns of density and distribution. Rarity is defined simply by Gaston (1994) 'as the state of having low abundance and/or a small range size'. Many authors have discussed the rarity of plants and in doing so have dealt with the concept variously (e.g., Harper, 1981; Rabinowitz, 1981; Palmer, 1986; Schoener, 1987; Fiedler and Ahouse, 1992). These studies have provided insights into the biology of rarity. Plants can be rare in different ways and several authors have tried to define different types of rarity (e.g. Rabinowitz, 1981; Fiedler and Ahouse, 1992). Essentially the different forms represent particular range, distribution and abundance patterns within different spatial (e.g. Rabinowitz, 1981; Gaston, 1994) and temporal scales (Fiedler and Ahouse, 1992).

There is generally a positive correlation between size of geographic range and local abundance (Brown, 1984, Gaston, 1996, Lawton, 1993). However there is a lot of variation such that individual taxa can be locally abundant but only have a small geographic range and others may be widespread but in consistently low population size (Rabinowitz *et al.* 1986). Rabinowitz (1981) designed a method of categorizing rarity with a dichotomously branching system using population size, habitat specificity and geographic range (Table 1).

Table 1: Seven forms of rarity (Rabinowitz, 1981)

Geographic distribution	**Wide**		**Narrow**	
Habitat specificity	**Broad**	**Restricted**	**Broad**	**Restricted**
Large population somewhere				
Small populations everywhere				

Table 1 has eight categories, but the one for plants with wide geographic distribution, broad habitat specificity and a large population somewhere are unlikely to be rare.

Fiedler and Ahouse (1992) simplified the spatial scale to one variable and introduced a temporal dimension (Table 2). They list a hierarchy of factors that may prove important in determining rarity for each category.

Table 2: Four categories of rarity in vascular plants (Fiedler and Ahouse, 1992)

Temporal persistence	**Short**	**Long**
Wide spatial distribution		
Narrow spatial distribution		

Numerous contributing factors have been identified as possible causes of rarity in studies conducted to date. The multiplicity of causes thought likely to contribute to the rarity of different species has led to the suggestion that all species may be idiosyncratic (Fiedler, 1986).

Small populations and extinction

'We need not marvel at extinction; if we must marvel, let it be at our own presumption in imagining for a moment that we understand the many complex contingencies on which the existence of each species depends'

C. Darwin (1872)

There are many reasons why a population may be small. Harper (1979) summarised them, as follows:

- The available sites are few and separated by distances beyond a species normal dispersal ability.
- The carrying capacity of the site is low.
- The habitability of the site is of short duration because of successional displacement.
- Colonisation is in its early stages, and full exploitation of the site has not occurred.
- Catastrophes of various kinds

Species with small populations are thought to have a greater chance of becoming extinct than others. However, there is still a large amount of variation in probability of extinction predictions (Gaston, 1994). Not all rare species exist in small populations, just as not all species with consistently small population size are considered rare. Plants have quite different life history characteristics than animals and the generalisations may not always apply. The diversity of mating systems from self-fertilisation to complete outcrossing, seed dormancy and the reliance upon animals for pollination and seed dispersal are some unique characters (Schemske *et al.* 1994). Plants that are self-compatible and that self fertilise are not susceptible to many of the factors that were identified by Soulé (1983) as possibly contributing to extinction of local populations (Table 3). As Table 3 suggests, many factors can affect extinction probabilities as well as small population size.

Table 3. Possible factors contributing to the extinction of local populations (from Soulé, 1983).

Rarity (low density)
Rarity (small, infrequent patches)
Limited dispersal ability
Inbreeding depression
Loss of heterozygosity
Founder effects
Hybridisation
Successional loss of habitat
Environmental variation
Long term environmental trends
Catastrophe
Extinction or reduction of mutualistic populations
Competition
Predation
Disease
Hunting and collecting
Habitat disturbance
Habitat destruction

Catastrophes are able to cause rapid population decreases that are often unpredictable. These include physical factors (hurricanes, droughts), biological factors (pest invasion, epidemics) or human induced changes (Mangel and Tier, 1994). Menges (1990) notes the importance of catastrophic events in a population viability analysis of Furbish's lousewort, finding that they dominate population viability estimates. Without catastrophes random variation in demographic parameters will see population numbers fluctuate over time. When population size is small, these population fluctuations may include zero (i.e. population extinction) (Rabinowitz *et al.* 1989).

It is expected that population density and geographic range would vary over time, hence the temporal component in species rarity. However, there is evidence suggesting range and abundance are persistent species characteristics i.e. some taxa are more prone to extinction than others (Lawton, 1993), the corollary of that being that some are more likely to persist than others. Density and range are considered linked to the species' fundamental niche breadth, an evolved species trait (Brown, 1984). A good example of this is found in New Zealand *Myosotis* species, as local density and range size are persistent species characteristics. This line of thought implies that there is a phylogenetic component to the types of rarity patterns found within genera and several researchers have studied this. Rare plants are under represented in some families (Rosaceae) and over represented in others (Scrophulariaceae, Lamiaceae) (Schwart, 1993). Another study found disjunct taxa within genera of herbaceous perennials had range sizes significantly correlated with each other (Ricklefs and Latham, 1992). A further study has found the families, that have more than an average number of rare species, are different in Australia, New Zealand and North America, thought to be due to different speciation histories in different landscapes (Westoby, pers. comm.). In New Zealand, our threatened species are mostly in the Boraginaceae, Scrophulariaceae, Asteraceae, Orchidaceae, and Poaceae (de Lange and Norton, 1998). Although genus and family size accounts for some of this pattern, not all can be explained in this way (see de Lange and Norton, 1998).

As well as the size of the population, other information can be used to assess extinction threats. Bond (1995) considered aspects of reproductive biology were critical in determining extinction probabilities. He found extinction risk to be greatest when the risk of pollinator or disperser failure, reproductive dependence on the process and demographic dependence on seeds are all high (Bond, 1995).

Persistence

Understanding adaptations rare species display that reduce their vulnerability to extinction is an area that requires research (Gaston, 1994). In studies of rare and common congeners, the differences found in the rare species are often considered adaptive traits that have evolved to cope with the state of rarity. These differences could be evolved adaptations to deal with rarity but there could also be other mechanisms involved (Kunin and Gaston, 1993). Often rarity is studied by ecologists and species are considered in ecological rather than evolutionary time; the species, though, is an evolutionary concept (Harvey, 1996). Species' success or lack of it is often studied in terms of their traits or characteristics which make them more or less successful under particular ecological circumstances but the traits are not randomly distributed. Therefore it is important to take phylogeny into account when making interspecific comparisons (Harvey, 1996). The newer phylogeny based tests used in conjunction with interspecific comparisons should be able to tell the difference but this requires knowledge of phylogeny and ancestral states (Maddison, 1990). Unfortunately this is not an easy undertaking, as there are very few groups with resolved phylogenies.

Sparse species (large range sizes and nowhere common) are possibly important in the study of persistence (Rabinowitz *et al.* 1986). Predicting the population size required to ensure high probability that a species will persist has limitations and different values can be obtained depending on the type of model involved (Stacey and Taper, 1992). Theorists work out probabilities and estimate times to extinction (e.g. Roughgarden, 1975) but in the real world there are some well documented cases of small populations persisting at very low densities e.g. some New Zealand birds (Craig, 1991) which are at odds with any of the theoretical projections. Stacey and Taper (1992) suggest two alternatives to the study of persistence; one is to follow the fate of a recently declining population due to human disturbance and determine population level parameters most sensitive, the other is to look at species that naturally occur in small, highly fragmented populations (i.e. sparse species). These species have managed to persist through time and have successfully solved the hazards of small population existence. As such, these may be model systems from which we can work out how small populations are viable (Stacey and Taper, 1992). This approach has been taken with woodpeckers (Stacey and Taper, 1992), bighorn sheep (Berger, 1990) and sparse prairie grasses (Rabinowitz and

Rapp, 1984). Rabinowitz and Rapp (1984) found sparse species are more likely to persist if they are good competitors. Further, sparse species were found to have a buffered reproductive response, considered to be a mechanism that offsets the demographic stochasticity, a hazard of small populations (Rabinowitz *et al.* 1989). Sparse species have been found to have a lower potential for rapid increase (Westoby, pers. comm.).

Conservation and rarity

Rarity and conservation are invariably connected as rare species are thought to have a greater chance than common species of becoming extinct. The ultimate fate of small populations, their persistence or extinction, is of primary concern to conservationists. A key issue in this regard is that species can be rare in different ways. Not all rare species are threatened with extinction. Individual species characteristics will affect the ability of the population to persist, but ultimately chance events may determine persistence. This knowledge has relevance to the process of categorising, classifying and prioritising rare species for threat assessment.

The reproductive biology of rare plants

An important aspect in the study of rare plants is the understanding of their reproductive biology. The mating system determines the genetic structure and evolutionary potential of plant populations (Allard, 1975). It is important initially, to identify the breeding system for the assessment of the status of any rare plant (Hamrick *et al.* 1991). Plants with different breeding systems are likely to have different population structures. Some species are highly selfing, some have mixed mating syndromes, and others are predominantly outcrossing. Self-pollinators can grow, reproduce and colonise a new area by the germination of just one seed. They can easily achieve reproductive isolation and do not require the presence of others to successfully reproduce and persist. Therefore small population size is not necessarily a sign that the species is rare or threatened. Obligate outcrossers or self-incompatible plants require local population densities at levels that ensure a supply of cross pollen and attract vectors, if required, to achieve pollination. Therefore, to be successful, they need population sizes considerably larger than selfing species.

Aspects of reproductive biology have been linked to rarity in several studies (e.g. Rabinowitz and Rapp, 1981; Karron, 1987a; Kunin and Shmida, 1997; Quinn *et al.* 1994). Breeding system, pollination ecology, dispersal ability and vegetative reproduction are important factors in determining plant distributions. Other factors, such as environmental constraints, and the evolutionary and recent history of populations will affect abundance and place limits on distributions. However, it is difficult to prove that particular factors have caused the rarity of species without conducting experimental manipulations and studies have generally provided correlational rather than causal explanations (Gaston, 1994).

Rare-common differences

A number of studies have focused on comparisons of life history traits between rare and common congeners. Despite the inherent problems associated with the study of any rare organism (i.e. lack of data) the comparisons have found systematic differences between these pairs of species (e.g. Rabinowitz, 1978; Rabinowitz and Rapp, 1981; Landa and Rabinowitz, 1983, Rabinowitz *et al.* 1986; Karron, 1987a & b; Kunin and Shmida, 1997). Recurrent patterns have emerged of species traits that are non-randomly distributed across a wide range of organisms (Kunin and Gaston, 1993). An entire book, *The Biology of Rarity: Causes and consequences of rare-common differences*, (Kunin and Gaston, 1997), has been produced on this topic alone. While the editors acknowledge a) that many different mechanisms can account for the similar patterns observed and b) that there are difficulties in determining the primary mechanism involved, these differences are consistently appearing in cross-species comparisons (Gaston and Kunin, 1997). Interestingly, when compiling these studies for meta-analysis, the different types of rarity are not taken into account and yet these patterns still appear. Whatever mechanisms are involved, rare species that have been able to persist are expected to have traits that allow them to do so in the rare state (Kunin and Gaston, 1993).

From these studies, some broad generalisations can be made regarding the reproductive traits of rare plants. Rare species are biased away from outcrossing and sexual reproduction (Kunin and Gaston, 1993). Kunin and Gaston (1993) reviewed the studies of rare and common species with respect to dispersal and reproductive effort from which they determined the following main points. Rare plants tend to have:

- lower levels of self incompatibility
- a bias toward asexual reproductive pathways
- lower overall reproductive effort
- poorer dispersal ability

Some problems have been identified with the methods used in some of the studies, such as the intercorrelation of traits (e.g. reproductive effort and dispersal distances are highly correlated) and non-independence of the traits of related species (Kunin and Shmida, 1997). Another problem cited by Kunin and Shmida (1997) was that only a single measure of rarity was used in each study. Plants can be rare in different ways and at different spatial scales. Some studies are of point endemics with dense local populations, while others are of sparse taxa with low densities and wide geographic ranges.

Breeding system and rarity

Population structure and mating system interactions have been studied in both experimental and natural populations of plants (Sampson *et al.* 1989) and reviews have been conflicting regarding any correlation between breeding system and rarity. For example Ellstrand (1992) makes the point that rarity of a species is not closely correlated with its breeding system, although many rare species are obligate outcrossers. Karron (1987b) found no significant relationship between breeding system and geographic range in the genus *Astragalus*. In Quinn *et al.* (1994), in a study of scarce British plants, obligate outcrossers were found to be significantly more aggregated than those able to self-pollinate. Pollination effects probably operate at small spatial scales (e.g. *Diplotaxis erucoides* in Kunin, 1992). The disparity of scales used to measure rarity in different studies was recognised by Kunin and Shmida (1997). They used three measures of rarity, local density, regional abundance and global distribution in a study of Mediterranean annual crucifers (Kunin and Shmida, 1997). They found breeding systems are related to regional- and global-scale measures only when local density is left out of the analysis so, although these measures are themselves related, they are not interchangeable (Kunin and Shmida, 1997). It may be that abundance measures have been made at different scales in different studies and this has caused the disagreements over the relationship between breeding system and rarity (Kunin and Shmida, 1997).

Pollination biology

Burd (1994) reviewed studies of pollen limitation in determining the role pollen limitation in fruit and seed production in flowering plants. He found significant pollen limitation at some times or at some sites in 62% of the 258 species included in the study (Burd, 1994). There was variation in the levels of pollen limitation observed at different times of the year and in different populations which suggests pollination events are not constant (Burd, 1994). Generally, self-incompatible species gain greater benefit from supplemental pollinations than self-compatible species (Burd, 1994). Burd (1994) suggests the best use of resources spread among ovules, pollen and pollinator attraction, is to invest in more flowers or ovules per flower than would normally be pollinated in order to take advantage of chance fluctuations that bring large amounts of pollen. This means that pollen limitation is likely and that flowering plants commonly mature fewer seeds and fruits than flowers and ovules produced (Burd, 1994).

A subsequent analysis of the same species was conducted by Larson and Barrett (2000) to determine whether pollen limitation was correlated with particular life-history traits and ecological conditions. Although herbaceous, nectariferous and temperate species were found to be significantly less likely to be pollen limited in the initial comparative analysis, when phylogeny was corrected for, although the trends remained the same, results were not significant (Larsen and Barrett, 2000).

In self-incompatible or obligate outcrossing species, the plants growing at low local density are more likely than those growing at high density to be pollination-limited in their reproductive success (Kunin 1992, 1993) and they are also more likely to be self-compatible (Kunin and Shmida, 1997). Theoretically population density and outcrossing rates are positively correlated (Karron *et al.* 1995).

Rare species may be more likely to evolve to adopting a species-constant pollinator since a generalist will bring largely inappropriate pollen (Kunin and Iwasa, 1996). However, Karron (1987a) found generalists pollinated restricted species of *Astragalus*. Several other restricted species have been found to be generalist-pollinated as well (Karron, 1987a). One possibility suggested was that pollinator specialisation is unlikely to either evolve or be maintained because a small population can only sustain a small number of individuals (Karron, 1987a).

Strong selection pressures would be expected for traits that attract pollinators both in terms of quality (i.e. specialist rather than generalist) and quantity (Kunin and Shmida, 1997). This has been confirmed in a study of crucifers, which found that self-incompatible species have particularly showy flowers compared to their self-compatible counterparts (Kunin and Shmida, 1997). Another way to attract pollinators is to have large floral displays. However, a study, which examined the response of insect pollinators to variation in flower number on *Myosotis colensoi*, and *Mimulus guttatus* found pollinators exploited flowers equally, regardless of size of display (Robertson and Macnair, 1995). Ohashi and Yahara (1998) found that the visitation rate of bumblebees to *Cirsium purpuratum* was a decelerating function of floral display (i.e. as floral display size increased, there was an increased visitation rate per plant but it was at a slower rate).

Self-fertilisation can be selected for in particular circumstances. In changeable environments, as population size varies and therefore possible mates, so too can pollinator availability such that when population size is low, selfing individuals are selected for (Karron, 1991). The ability to self-pollinate may be advantageous when pollinators are scarce and disadvantageous when they are abundant if there is high inbreeding depression (Robertson *et al.* 1994). In a study of annual crucifers the rare species tended to display more extreme values for floral traits than common ones with the same breeding systems (Kunin and Shmida, 1997), which does imply disruptive selection is occurring.

Dispersal ability

The dispersal ability of species is expected to have an effect on their distributions. Rabinowitz (1978) found diaspore weight and abundance are positively correlated in prairie grasses, and suggested the most likely reason was that rare species seeds are adapted for longer-distance dispersal as they are colonisers of spatially and temporally rare microsites. Further work conducted in this respect found dispersal ability negatively correlated with abundance (Rabinowitz and Rapp, 1981). Oakwood *et al.* (1993) found a positive relationship between dispersal ability and range size in three ecological regions of Australia. Quinn *et al.* (1994) also found dispersal ability was correlated with distribution pattern of scarce British plants. However, this has since been refuted by Thompson and Hodgson (1996) who, using the same taxa, a different classification of dispersal in space (i.e. how far they disperse), and new data on dispersal in time (i.e. persistence in the seed bank), found no relationship between distribution and dispersal ability. Thompson and Hodgson (1996) believe habitat loss is the major determining factor in the distributions of plants in Britain. Further investigation is required as there are other factors involved in determining distributions. There may be different regimes operating on the floras of different countries. There is a widely held view amongst population biologists that plant population growth is not limited by seed production. Crawley (1990) concluded that plant population growth is more likely to be limited by environmental factors such as microsite availability than by seed production. Bawa and Beach (1981) believe plant reproductive success is rarely limited by pollination events. However, a recent review of seed limitation studies found 50 % of augmentation studies and 53 % of introduction studies showed evidence of seed limitation occurring (Turnbull *et al.* 2000).

Genetics

The genetic theories of Sewell Wright (1931) stimulated concepts that highlighted the genetic diversity or homogeneity of rare species. The individuals of rare species were thought to have less genetic variability, more homogenous genomes and consequently the spread of deleterious genes will be more rapid within their small populations (e.g. Wright, 1956, Huxley, 1963).

Conserving the evolutionary capacity of species by preserving their natural levels of genetic diversity is one goal of conservation biology. A primary focus of conservation genetic research has been to determine the levels of genetic variation in rare species. Mutation, natural selection, migration and random genetic drift are the four evolutionary forces which interact with an organism's recombination system that account for the way in which genetic variation is distributed among individuals within populations and among populations within regions (Barrett and Kohn, 1991).

Theory predicts that widespread, long-lived, wind pollinated, outcrossers maintain more genetic variation within their populations than species with other trait combinations (Hamrick *et al.* 1991). Data has been gathered and analysed from studies of genetic diversity to see if any generalisations can be made. Genetic diversity within and among populations has been found to be significantly influenced by the breeding system

(Hamrick *et al.* 1979; DeMauro, 1993). Species with different breeding systems, seed dispersal mechanisms, geographic ranges, taxonomic status and life forms generally have different levels of diversity within and among their populations (Hamrick and Godt; 1996). Life form, and breeding system in particular, has highly significant influences on levels and distribution of allozyme genetic diversity in seed plants (Hamrick and Godt, 1996). Specifically, outcrossing and woody plants had more overall genetic diversity and less interpopulation variation (Hamrick and Godt, 1996).

It is not surprising that these life history traits have been found to influence genetic diversity. There are well known associations between breeding system and life form i.e. between selfers and the annual habit and between outcrossers and the perennial habit (Clegg and Brown, 1983). Kunin and Shmida (1997) found significant interaction effects between breeding system and geographic range. There was a positive correlation between geographic range and local abundance in a wide range of organisms (Brown, 1984). Therefore, many of these traits may themselves be highly correlated. This highlights both the complexity and importance of understanding breeding system interactions with the state of rarity.

Genetic threats

Generalisations aside, genetic considerations are important in a couple of areas. Self-incompatible plants can become endangered through the loss of alleles at self-incompatibility loci that can cause direct threats to population persistence through reproductive failure (De Mauro, 1993). If a common, sexually compatible species is sympatric with a rare species, then the rare taxa may be vulnerable to extinction through hybridisation (Ellstrand, 1992).

Inbreeding depression

Although some rare plants occur in large populations, many exist in small, sometimes isolated populations. One of the possible effects of small population size is inbreeding depression which is a comparative term given to the relative reduction in fitness of selfed compared with outcrossed offspring (Barrett and Kohn, 1991). The most famous product of the genetic emphasis on conservation was the "fifty/five hundred" rule (Franklin, 1980). This was considered the minimum population size required to ensure short- and long-term viability, (N_e of 50 and 500 respectively), determined by calculating inbreeding depression coefficients (Holsinger and Vitt, 1995). Inbreeding effects on fitness in plants will depend on the diverse array of breeding systems (Charlesworth and Charlesworth, 1987) and may not depend on population size. Species that occur naturally in small, isolated populations may be adjusted to inbreeding effects in their genetic make up. Their mating systems may have adjusted to allow for the genetic consequences of rarity (Barrett and Kohn, 1991). When historical evolutionary patterns are very different from recent ones, as for those that have naturally large populations that have suffered recent declines, problems may arise (Huenneke, 1991). Individuals may be unable to adjust quickly enough to their altered circumstances.

There have been a lot of theoretical and experimental investigations into the significance of inbreeding and heterosis on fitness. Generalisations are that inbreeding depression usually occurs in outcrossing plants, is less severe in partially selfing species and can be absent in species with high selfing rates (Lande and Schemske, 1985; Barrett and Kohn, 1991). However, actual tests of the theory do not always prove the case. In some studies, the theory was confirmed but in others inbreeding depression was found to occur in species that frequently self-fertilise (Barrett and Kohn, 1991).

Mating system evolution has largely been considered within the realm of population genetics (Holsinger, 1996). The maintenance of outcrossing individuals in populations is thought to be due to high levels of inbreeding depression (Charlesworth and Charlesworth, 1987). Therefore, inbreeding carries out an important genetic function in maintaining the range of plant-mating systems. The means by which genetic transmission is achieved in outcrossers is via their pollinators and, subsequently, their offspring. Plant-pollinator interactions and their consequences for seed set are generally studied from an ecological rather than genetic perspective (Holsinger, 1996; but see Lloyd, 1979). The pollen transfer patterns will be interacting with, and influencing the means by which genetic transmission is accomplished within different mating systems (Holsinger, 1996). Further knowledge gathered through empirical studies of pollination biology will assist our understanding of mating system evolution.

There have been a great number of genetic studies in recent times, with the development of biochemical and molecular techniques that have enabled levels of genetic diversity to be quantified. The idea that rare plants are genetically depauperate and the theory behind it, still stands, although results have not universally supported the theory. Population size, while an important factor, clearly is not the only factor determining genetic diversity. The evolutionary history of a population will have impacted on the level of genetic diversity within it. The level of genetic variation within a species provides the potential for evolutionary change (Huenneke, 1991). The breeding system is the mechanism for passing on this variation. Levels of genetic diversity are significantly influenced by the breeding system (Hamrick *et al.* 1991).

Similarly, current theory regarding the effects of habitat fragmentation predicts that reduced size and increased isolation of populations will lead to reduced genetic variation through genetic drift, inbreeding, lower levels of gene flow and local population extinctions (Gilpin, 1991). A review of recent studies of the population genetic consequences of habitat fragmentation for plants has found that although the data generally confirm reduced population size is accompanied by reduced genetic variation, not all fragmentation events lead to genetic losses and in some cases fragmentation appears to increase gene flow among remnant populations (Young *et al.* 1996).

After a couple of decades of research into plant conservation genetics and population viability, there are few generalisations that can be made about the genetics of rare plants. Genetic considerations are important for endangered self-incompatible plants because the loss of alleles at self-incompatibility loci may pose direct threats to population persistence (DeMauro, 1993). Hybridisation of endangered species with common relatives may result in genetic assimilation and loss of the rare taxon (Ellstrand, 1992; Rieseberg, 1991). However apart from these two situations, genetic concerns may be largely inappropriate when assessing the status of rare plants. If populations are ecologically viable, then they may be large enough to handle any risks of loss of genetic diversity (Lande, 1988). This line of thought has lead to the suggestion that there is no need to be concerned about genetic threats to the persistence of rare species that have always been rare (Holsinger and Vitt, 1995). The populations that have suffered recent losses, particularly those with previously widespread distributions, are likely to be relatively more at risk than those that have always had small populations.

Vegetative reproduction

Rare plants are biased toward having asexual reproductive pathways (Kunin and Gaston, 1993). When population size is low or variable, selection for individuals with the capacity to reproduce vegetatively may be similar to that for selfing mechanisms. There may be the advantage that the sprouting of a rosette from an existing plant, which effectively increases the plants' size and reproductive capacity, may improve its ability to attract pollinators and sexually reproduce. It also may simply be a safer means of reproduction.

In conclusion, the overriding importance of understanding the reproductive biology of rare plants is emphasized. In particular mating system and pollination biology are major factors determining vulnerability of rare plants in different situations. Genetic considerations of endangered species are required in only a few cases.

New Zealand *Myosotis* L. (Boraginaceae)

Forget-me-nots, *Myosotis* species, belong to a cosmopolitan genus within the Boraginaceae and have a worldwide temperate distribution. Current references for the total number of species are inaccurate. Mabberly (1987) puts the worldwide distribution at 50 with 41 of these occurring in Europe. However, there are 34 New Zealand species described in the Flora of New Zealand (Allan, 1961), although Webb *et al.* (1990) put the number of New Zealand species as 47 and the worldwide total at about 100. This may still increase as the numerous undescribed taxa are given taxonomic treatment. With the inclusion of all undescribed taxa, Druce (1993) inflates the New Zealand total to about 60 species.

The most recent taxonomic treatment of the New Zealand species is in the Flora of New Zealand by Dr Lucy Moore (Allan, 1961). Previous classifications of the genus initially divide it on the basis of anther position in relation to corolla scales (see Robertson, 1989 for a review). Moore (Allan, 1961) makes the comment that it is not necessarily a natural division (p. 807), but retains it as the initial division of the genus. Some taxa are recognised on this basis with little else differentiating them. For example, it is the only readily distinguishable character difference between *Myosotis venosa* and *Myosotis forsteri* (Robertson, 1989). *Myosotis lyallii* may be synonymous with *Myosotis elderi* for the same reason (Robertson, 1989).

Grau and Schwab (1982) proposed a new infrageneric classification based on morphological characters of pollen, stigma, corolla scales, and anther tips. They split the genus into two sections, Myosotis and Exharrhena. The Exharrhena section is split into two groups, the discolor group, which contains some European and one African species, and the austral group, which contains the New Zealand taxa. New Zealand is the southern hemisphere centre of diversity for the genus (Winkworth *et al.* 1999). In addition to the New Zealand taxa the austral group contains four other species; two in Australia, one in South America and one in New Guinea.

Floral morphology and pollination biology in New Zealand *Myosotis*

New Zealand *Myosotis* species studied in detail have been found to have different mating types that depend, in part, upon the degree of anther exertion displayed and length of the style in relation to the position of the anthers (Robertson and Lloyd, 1991). Moore (in Allan, 1961) states that "...exserted anthers are little known in the genus outside NZ". The general character of the genus here differs in many respects from the Northern Hemisphere relatives. Several aspects of the reproductive biology of selected taxa have been studied. All species studied so far have been found to be fully self-compatible, but differ in their ability to set seed autonomously (Robertson, 1989). With the exception of *M. pygmaea* var. *minutiflora*, which was not dichogamous, all species studied to date have incomplete dichogamy in the form of protogyny (Robertson and Lloyd, 1991). One species, *Myosotis monroi*, has precocious buds (*sensu* Robertson, 1989), in which stigmas are exserted before the bud opens.

Flowers are hermaphroditic, carried on scorpioid cymes and each is able to produce up to four nutlets. Three main types of flowers are recognized in New Zealand *Myosotis*; tube, funnel and brush blossoms (Robertson, 1989).

Tube blossom: These flowers usually have a narrow corolla tube of variable length. The tube is framed by conspicuous corolla scales, with flat, free corolla lobes (fig 1, left). Filaments are more or less short carrying anthers near the top of the tube, but the degree of inclusion/exertion varies.

Funnel blossom: These flowers have a wider corolla tube that is more or less funnel shaped. The corolla lobes are spreading rather than flat and often fused above the scales giving the flower a funnel shape (fig 1, centre).

Brush blossom: These flowers have a narrow corolla tube, with a rim of conspicuous scales and free flat corolla lobes, but differ from the tube blossom type in having anthers and stigma on long exserted filaments and styles (fig 1, right).

Figure 1: Examples of the three floral forms recognised within New Zealand *Myosotis*. *M. traversii* var. *traversii* (left) has tube blossom flowers, *M. arnoldii* (centre) has funnel blossom flowers and *M. brockiei* has brush blossom flowers. Photos by Bruce Sunnex, Bill Malcolm, and Alastair Robertson.

CONSERVATION

Within the New Zealand group there are a large number of extremely localized taxa, which are often restricted to a single mountain or range, and the genus contains some very rare species. By contrast, other species have very sparse distributions and although they are relatively widespread, they are never common where they occur. Several different abundance and distribution patterns or rarity patterns are evident within the New Zealand taxa. For example *Myosotis forsteri* is found within about three quarters of New Zealand's land area south of latitude 38° S (Landcare Research Herbarium, Lincoln, hereafter referred to as CHR; Allan, 1961). While having a widespread distribution, it is often found as a single individual or as two or three plants in a population and has only occasionally been seen in populations as large as 30. Although never common where it occurs, it is not considered threatened in any way. By contrast, *M. concinna*, an endemic of the Marino Mountains in north-west Nelson, occurs in a very restricted geographic area. Although confined to specific habitats within the alpine zone, it is quite common where it occurs.

A large proportion of the New Zealand taxa in the genus are considered threatened, with 33% featuring on the latest list (de Lange *et al.* 1999), but are often not well enough known in the field or are of uncertain taxonomic status (Molloy and Davis, 1994;

Cameron *et al.* 1995). Priority setting and management decisions for their conservation are impeded by this lack of knowledge. Six of the Nelson taxa are currently listed as threatened in some way. *M. laeta* has been classified as Vulnerable (Cameron *et al.* 1995) but the reappraisal to the newly erected Range Restricted category (de Lange *et al.* 1999) acknowledges recent survey results; *M. brockiei* has been classified as Rare (Cameron *et al.* 1995), I (Insufficiently known) (Dopson *et al.* 1999) and is now classified as Range Restricted (de Lange *et al.* 1999); *M. angustata*, *M. arnoldii* and *M. concinna* were on the local plant list (Cameron *et al.* 1995) and are now classified as Range Restricted (de Lange *et al.* 1999). *M. petiolata* var. *petiolata* has been classified as Insufficiently Known since 1993 (Cameron *et al.* 1993; Molloy and Davis, 1994; Cameron *et al.* 1995; de Lange *et al.* 1999). Due to the large number of threatened species within the genus, the Department of Conservation has assigned high priority to research in the genus, and have part funded this research in order to find out more about this group of rare plants. A report was produced for the Nelson/Marlborough Conservancy of the Department of Conservation (Appendix 1).

The aims of the thesis were to investigate several aspects of rarity in New Zealand *Myosotis*. The reproductive biology of several species with a range of floral morphologies and rarity types were studied. Mating types were examined in order to determine whether pollen limitation was occurring and whether this related to rarity patterns. Reproductive failure and population extinction due to Allee effects is a possible consequence of low population density and size. The influence that local flower density and population size had on reproductive output in out-crossing species was compared with selfing taxa. The effect on population dynamics was considered. Precocious stigma presentation was investigated in a geographically restricted species, *M. monroi*. The effect of precocious bud development and a prolonged initial female phase on pollination success was considered. As the genus contains several different types of rarity and many threatened species, one of the aims of this thesis was to investigate whether certain morphological traits had any association with the range of rarity patterns seen. Species distributions, population disjunctions and local densities were compared with floral morphologies, pollination requirements, potential dispersal aids (seed size and some calyx properties), and life-history traits (e.g. how long lived each species is). Species limits in the *M. forsteri*/*M. venosa* species complex were explored to critique Moore's treatment of the genus (in Allan, 1961). Specifically, the use of anther exertion as an appropriate taxonomic character with which to split the genus was explored.

CHAPTER TWO

Mating types, herkogamy and rarity in six species of New Zealand *Myosotis* L. (Boraginaceae)

Abstract

The reproductive biology of six species of *Myosotis* was studied. New Zealand *Myosotis* species studied to date fall into either one of two mating types based on the degree of herkogamy exhibited by the flowers. Species with flowers that are herkogamous throughout the life of the flower require pollinators to set seed while those that are not herkogamous at some stage during anthesis are able to self-pollinate autonomously without the need of a vector. Details of the mating types operating in six species of *Myosotis* are presented. Hand pollination tests showed all six species are fully self-compatible. The four species with brush or funnel blossom flowers, *Myosotis monroi, M. brockiei, M laeta* and *M. macrantha,* required pollinators to transfer pollen and to set seed. The two tube blossom species, *M. forsteri* and *M. tenericaulis* were able to set seed autonomously without pollinators. These mating types were directly related to the degree of herkogamy displayed. Pollen limitation was found to occur in the four pollinator-requiring species but not in the two autonomous species. The association between mating types and rarity patterns are discussed. Results are examined in the context of theories regarding mating system evolution with respect to New Zealand *Myosotis.*

Introduction

New Zealand *Myosotis* L. (Boraginaceae) is a genus of relatively short-lived, herbaceous species that occurs from the salt turfs of the coastal zone to the fellfields of the high alpine zone. Within the group there are many rare species, encompassing several different types of rarity, and a range of floral forms. Three main types of floral syndrome have been recognized in New Zealand *Myosotis*; tube, funnel and brush blossoms (Robertson, 1989) (Fig. 1, Chapter one). The brush blossom taxa are unique to New

Zealand as Moore (in Allan, 1961) states, "...exserted stamens are little known in the genus outside (New Zealand)". Robertson (1989) has investigated the reproductive biology of six species of New Zealand *Myosotis; M. colensoi, M. forsteri, M. uniflora, M. australis* var. *lytteltonensis, M. pygmaea* var. *minutiflora* and *M. spathulata.* In some species vectors are required for pollination to be carried out. In others, the receptive stigma is in contact with the anthers at least some of the time. A variety of forms of herkogamy and incomplete dichogamy were found which influences the parentage of seeds and represents different stages along the outcrossing-selfing continuum (Robertson and Lloyd, 1991). Species have been found to have different mating types that depend, in part, upon the degree of anther exertion displayed and/or the length of the style in relation to the anthers (Robertson and Lloyd, 1991). A range of mating types from complete autonomy to complete pollinator-dependence is found within the tube-flower group (Robertson and Lloyd, 1991). The species included in this study encompass all three blossom types: the brush, funnel and tube.

An important aspect in the study of rare plants is to understand their reproductive biology. The mating system determines the genetic structure and evolutionary potential of plant populations (Allard, 1975). Wright (1938) discussed the evolutionary importance of breeding system and population size on speciation. Plants with different breeding systems and population structures are under very different selection regimes. In predominantly outcrossing species, individuals that occur in small isolated populations are likely to undergo more rapid evolutionary change than when they occur in large populations (Wright, 1939). Selection that occurs in selfing species on the other hand, is not affected by size or isolation of population and results in groups of basically genetically identical individuals (Wright, 1939).

Herkogamy and Dichogamy

There are two opposing forces at work on hermaphroditic flowers that influence their morphology. On the one hand there is a strong advantage in having the two pollination surfaces in the same position, thereby increasing efficiency of pollen transfer, while on the other hand, there is the opposing need to avoid interference between receiving and exporting pollen (Webb and Lloyd, 1986). Dichogamy, the temporal separation of anthers and stigmas, and herkogamy, the spatial separation of anthers and stigmas are two methods hermaphrodites use to avoid interference during the reproductive season which have the additional utility of improving outcrossing potential (Webb and Lloyd, 1986). The degree of dichogamy and/or herkogamy a flower exhibits influences the parentage of seeds (Robertson and Lloyd, 1991). For species that are fully self-compatible, physical methods such as herkogamy and dichogamy can reduce levels of self-pollination (Garnock-Jones, 1976). There are a range of types of dichogamy and herkogamy described to date (see Lloyd and Webb, 1986 and Webb and Lloyd, 1986). Only herkogamy allows both the receipt and dispatch of pollen during a single insect visit whereas dichogamous blossoms usually require at least two visits (Webb and Lloyd, 1986).

The six species of *Myosotis* with tube blossom type flowers that were studied by Robertson (1989) were assigned to three categories - 'always herkogamous', 'initially herkogamous' and 'never herkogamous' (Robertson and Lloyd, 1991). The flowers of plants always exhibiting herkogamy will require a vector of some sort to achieve pollination and fertilization. Of the *Myosotis* species studied by Robertson (1989), the flowers of those that were 'always herkogamous' exhibited 'approach herkogamy', in which the stigma protrudes beyond the level of the anthers, and is therefore more likely to be contacted first by pollinators, making self-pollination less likely (Robertson and Lloyd, 1991). The species of *Myosotis* studied by Robertson (1989) that were herkogamous at least some of the time, also exhibited incomplete dichogamy in the form of protogyny (presentation of stigma before pollen). Protogyny is not as common as protandry in angiosperms (Lloyd and Webb, 1986). Webb and Lloyd (1986) concluded that protogyny is a more successful means of avoiding self-fertilization than protandry and will more likely be the type of dichogamy that evolves when the avoidance of self-fertilization is the selective force. Therefore, the combination of approach herkogamy and protogynous dichogamy exhibited by *Myosotis* species that require vectors to achieve pollination will improve their chances initially, at least, of receiving outcross pollen.

Rarity patterns

Looking at the New Zealand species in the genus there are several different abundance and distribution patterns evident. Many *Myosotis* species are rare and local, often occurring in single, isolated populations, sometimes in large numbers while others are widespread but never common where they occur. For example *Myosotis forsteri* is found within about three quarters of New Zealand's land area south of latitude 38° S (Allan, 1961). While having a widespread distribution, it is often found as a single individual or as two or three plants in a population and has been seen occasionally in populations as large as 30. By contrast, *Myosotis monroi*, an ultramafic endemic to mountains east of Nelson, New Zealand, occurs in a very restricted area. Although confined to a very small range size, it is locally common where it occurs and is usually found in relatively large populations of more than 500 flowering individuals. In between these two extremes is *Myosotis brockiei*, which has a small range size, but typically occurs in small populations of up to 50 flowering individuals.

The range of rarity types is quite well covered by the genus as are a variety of threat classifications. Of the six species in this study two are listed as threatened in some way. *Myosotis laeta* and *M. brockiei* are classified as Range Restricted (de Lange *et al.* 1999); *M. monroi, M. forsteri, M. tenericaulis* and *M. macrantha* are not considered threatened.

The aims of this chapter are to determine what mating type is operating in each of the taxa by carrying out various pollination experiments. Where some populations are not producing the maximum amount of seed possible, hand pollinations are carried out to distinguish between resource and pollen limitation in the field. Results are examined with regard to rarity patterns and mating type evolution.

Materials and Methods

Study taxa, field sites and treatments

Six species of *Myosotis* were chosen to study their pollination requirements. Flowers are hermaphroditic carried on scorpioid cymes and are each able to produce up to four nutlets.

The species chosen and populations studied were:

Myosotis brockiei L. Moore et M. Simpson

Cobb Valley and Flora Valley, Nelson, New Zealand

Figure 1: *Myosotis brockiei*, Cobb Reservoir, Nelson, New Zealand. Photo by Bruce Sunnex.

This species is confined to a small area centred in the Cobb Valley. Three populations were used for this study, as no single population was large enough to enable all treatments to be carried out. Plants are usually found on ultramafic or limestone outcrops often within forest or at river gorge margins and usually below treeline. All treatments were carried out on separate plants. Flowers are of the brush blossom type. It is currently classified as Range Restricted (de Lange *et al*. 1999). Members of the Acroceridae were observed pollinating flowers at populations near the Cobb Reservoir.

Myosotis laeta Cheesm.

Red Hills Plateau, Richmond Range, Nelson, New Zealand.

Figure 2: *Myosotis laeta*, Red Hills Plateau, Nelson, New Zealand. Photo Alastair Robertson.

M. laeta occurs in tussock and manuka shrubland of the mineral belt, east of Nelson. The population studied is restricted to a relatively small area of the plateau, previously burnt, in amongst regenerating manuka. All treatments were carried out on separate plants. Flowers are of the brush blossom type. It is currently classified as Range Restricted (de Lange *et al.* 1999). Members of the Halictidae were observed pollinating flowers at the Red Hills Plateau population.

Myosotis monroi Cheesm.

Dun Mountain, Bryant Range, Nelson, New Zealand.

Figure 3: *Myosotis monroi*, Dun Mountain, Nelson, New Zealand. Photo Alastair Robertson.

M. monroi is an ultramafic endemic of the mineral belt, which appears sporadically along the ranges east of Nelson, from D'Urville Island in the north through to the Red Hills in the south. At Dun Mountain, populations occur on the exposed, rocky, treeless fellfield, between 880 and 1100 meters. Few other plants are able to tolerate the conditions of the serpentine environment (Lyon *et al.* 1970). All treatments were carried out on separate plants. Flowers are of the brush blossom type. Very little pollinator activity was observed at the Dun Mountain populations. Some rare visits by bumblebees (*Bombus* sp.) were seen. At the Red Hills Ridge, however, populations of which flower about six weeks later in the season, members of the Halictidae, Syrphidae and black scree butterflies were observed frequently visiting flowers.

Myosotis macrantha (Hook. F.) Benth. et Hook. f.

Mt Mytton, Peel Range, Nelson, New Zealand.

Figure 4: *Myosotis macrantha*, Lake Peel, Nelson, New Zealand. Photo Alastair Robertson.

M. macrantha is a South Island endemic occurring mainly west of the divide (Allan, 1961). It occurs on a range of geological substrates but is quite particular regarding altitude, aspect and habitat. The Mt Mytton population is restricted to alpine, limestone outcrops, which are surrounded by tussock grassland. Due to site conditions and plant size three pollination treatments were carried out on the same plant, (i.e. self, cross and pollinator excluded) while the control plants were separate. Flowers are of the funnel blossom type. Robertson (1989) observed moths carrying out pollination at a population of this species. Small Diptera were observed in flowers at the Mt Mytton population and were also observed in flowers at Lake Peel.

Myosotis forsteri Lehm.

Myttons Creek, Cobb Valley, Nelson, New Zealand.

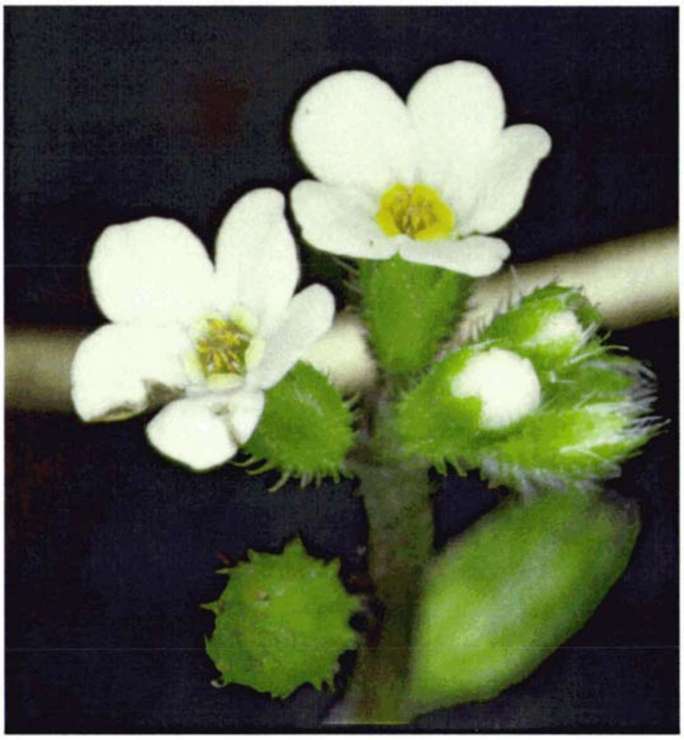

Figure 5: *Myosotis forsteri*, Cobb Valley, Nelson, New Zealand. Photo Alastair Robertson.

This species is found in a variety of habitats and geological substrates in forest or at forest margins. This species has been found to be 'initially herkogamous' when the reproductive biology of plants from a Central Otago population was studied (Robertson and Lloyd, 1991). As plants self themselves, self-pollinations were not carried out. All other treatments were carried out on separate plants. Flowers are of the tube blossom type. No insects were seen visiting flowers at any of the populations.

Myosotis tenericaulis Petrie

Flora Limestone, Flora Valley, Nelson, New Zealand.

Figure 6: *Myosotis tenericaulis*, Flora Valley, Nelson, New Zealand. Photo Bruce Sunnex.

These plants occur in a variety of habitats but are often found under limestone overhangs and at cave entrances. Anthers and stigmas are in close contact during anthesis, which indicates self-pollination will occur. Therefore the selfing treatment was not carried out. All other treatments were carried out on separate plants. Flowers are of the tube blossom type. No insects were observed visiting flowers.

Treatments

Individual plants were either caged to exclude pollinators while flowers were in bud or left open in the field. As and when flowers opened treatments were carried out.

For all species except *M. macrantha*, plants were assigned to one of four treatments:

1. Pollinator-excluded (caged) and self-pollinated
2. Caged and cross-pollinated
3. Caged and unmanipulated
4. Open and unmanipulated = control

Since *M. macrantha* usually has multiple inflorescences, I decided to place treatments 1 to 3 all within one plant, but a different plant was used for treatment 4, as I was worried that the bags used in the other treatments might deter flower visitors. Flowers of the two selfing species, *M. forsteri* and *M. tenericaulis*, assigned to the cross-pollination treatment had anthers pinched off with forceps prior to anthesis. It was not necessary to remove anthers in the remaining species, as they do not contact stigmas.

Two to three weeks after treatment, seed set was counted. The developing seeds are easily observed in maturing calyces.

Herkogamy

Herkogamy was measured in the field using calipers to the nearest 0.1 mm. The distance between the stigma and the centre of a randomly chosen anther was measured on flowers of each species.

Data analysis

In each case seed set was expressed as a proportion set out of the total possible, then arcsine transformed to better fit the assumptions of the tests performed. ANOVAs were performed on seed set results to compare treatments for each species. In *M. macrantha*, where the three hand-treatments were performed within a plant, I performed two analyses. In the first, I used each plant as a block and compared only the three treatments these plants received. In the second analysis, I pooled the hand-cross and hand-selfed results and compared these to the open-pollinated plants. Multiple comparisons were then carried out to determine which treatments were significantly different.

Using *S-Plus*, box plots were generated from the seed set data to graphically show the results from each treatment and to examine variation. The box plots depict the median (central line in each box), 25% and 75% quartiles (upper and lower limits of each box), the maximum point within 1.5 times the interquartile range from the quartiles (indicated by the whiskers), and the outliers (i.e., points greater than 1.5 times from the quartiles) indicated by lines outside whiskers.

Results

Pollination requirements

All species with brush or funnel blossom type flowers were unable to set seed when pollinators were excluded (Fig. 7; Table 1). *Myosotis brockiei, M. laeta, M. monroi* and *M. macrantha* set very little seed when exclusion caged, and therefore require a pollinator to achieve much seed set. The two species with tube blossom flowers (*M. forsteri* and *M. tenericaulis*) were able to set high levels of seed when pollinators were excluded and do not require a pollinator to achieve seed set (Fig. 8; Table 1).

Three populations were used for the study of *M. brockiei*. Therefore the population was included as a block in the ANOVA, and there was a small but significant population effect (p=0.01; Table 1) as well as a treatment difference but no population by treatment interaction.

All six species in this study are fully self-compatible, at least to the seed set stage. Flowers set as much seed when pollinated with self or outcross pollen. I did not test viability or vigour of seeds produced from self compared with outcross pollinations, therefore can only state with certainty that these flowers are self-compatible to the seed production phase.

Pollen limitation

The four vector-requiring species, *M. brockiei, M. laeta, M. monroi* and *M. macrantha*, all showed significant differences between seed set by unmanipulated plants and hand-pollinated plants (Fig. 7; Table 1). Flowers of unmanipulated plants set significantly lower levels of seed than flowers that had been hand pollinated, which suggests pollen limitation is occurring. The two autonomous species, *M. forsteri* and *M. tenericaulis*, were never pollen limited.

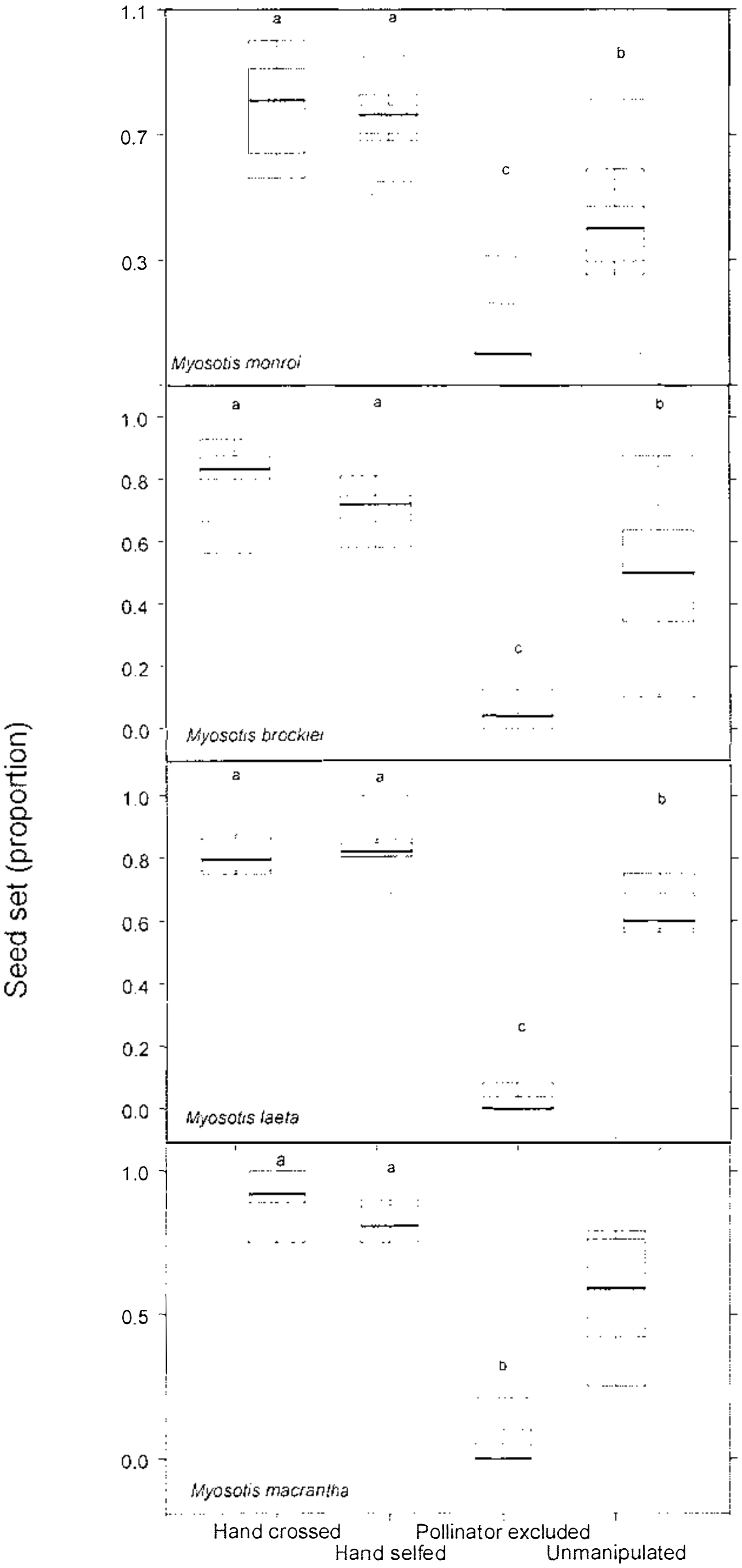

Figure 7: Box plots of resultant seed set from the pollination treatments carried out on each pollinator-requiring species. Treatments that, within a panel, share a letter are not significantly different ($p<0.05$).

Table 1: Results of the analysis of the pollination treatments.

Species	Factor	df	F value	*P*
Myosotis brockiei	Population	2	3.48298	0.0417
	Treatment	3	18.36173	<0.0001
	Pop:trt	6	0.25232	0.9550
	Residuals	35		
Myosotis laeta	Treatment	4	62.30722	<0.0001
	Residuals	35		
Myosotis monroi	Treatment	3	62.94387	<0.0001
	Residuals	44		
Myosotis macrantha	Plant	6	0.47704	0.8071
(within-plant treatments)	Treatment	2	87.97845	<0.0001
	Residuals	7		
(among-plant treatments)	Treatment	1	17.61343	0.0012
	Residuals	12		
Myosotis forsteri	Treatment	2	0.1494216	0.8621
	Residuals	22		
Myosotis tenericaulis	Treatment	2	0.1671392	0.8472
	Residuals	21		

There is a greater variance in seed set by vector-requiring species than autogamous species when plants are open pollinated without manipulation. Variation decreases for these species when flowers are caged to exclude pollinators. There is no difference between treatments in variation for the autonomous species. The greater variance in seed set by unmanipulated plants suggests individual plants are subject to differing degrees of pollen limitation.

The results for the experiments carried out on *Myosotis macrantha* were analysed differently from the other species. The cross, self and pollinator-exclusion treatments were carried out on the same plant while the control data were collected from separate plants. The analysis was carried out in two parts; firstly, the three treatments carried out on each plant were analysed separately in order to test for any plant effects (results of which are represented by letters above the box-plots). There was not a plant effect. Secondly, as there was no significant difference between cross- and self-pollination treatments, the results were pooled to test hand versus open pollinated seed set for the difference (which was significant).

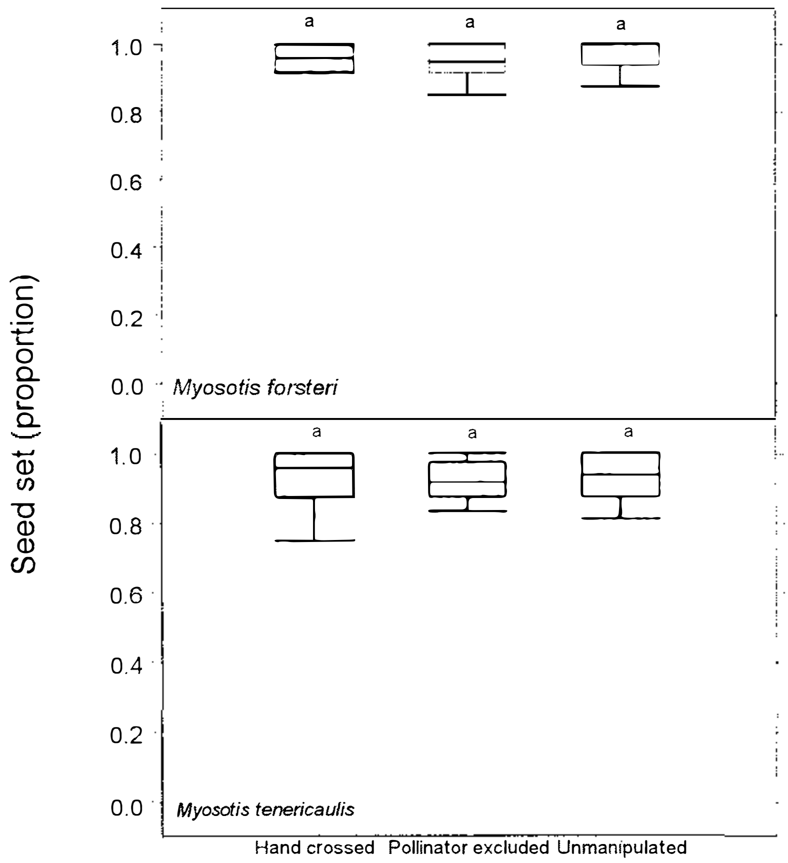

Figure 8: Box plots of resultant seed set from the pollination treatments carried out on each of the autonomous species. Letters indicate significant differences found in statistical analyses ($p<0.05$).

Herkogamy and selfing rates

These results show that herkogamy has a close relationship to selfing rates in these species. In order to look at this I have tabulated the herkogamy and selfing rates for those taxa for which it has been measured (Table 2). The autonomous selfing rate of species (seed set rate by flowers from which pollinators have been excluded, expressed as a proportion) is closely related to the degree of herkogamy (spatial separation of stigma and anthers) (Fig. 9). Poor seed set occurs when the herkogamy is greater than 0 mm.

Table 2: Herkogamy and mean seed production in pollinator excluded flowers

Species	Herkogamy (mm)	Selfing rate (proportion)
Myosotis brockiei	3.755	0.042
Myosotis macrantha	3.556	0.042
Myosotis colensoi *	3.52	0
Myosotis petiolata	3.5	0.02775
Myosotis monroi	1.688	0.039
Myosotis laeta	1.378	0.02
Myosotis venosa	0.7	0.125
Myosotis forsteri (Nelson)	0	0.95
Myosotis tenericaulis	0	0.92
Myosotis 'lytteltonensis' *	0	0.51
Myosotis forsteri (Central Otago) *	0	0.8575
Myosotis spathulata *	0	0.9175
Myosotis 'minutiflora' *	0	0.9825

* data from Robertson (1989)

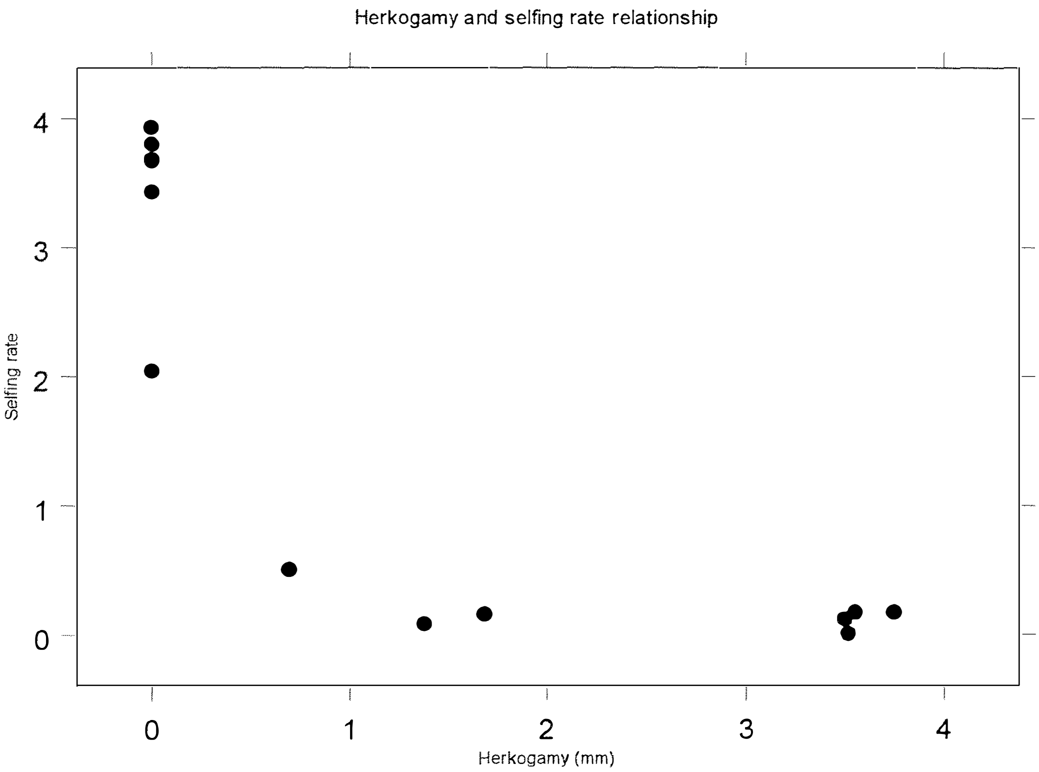

Figure 9: Herkogamy (mm) graphed against autonomous selfing rates (selfing rate = seed set/flower when pollinators are excluded) for thirteen species for which we have this data.

Discussion

Mating types

While several floral forms exist within the New Zealand taxa of *Myosotis*, species studied to date separate into one of two distinct mating types; those able to set seed themselves (autonomous) and those unable to set seed without a vector (non-autonomous). These types can be predicted by measuring herkogamy or spatial separation between anthers and stigma. Those species that are 'always herkogamous', (*sensu* Robertson and Lloyd, 1991), require vectors to achieve pollination. Those that are 'initially herkogamous' or 'never herkogamous' (*sensu* Robertson and Lloyd, 1991) are able to self-pollinate autonomously.

The ease by which herkogamy can be measured in the field as opposed to conducting the type of detailed experiments carried out in this study could be made more use of in determining pollination requirements for other rare *Myosotis* species. Simply measuring herkogamy in a range of flowers can determine the degree of autonomous selfing a particular species may experience. The population dynamics of species will be affected by the level of autonomous selfing operating, knowledge of which, can assist in predicting a populations' vulnerability to extinction. The density dependence for reproduction has been found to occur in all pollinator-requiring species studied so far (see Chapter three). These species were pollen limited in low-density patches. A small, thinly scattered population of a species capable of self-pollination is not necessarily a sign of threat, but could provide a warning for a pollinator-requiring species.

Results for *M. brockiei* showed there was also a significant population effect on seed set. This was the only species where more than one population was used to collect this data. In another study, data were collected on seed set by open pollinated plants from several populations and although local density had strong effects on seed production, there was no population size effect (see Chapter three). Two of the three sites of *M. brockiei* were on ultramafic outcrops, which were quite similar in terms of substrate and aspect, while the other population sampled from was on tertiary limestone in a very different habitat.

Self-compatibility and pollen limitation

There was no significant difference in seed set by flowers receiving self- versus cross-pollination treatments for any of the species in this study. All species of New Zealand *Myosotis* studied to date have been found to be fully self-compatible, but differ in their ability to set seed autonomously. Self-compatibility is thought to be the usual state for New Zealand genera (Godley, 1979) although the true level of self-incompatibility in our flora is still not very well known (Webb and Kelly, 1993). Other genera for which self-compatibility is the usual state are *Parahebe* (Garnock-Jones, 1976), *Cardamine* (Pritchard, 1957), *Epilobium* (Brockie, 1959), *Cotula* (Lloyd, 1972) *Gentiana* (Webb and Littleton, 1987) and *Peraxilla* (Robertson *et al.* 1999). Self-incompatibility has been confirmed in only a few New Zealand plants e.g. *Pseudowintera*, *Corokia*, *Cordyline* and *Discaria,* but it is thought that not enough is known to assess true levels of self-incompatibility in the New Zealand flora (Webb and Kelly, 1993).

For non-autonomous species, seed set by hand-pollinated flowers was found to be significantly higher than seed set by unmanipulated flowers. In addition, there was a large degree of variation in the amount of seed set by the unmanipulated flowers compared to those hand pollinated. Hand-pollinations show that the variation in seed set by these species is not due to resource limitation as flowers given hand pollination treatments were invariably able to average significantly more seed than that set by unmanipulated flowers. The variation in seed set was not due to inbreeding depression, as there was no significant difference between self and cross pollination in terms of seed set; inbreeding depression is therefore not seen at least at the level of seed set. I can conclude that the significant difference in seed set between hand-pollinated and unmanipulated flowers in non-autonomous species is due to pollen limitation. Pollen limitation and the resultant lower seed output can cause problems for plant populations when recruitment is unable to keep up with mortality. There is a widely held view among population biologists (e.g. Crawley, 1990) that plant population growth is usually not limited by seed production but by microsite availability. Therefore lower seed output does not affect demography. However, Turnbull *et al.* (2000) have reviewed the literature on seed limitation studies and around half of those reviewed showed evidence of seed limitation.

There is a possible third mating type operating in New Zealand *Myosotis*. A preliminary study carried out on the rare, southern New Zealand endemic, *M. oreophila*, in the northern Dunstan Mountains, found both pollination systems operating within the same plants within the population (Appendix 2). Some flowers were able to set seed autonomously while others required a vector to set seed. This was due to variation in style height. This was also observed at Mount Arthur, in a population of the Nelson endemic, *M. angustata*. In the initially herkogamous *M.* 'lytteltonensis', the large variation in pistil length can result in both selfing and non-selfing phenotypes within the same population (Robertson, pers. comm.). Further study of these taxa is required to determine more about this third type of syndrome in which both selfing and vector-requiring morphologies coexist in the same population. Populations of plants with mixed mating systems can be at an advantage in certain circumstances. In changeable environments, as population size varies and therefore possible mates, so too can pollinator availability such that when population size is low, selfing individuals are selected for (Karron, 1991). A positive feedback loop can establish, as seed production by self-pollinating individuals is generally high and constant, recruitment chances are improved by higher seed production that ultimately results in an increase in population size. At times when population size is large enough to attract pollinators, the presence of outcrossing phenotypes in the population can then be beneficial, particularly if inbreeding depression is occurring. In fact the high and consistent seed production of self-fertile plants could allow for considerable selection in the offspring without affecting demography at all (Luitjen *et al.* 1998). Wright (1939) considered such a situation to be advantageous to populations in which reproduction is predominantly selfing. He considered occasional crossing could allow for effective selection by genotypes in a "continuously restored field of variability" (Wright, 1939). The mixed mating strategy therefore allows populations to persist in difficult circumstances and then take advantage of more favourable conditions when they occur.

Habitats, distributions and rarity

These results are in contrast to other studies of breeding systems in relation to the environment in which they live. Vector-requiring species usually have the more lowland, widespread distributions by comparison with their autonomous counterparts, which tend to have alpine and restricted distributions (e.g. Lloyd, 1965; Arroyo, 1973; Garnock-Jones, 1976; Webb and Pearson, 1993). Selfing is expected to be more frequent in unpredictable environments due to pollinator paucity and small population size (Lloyd, 1980). Explanations were provided in terms of selfing (considered the derived condition) evolving in response to the late start or to reduced levels of insect activity during cold, windy weather and the short alpine summer (Garnock-Jones, 1976; Webb and Pearson, 1993). In New Zealand *Myosotis* however, the selfing taxa generally have more lowland and widespread distributions (e.g. *M. forsteri*), while the alpine and geographically restricted taxa tend to be the vector-requiring ones (e.g. *M. monroi*). Species that require a vector to achieve pollination have all been found to exhibit inverse density-dependence for reproduction (Chapter three). These species require population densities at levels that attract pollinators. It is more difficult for these taxa to establish new populations and colonise new territory. Therefore, we expect to see those species occurring with more geographically restricted distributions. For these species, individuals are more successful at reproduction when in dense patches rather than sparse ones. Therefore, we might expect those species to have larger, more abundant local populations. This provides a mechanism by which the observed patterns of density and distribution are maintained and could only maintain such patterns if seed production is important to the demographics of such populations (see Chapter three). If a propagule from an autonomous type disperses into a new area, a plant can grow, reproduce and colonise a new area by the germination of one seed. On the other hand, if such a propagule from a non-autonomous type disperses into a new area, grows, flowers and dies without pollination occurring it may never set seed. Therefore colonisation will not occur. By this means, individuals with autonomous morphologies can become more widespread and colonise new ground.

Mating system evolution

Within any taxon exhibiting an array of breeding systems from obligate outcrossing through to autogamy, the direction of evolution is generally considered to go from outcrossing to selfing (e.g. Arroyo, 1973; Garnock-Jones, 1976; Barrett *et al.* 1996). This implies that the ancestors of the New Zealand *Myosotis* taxa are or were outcrossing. However, most species that establish on remote islands tend to be self-fertilizing species as such individuals can start a reproducing colony by the arrival of just one individual (Lloyd, 1980; Pannell and Barrett, 1998). Since much of the extant flora of New Zealand, including *Myosotis*, is thought to have arrived via recent long distance dispersal (e.g. Winkworth *et al.* 1999), selfing is the more likely ancestral state for these taxa. While a large percentage of most island floras is made up of self-fertilizing taxa, New Zealand does not follow this trend and has a high frequency of unisexuality among its flora (Lloyd, 1980). This is thought to be due to subsequent speciation that has occurred into new environments with outcrossing types evolving secondarily (Lloyd, 1980). Therefore the ancestral state for New Zealand *Myosotis* is likely to have been a self-fertilizing species. The full range of mating types observed occurs within one floral morphological class, i.e. the tube (Robertson and Lloyd, 1991). This is due to variation in style and filament lengths, indicating plasticity for these traits exists within the genomes of these taxa. Studies in the Polemoniaceae, which comprises a range of taxa with a variety of reproductive modes, show phylogenetic constraints do not limit opportunities when ecological conditions demand shifts in pollination and mating systems even though autogamy does tend to occur terminally in the phylogeny (Barrett *et al.* 1996). As brush blossom floral forms are not found outside New Zealand, this floral form has likely evolved here. As filament length is the main feature of brush blossom flowers that distinguishes them from tube blossoms and plasticity for filament and style length are known, then brush blossom has possibly evolved from the tube blossom type.

The current distributions of the New Zealand species of *Myosotis* tend to support the theory that outcrossing types have evolved secondarily. The brush blossom taxa, in particular, have very restricted distributions. The self-fertilising species tend to have the widespread, lowland distributions, while the vector-requiring species tend to have more restricted distributions.

CHAPTER THREE

Allee effects in plant reproductive performance: Local density, population size, rarity and reproductive success in natural populations of *Myosotis* L. (Boraginaceae)

Abstract

Reproductive failure due to Allee effects is a possible consequence of low population density and small population size. Data were collected from natural populations of five species of *Myosotis* L. (Boraginaceae) with different pollination requirements and rarity patterns in the Nelson region of New Zealand. Seed set per flower was measured in populations of varying density and size. For pollinator-requiring species, *Myosotis monroi*, *M. macrantha*, *M. laeta* and *M. brockiei*, local population density had strong effects on seed set, while population size had no effect. These plants were always pollen limited in low-density patches. For a self-fertile species, *M. forsteri*, seed set was always high and unaffected by either local density or population size and pollen was never limiting. These results indicate reproductive success in pollinator-requiring species of *Myosotis* is subject to Allee effects and these effects occur at a very local scale. This density-dependent process can explain how the different patterns of density and distribution observed are maintained for each species. These results have implications for the assessment and management of threatened *Myosotis* species in particular and rare plants generally. It is essential to know the pollination requirements and levels of density dependence for reproduction both for the assessment of threat and for determining management strategies for threatened plants.

Introduction

A central issue in conservation biology concerns management of populations of threatened species. Species with small populations are thought to have a greater chance of becoming extinct than those with large populations (e.g. Mace and Kershaw, 1997). However, there is still a large amount of variation in probability of extinction predictions

(Gaston, 1994) and there are some well documented cases of small populations persisting in very low numbers, for example some New Zealand birds (Craig, 1991), which are at odds with the theoretical projections. Threatened plant populations are represented by several different types of rarity (*sensu* Rabinowitz, 1981). Some species are known only from a single site, such as the extreme example of the New Zealand plant *Tecomanthe speciosa*, known only as a single individual in the wild, while others occur in typically small but widely scattered populations, such as *Carex uncifolia* (de Lange *et al.* 1999).

Due to the large number of threatened plant species worldwide, their conservation will have to be achieved largely *in situ* (Ellstrand and Elam, 1993). Determining critical thresholds in processes essential to population persistence, such as levels of density dependence for reproduction, is an important part of ensuring conservation efforts are effectively targeted and can contribute to our understanding of plant population dynamics. Plants make ideal study subjects to research the effects of local density and population size as such variables can easily be measured.

It is important, initially, to identify the breeding system for the assessment of threat status of any rare plant (Hamrick *et al.* 1991). Plants with different breeding systems are likely to have different population structures, sizes and degrees of density dependence. Self-fertile species can grow, reproduce and colonise a new area by the germination of just one seed. They do not require the presence of others to successfully reproduce and persist. Therefore a small population size of low density is not necessarily a sign that the population is threatened. On the other hand, self-incompatible plants or those that require a vector to achieve pollination need local population densities at levels that ensure a supply of cross pollen and/or attract vectors. Population density and outcrossing rates are likely to be positively correlated (Karron *et al.* 1995). Therefore, to be successful, they probably need population sizes considerably larger and denser than selfing species.

The generally accepted, textbook theory on density dependence maintains that there is an upper density limit (the carrying capacity) above which population growth is adversely affected by over-crowding, competition and/or increased predation (e.g. Begon and Mortimer, 1986). What happens at lower than optimal densities, however, is not well understood and the various models in current usage show a variety of possible outcomes as density decreases (Kunin, 1997a). In his review on density dependence in insect-plant interactions, Kunin (1997a) stresses the importance of understanding the behaviour of populations at low densities to enable us to focus our conservation efforts productively on appropriate management techniques.

Levels of density dependence will vary with the pollination requirements of species. For animal pollinated plants reproductive success is likely to depend on density and population size. For populations of these species, plants growing at low local density are more likely than those growing at high density to be pollen-limited in their reproductive success (Kunin 1992, 1993). These species may find it difficult to colonise new ground and therefore be less likely to be found in small populations. Small, isolated populations would be less likely to persist if unable to attract pollinators. This inverse density-dependent process, which is also known as the 'Allee effect' (Allee, 1951) occurs where rates of reproduction decrease disproportionately in response to decreasing population density or size. Several researchers have studied Allee effects on reproductive success from natural populations (e.g. Silander, 1978; Kunin, 1992), experimental populations (e.g. Feinsinger *et al.* 1991; Groom, 1998; Kunin, 1993) and theoretically (e.g. Dennis, 1989; Kunin and Iwasa, 1996; Amarasekare, 1998). Generally, for self-incompatible plants, reproductive success decreases as local population density decreases. Population size, which is often correlated with and sometimes not differentiated from local density (Gaston, 1994), would be expected to also have a similar effect on reproductive success. Several studies have found such population size effects on fruit and seed set when comparing small to large populations (e.g. Aizen and Feinsinger, 1994; Ågren, 1996). However, there are also several studies that have found population size had no effect on reproduction (Van Treuren *et al.* 1993; Kunin, 1997b; Molano-Flores *et al.* 1999). When local density and population size have been differentiated, results have shown very different effects on species interactions and population dynamics (Van Treuren *et al.* 1993; Kunin, 1997b; Molano-Flores and Hendrix, 1999). Yet the concept that small populations face increased extinction risk is an integral component of threat assessment and conservation management. Understanding small population dynamics is crucial in our effort to conserve at risk populations.

I examined the effects of population density and size on seed set by flowers of five species of *Myosotis* (L.) (Boraginaceae). All are short-lived, fully self-compatible, more-or-less rare herbs endemic to New Zealand. For these species I wanted to test the hypotheses that the variation in seed set found in natural populations could be due to local density and population size.

Material and Methods

Study taxa

Myosotis L. (forget-me-nots) is a cosmopolitan genus of the Boraginaceae and has a worldwide temperate distribution. New Zealand is the Southern Hemisphere centre of diversity for the austral group of the genus (Grau and Schwab, 1982; Winkworth *et al.* 1999). In addition to the New Zealand taxa, the austral group contains four other species; two in Australia, one in South America and one in New Guinea. Within New Zealand there are 43 named species and varieties (Allan, 1961) but an additional 25 tag named entities exist (Druce, 1993) and the genus requires revision. The New Zealand species in the genus display a wide variety of rarity types with about 33% (including tag-named taxa) considered threatened in some way (de Lange *et al.* 1999). This has contributed to the taxonomic difficulties as they are rarely encountered in the field and are simply not that well known.

Within the New Zealand species, several different abundance and distribution patterns are evident. Many *Myosotis* species are rare and local, often occurring in single, isolated populations, sometimes in large numbers while others are widespread but never common where they occur. This provides an opportunity to study local abundance and distribution patterns or rarity patterns within a single genus. For example *Myosotis forsteri* is found within about three quarters of New Zealand's land area south of latitude 38° S (Allan, 1961). While having a widespread distribution, it is often found as a single individual or as two or three plants in a population and has been seen occasionally in populations as large as 30 individuals. Although sparsely distributed, it is not considered threatened in any way. By contrast, *M. monroi*, an ultramafic endemic to mountains east of Nelson, New Zealand, occurs in a very restricted area. Although confined to a very small range size, it is locally common where it occurs and is usually found in relatively large populations of more than 500 individuals.

The range of rarity types is quite well covered by the genus as are a variety of threat classifications. Of the five species in this study two are classified as threatened in some way. *M. laeta* is classified as Vulnerable (Cameron *et al.* 1995) and Range Restricted (de Lange *et al.* 1999); *M. brockiei* is classified as Rare (Cameron *et al.* 1995), I (Insufficiently known) (Dopson *et al.* 1999) and Range Restricted (de Lange *et al.* 1999); *M. monroi*, *M. forsteri* and *M. macrantha* are not considered threatened.

Flowers are hermaphroditic, carried on scorpioid cymes and each is able to produce up to four nutlets. Species have been found to have different mating types that depend on the length of the style in relation to the position of the anthers and length of the filaments (Robertson and Lloyd, 1991). Moore (in Allan, 1961) states that "...exserted anthers are little known in the genus outside (New Zealand)". I have studied several aspects of the reproductive biology of selected taxa. All species studied to date have been found to be fully self-compatible, but differ in their ability to set seed autonomously (Robertson, 1989; Chapter two). While several floral morphologies exist within the genus, the species studied to date separate into one of two distinct mating types; those able to set seed themselves (autogamous) and those unable to set seed without a vector (non-autogamous). These types can be distinguished by measuring herkogamy (the spatial separation between anthers and stigma) (Robertson and Lloyd, 1991). In the wild in the pollinator-requiring species, seed set by unmanipulated flowers is usually quite variable (Chapter two).

For this study I have concentrated on five species with a variety of abundance and distribution types and both mating types (Table 1). All species are endemic to New Zealand. *Myosotis forsteri*, the only autogamous species in the study, is widely distributed and is everywhere sparse. The other four species are non-autogamous and cannot set seed when pollinators are excluded (Chapter two). *M. macrantha* occurs throughout the South Island (42 - 46 ° S). Although particular with respect to aspect and habitat, it is usually locally common where it occurs. *M. monroi* is found on an ultramafic mineral belt that extends from D'Urville Island in the north to the Red Hills ridge in the south (40.8 - 41.5 ° S). Although range restricted it is very common where it occurs on the weathered, treeless fellfield of the mineral belt. *M. laeta* is known from two locations within the same mineral belt, at the southern end on the Red Hills Plateau where it is very common and on the lower flanks of Mt Starveall where known populations are much smaller. This species was studied at one site only. *M. brockiei* usually occurs in small populations and is found in a very restricted area geographically, centred in the Cobb Valley, Nelson (14 km^2 at about 41° south). It is a basicole as it occurs on both ultramafic outcrops as well as on limestone.

Table 1: Summary table of the species distributions, typical abundance, herkogamy and mating type

Species	Range size	Population sizes	Herkogamy	Pollination type
Myosotis forsteri	Widespread	1-38	0	Autogamous
M. macrantha	South Island	9-1000	3.6 mm	Non-autogamous
M. monroi	Restricted (Bryant Ecological District	22-2000	1.8 mm	Non-autogamous
M. laeta	Restricted (Bryant Ecological District)	24-2000	1.4 mm	Non-autogamous
M. brockiei	Restricted (Cobb Valley – Mount Arthur Tablelands)	3-90	3.2 mm	Non-autogamous

Data on abundance were gathered from 1997-1999. Distribution were obtained from the most recent treatment in Allan (1961) and from herbaria records (CHR[2], WELT[3], WELTU[4] and AK[5]). These are summarised in Table 1.

Local density and population size effects on seed set - sampling procedure

Seed set counts were made for plants growing in a range of densities and population sizes. The number of seed set by each flower was made as a count (0 – 4), and then converted to a proportion of ovules set by each plant. Local density was measured as number of inflorescences in a three-meter radius around the focal plant for which seed set counts were made. Population or patch size was counted as number of adults (flowering plants) in the population or patch.

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[2] CHR: Landcare Research Herbarium, Lincoln

[3] WELT: National Museum Herbarium

[4] WELTU: Victoria University of Wellington Herbarium

[5] AK: Auckland Museum Herbarium

Pollen limitation experiment

Areas where *Myosotis* plants are less dense might be less suitable for the species than more densely populated areas and plants could be setting less seed for that reason. To check this, pollen supplementation was carried out on flowers of plants in a representative range of densities to discriminate between resource and pollen limitation. Pollen from neighbouring plants was applied by hand to stigmas. Control flowers were left unsupplemented on the same plant. The resultant seed set was compared.

Data analysis

Three analyses were carried out. The seed set data gathered were converted into a proportion (of the total possible) and arcsine square root transformed. Firstly, the proportions of seed set by unmanipulated flowers were fitted against local flower density, using a Generalised Linear Model (GLM). Secondly, the same analysis was carried out on the seed set by flowers that had been hand pollinated, which were also fitted against local flower density. Thirdly, to test for population size effects, the least squared means obtained from the first analysis for each population were then correlated against population size, using a GLM.

These analyses were all performed using *S-PLUS* version 4.5 (MathSoft Inc. 1998).

Results

Myosotis brockiei, Myosotis macrantha, Myosotis monroi and *Myosotis laeta*

Local density had strong effects on seed set in natural conditions in all four species and there were population effects for all species with more than one study population (Fig. 1, Table 2). *M. laeta* was studied at just one population so population size effects could not be tested for. Populations varied significantly, but the variation was not due to population size. When means for each population were adjusted for local density the population effect disappeared (Fig. 2, Table 3). Local density had no effect on seed set by pollen-supplemented flowers (Fig. 1, Table 2). Two of the species, *M. macrantha* and *M. laeta*, had low seed set results by some of the pollen-supplemented flowers at low flower density sites (Fig. 1).

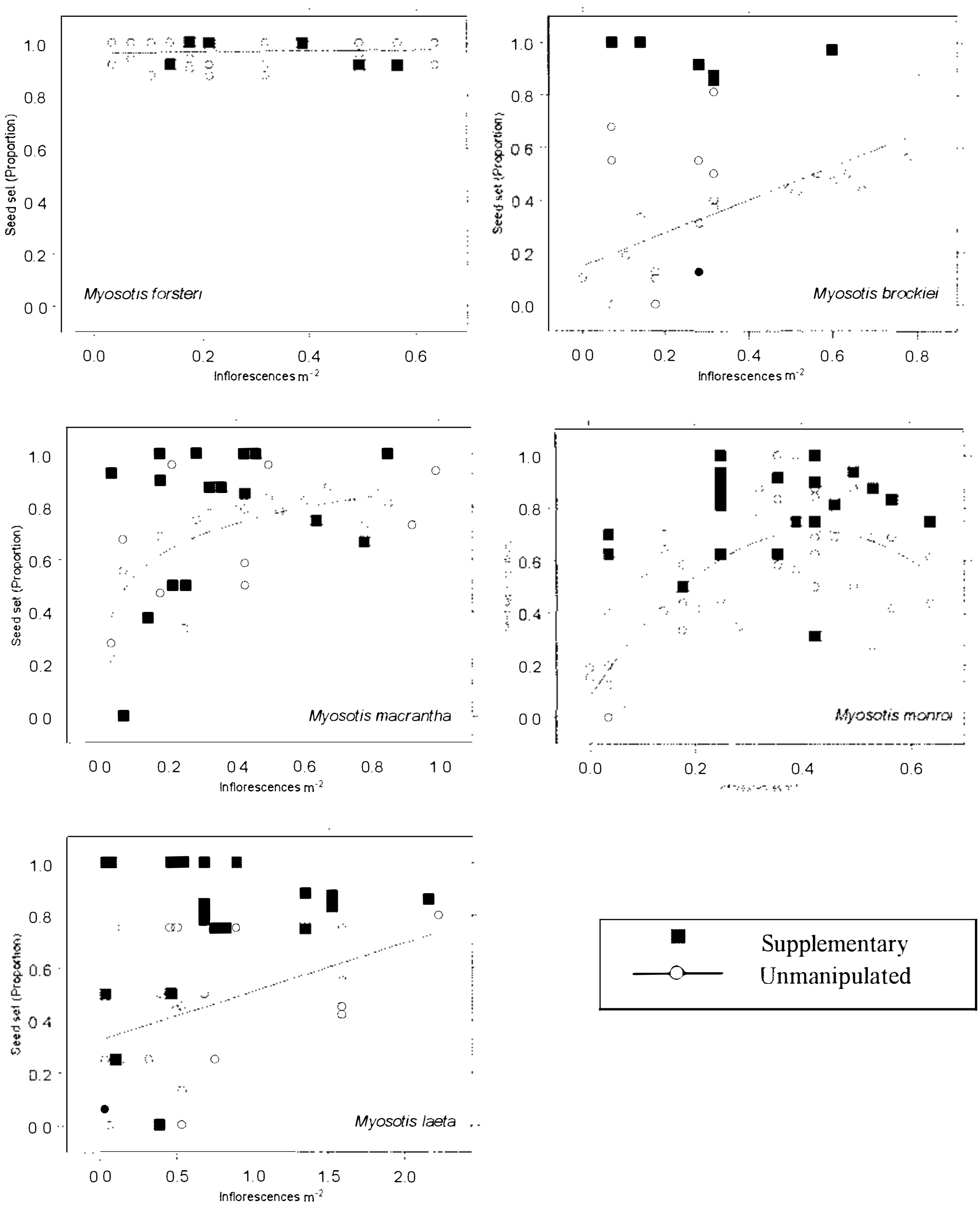

Figure 1: Local flower density effects on seed set by unmanipulated flowers and pollen supplemented flowers graphed for each species *M. forsteri*, *M. brockiei* and *M. laeta* had linear curves fitted. *M. macrantha* had a $\log_{10}$ curve fitted. *M. monroi* had a polynomial curve fitted. Each data point represents the mean seed set for each plant. All supplementation flowers had paired unmanipulated flowers seed production recorded. Lines are fitted to the unmanipulated points. See Table 2 for details of the statistics.

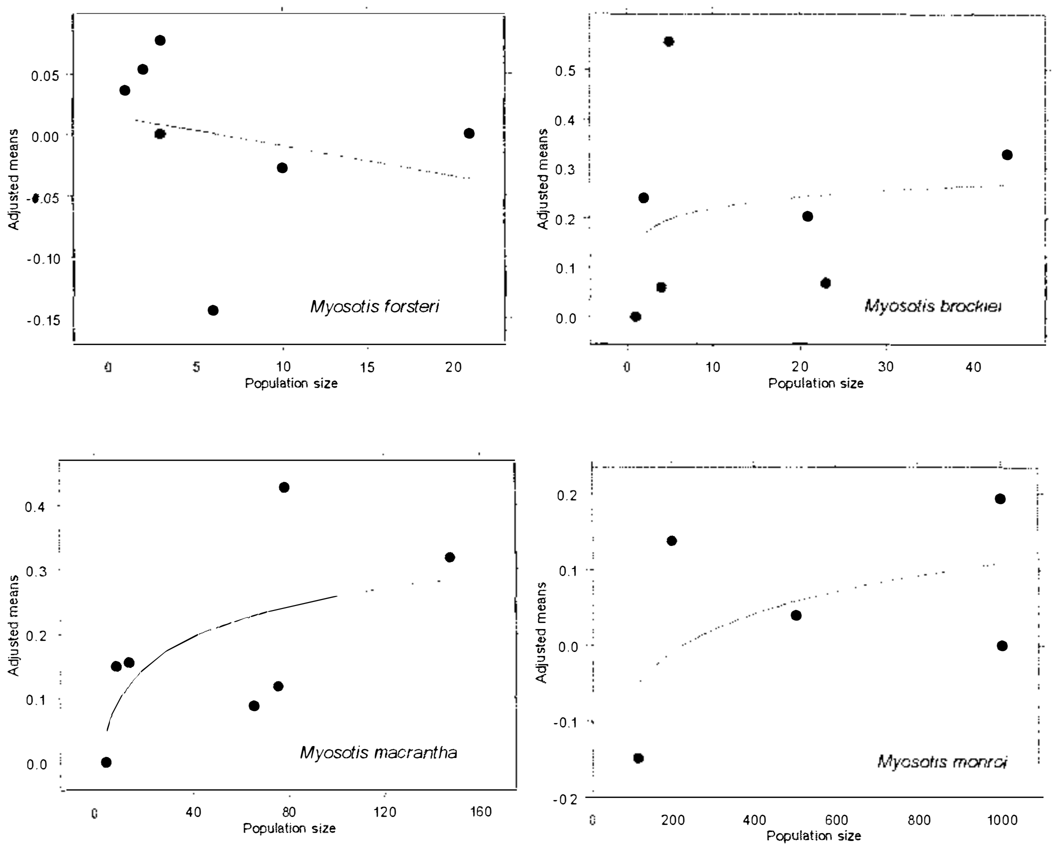

Figure 2: The relationship between population size and seed set by flowers on unmanipulated plants using means from each population. Means are adjusted for local density. None of these relationships were significant.

Myosotis forsteri

Local density had no effect on seed set in natural conditions and there was no population effect (Fig 1, Table 2). When means for each population are adjusted for local density there was no population effect (Fig. 2, Table 3). Local density had no effect on seed set by pollen-supplemented flowers (Fig. 1, Table 2).

Table 2: Density effects on seed set in natural conditions and with pollen supplementation of flowers

Species	Factor	Df	F value	P	% explained
Myosotis forsteri	Population	6	0.5353	0.7758	12.23
Natural conditions					
	Flower density	1	0.0361	0.8509	0.14
	Residuals	23	0.0514		
Pollen supplementation	Flower density	1	0.929	0.3897	18.85
	Residuals	4			
Myosotis brockiei	Population	6	2.2827	0.0791	29.51
Natural conditions					
	Flower density	1	13.7149	0.0015	29.55
	Residuals	19			
Pollen supplementation	Population	2	17.65155	0.0536	94.46
	Flower density	1	3.07135	0.8144	0.19
	Residuals	2			
Myosotis macrantha	Population	6	2.2748	0.0736	26.43
Natural conditions					
	(log$_{10}$)flower density	1	15.9817	0.0006	30.96
	Residuals	22			
Pollen supplementation	Population	3	1.076833	0.3907	15.91
	Flower density (log10)	1	0.074302	0.1014	15.14
	Residuals	14			
Myosotis monroi	Population	4	3.12735	0.0253	13.81
Natural conditions					
	Polynomial (flower density, 2)	2	19.53660	0.0000	43.14
	Residuals	39			
Pollen supplementation	Population	1	3.3058	0.0827	12.53
	Flower density (log10)	1	1.0723	0.3117	4.06
	Residuals	22			
Myosotis laeta	Flower density	1	10.7457	0.0034	32.82
Natural conditions					
	Residuals	22			
Pollen supplementation	Flower density	1	0.0744466	0.7880749	0.42
	Residuals	18			

Table 3: Population size effects after local density is adjusted for.

Species	Factor	Df	F value	P	% explained
Myosotis forsteri	Population size	1	0.3002	0.6073	5.68
	Residuals	5			
Myosotis brockiei	Log$_{10}$ Population size	1	0.2922	0.6120	5.74
	Residuals	5			
Myosotis macrantha	Log$_{10}$ Population size	1	3.1783	0.1347	38.84
	Residuals	5			
Myosotis monroi	Log$_{10}$ Population size	1	1.183	0.3563	28.22
	Residuals	3			

Discussion

These results demonstrate that knowledge of breeding system is important in conservation risk assessment of plants. Here, I have shown a selfing species is not threatened with recruitment restriction when found in small populations. In the selfing species, *Myosotis forsteri*, local density had no effect on seed set, there was no density effect of pollen supplementation on seed set and there were no population size effects. These results are not surprising. For self-compatible, autonomous species, small population size is not a sign of threat on its own. The need for large, dense populations to get adequately pollinated is not there for those that are able to self-pollinate.

For those species that required pollinators, however, the local flowering plant density had a large impact on seed set. There are three possible reasons why low local density could have such an impact on reproductive success. Firstly, the quality of pollen deposited on their stigmas may be poorer in low-density patches. There may be more geitonogamous (within-plant) pollen transfers or movement between closely related individuals at low plant densities, thus low seed set by these plants may be indicative of inbreeding depression (Bosch and Waser, 1999). However, all species in this study have been found to be fully self-compatible and there is no significant difference in seed set by hand self- and cross-pollinations (Chapter two). Inbreeding depression, therefore, does not appear to be operating at the level of seed set at least.

Secondly, local plant densities may be influenced by the local site conditions within each population. Availability of resources such as soil moisture, nutrients and solar radiation levels can influence germination and growth of new plants (Bosch and Waser, 1999) as well as limiting the number of ovules that can develop into seed even if flowers are well pollinated. However, I found no density effects in the autonomous species, *Myosotis forsteri*, and, therefore no evidence that local conditions affect seed set in that species. In general, population effects on seed set, if they occur at all, are small. If resource availability is an important limiting factor, I would expect some population effects as some populations will be in more suitable places than others. In the pollinator-requiring species, density had no effect on seed set in flowers that received supplemental pollen suggesting that plants were pollen limited rather than resource limited in low density patches.

Thirdly, it could be that lower levels of pollen are being deposited on stigmas in low-density sites and that this causes an impact on seed production. As I have eliminated the other two causes, I conclude that it is the quantity of pollen receipt, which is limiting seed production at low plant densities in the four pollinator-requiring species studied.

For the two species *M. monroi* and *M. macrantha*, in some populations local flower densities reach high levels, such that pollen limitation is eliminated. For *M. monroi*, a quadratic curve provides a better fit of the data, and optimal densities are intermediate. These optimal levels do not appear to be reached in either *M. laeta* or *M. brockiei*. There may be resource limitation from intraspecific competition affecting seed production in *M. monroi* at those higher flower densities.

For the two species *M. monroi* and *M. laeta*, some of the hand-pollinated flowers set low seed levels at low density sites, although it was not significant in the analysis. This could be due to the lower density sites containing more closely related individuals with poorer quality pollen.

Although there were no detailed, systematic pollinator observations to accompany these data, by eliminating other possible mechanisms for poorer seed set by low density plants, inferences can be made regarding pollinator behaviour to explain how pollen receipt is limiting seed production. Visitors to plants growing at low density are expected to be fewer and less flower constant than visitors to plants in high-density patches (Kunin and Iwasa, 1996). Kunin (1997b) and Groom (1998) found plants growing at low densities had fewer visits by pollinators than individuals growing at high densities. Rare, self-compatible plants are often pollinated by generalists. Therefore visitors to less dense plants may be carrying pollen from other species (Groom, 1998). Kunin (1997b) found pollinators visiting sparse plants were less flower-constant, and are therefore are likely to carry a higher percentage of inappropriate pollen to plants of low density.

In this study, the size of the population had no effect on seed set (though I have less statistical power to detect population size effects as only a few populations of each species were sampled). Three species, *Myosotis brockiei*, *M. macrantha* and *M. monroi* show population differences in seed set in their original ANOVAs. However, there is no significant correlation between population size and seed set once the means are adjusted for local density. While the very smallest populations of these species have the lowest values for adjusted seed set, the reproductive output of the other populations does not appear to be influenced by population size. Although insufficient populations were studied, it is possible that there may be a minimum size necessary to attract sufficient pollinator service.

For these pollinator-requiring taxa then, low local density is a potential problem. Low reproductive success can create difficulties in establishing new populations and limit successful colonisations (Groom, 1998) thereby impacting on members of established populations. Species that have been found to suffer Allee effects in small populations tend to have bimodal patch occupancy patterns (i.e. either full or empty). This suggests there is an extinction threshold at low habitat occupancy (Amarasekare, 1998), and may explain the pattern of abundance and distributions of *Myosotis* species seen in the field. So while there were no population size effects detected in this study, the density dependence of reproduction is likely to influence both the population structure and the size of existing populations, while eliminating those populations that are too small to attract pollinators.

The positive relationship between local density and/or population size and plant fecundity, which has now been found in several other studies (e.g. Kunin, 1992, 1997b; Lamont *et al.* 1993, Ågren, 1996; Groom, 1998), is likely to affect the population dynamics of these species. The demographic effects seen, at least in this study, are explained if low seed production in low-density patches is limiting population growth and causing local extinction at those sites. It explains why the populations of the pollinator-requiring species are usually denser and larger than those of the selfing species. It is expected that population density and geographic range would vary over time. However there is growing evidence across several taxonomic groups, suggesting range and abundance are persistent species characteristics (Lawton, 1993). Density and range are considered linked to the species fundamental niche breadth, an evolved species characteristic (Brown, 1984). These results are consistent with this body of thought, as the pollination ecology of each species, an evolved trait, shapes the type of population structure that is successful for each species.

There is a widely held view among population biologists that plant population growth is often not limited by seed production. Crawley (1990) concluded that plant population growth is more often limited by microsite availability than by seed production. Some pollination biologists, such as Bawa and Beach (1981), believe plant reproductive success is rarely limited by pollination events (Kunin, 1992). Seed-limited recruitment has very rarely been studied, (but see Eriksson and Ehrlén, 1992; Maze and Bond, 1996) but it is thought that the persistence of plant populations will depend on the growth form of the species (e.g. longevity) and on the habitat type that it occupies (Crawley, 1990). A recent review of seed limitation studies found 50% of augmentation studies and 53% of introduction studies showed evidence of seed limitation (Turnbull *et al.* 2000). My study was conducted on short-lived herbaceous rare plants that occur in a range of different population types. Generally species with density-dependent reproduction tend to have clumped distributions while those that do not have density-dependent reproduction occur in sparse, small populations. The pollinator - plant interactions for the vector-requiring species of *Myosotis* shown with this data set are very sensitive. Loss of this interaction will lead possibly lead to loss of plant populations over time. At low densities, it may be hard to attract and ultimately maintain pollinator populations. Eventually these plants may die and if they've only rarely set seed it is hard to imagine how populations can persist in the long term under these circumstances.

An important means by which these high densities may be maintained is through selection against over-dispersal (Kunin, 1992). Kunin (1992) introduced the concept that density-dependent fertilization may select against over-dispersal. Mechanisms that improve dispersal ability are usually viewed as positive attributes to a species as the ability to disperse into otherwise unoccupied sites has the potential to increase fitness and will therefore be favoured by selection (e.g. Webb, 1998). However, such dispersal events will be unsuccessful for many species particularly those that are restricted to particular, insular habitats or those that are not self-fertile. Cody and Overton (1996) found evidence for reduced dispersal potential within just a few generations in island plant populations of weedy, short-lived, wind-dispersed plants when compared to mainland populations. In this system such evolution could involve an increase in seed size. This idea is investigated further with respect to all New Zealand species (see Chapter five).

These results are consistent with the research carried out to date which, with a few exceptions, are generally showing low density plant populations have reduced pollination success (Kunin, 1997b). These exceptions are generally from studies of self-compatible species (e.g. Klinkhamer and de Jong, 1990; Molano-Flores *et al.* 1999), so this study is one of a growing number of studies finding these effects in fully self-compatible xenogamous (vector-requiring) species (e.g. Fritz and Nilsson, 1994; Groom, 1998).

These results also have implications for studies of the evolution of floral form, some of which have focused on pollinator-mediated selection on floral traits. Typically these studies make phenotypic measurements of floral characters, such as corolla size, which are then compared to seed or fruit set and are explained in terms of pollinator visitation rates to determine the effect of floral morphology on female fitness (e.g. Galen, 1996). There is a large amount of variation in seed set in natural populations some of which I have found is due to local density. I found that about 30% of the variation in seed set is explained by local flower density in the four vector-requiring species (range 29.55 – 43.1) (Table 2). It may be useful in future studies to include local density, which is very easy to measure, as a variable to account for any density effects that would otherwise be perceived as unexplained noise and thus improve the chance of detecting phenotypic effects on seed set.

CHAPTER FOUR

Precocious bud pollination: Maximizing chances for cross pollination in the ultramafic endemic *Myosotis monroi* Cheesm. (Boraginaceae).

Abstract

Precocious stigma presentation was investigated in the geographically restricted, serpentine endemic forget-me-not *Myosotis monroi*. Stigmas collected from precocious buds, in which the style and stigma protrude out of the bud before the petals open, showed some pollination was taking place at the precocious phase. 26.2 % of the precocious stigmas collected had received pollen, and 11.9 % had received at least 5 pollen grains. This contrasts with stigmas collected from flowers at the end of the male phase, where 88.1 % had received pollen, 75.81 % with at least 5 pollen grains. Hand pollinations confirmed precocious stigmas were receptive but seed set by hand-pollinated open flowers was significantly higher. Stylar precocity in *M. monroi* effectively lengthens the female phase of this protogynous species. *M. monroi* also shows far greater phenological synchrony of within plant flowering than five other species of New Zealand *Myosotis* and this results in a much larger flowering display. Large floral displays, while attracting pollinators, have the disadvantage of increasing chances for geitonogamous pollinations. It is hypothesized that the impact of a large floral display on levels of self-pollination in *M. monroi* is alleviated to some degree by the relatively long, initial female-only phase.

Introduction

Floral traits that influence outcrossing rates, such as the separation of female and male functions, are common in angiosperms (Faegri and van der Pijl, 1979). For individual hermaphrodite flowers, the separation of pollen and stigma presentation in time (dichogamy) and space (herkogamy), are traits that have evolved to avoid interference between these two functions, which have the additional utility of improving outcrossing potential (Webb and Lloyd, 1986). Although treated as separate functions by Webb and

Lloyd (1986) and Lloyd and Webb (1986), these mechanisms (herkogamy and dichogamy) can occur together in many different flower types (Luijten *et al.* 1999; Robertson and Lloyd, 1991). For self-compatible plants, the herkogamy and dichogamy exhibited by flowers are the main means by which chances for cross pollination events can be influenced. Factors that increase pollen carryover or cause pollinators to make more frequent interplant movements are also potentially important. The degree of synchrony within the plant and the degree to which it is complete within a flower are two aspects of dichogamy that Lloyd and Webb (1986) recognized as important to the breeding system. In biotically pollinated flowers, protandry (the presentation of stigma after the pollen) is more common than protogyny (the presentation of pollen before the stigma) (Lloyd and Webb, 1986) although it is thought that protogyny gives better protection against self-pollination (Robertson and Lloyd, 1991). The particular set of dichogamous and herkogamous traits that occur in a self-compatible species will influence levels of self-pollination. Webb and Lloyd (1986) described three main classes of herkogamy found in angiosperms: interfloral (i.e. monoecism); reciprocal (i.e. heterostyly) and homomorphic, in which flowers are hermaphroditic, of one form and pollinator contact is unordered or ordered. In ordered herkogamy, pollen and stigma presentation relates to the pathway of the reward-seeking pollinator (Webb and Lloyd, 1986). Approach herkogamy, a type of ordered herkogamy, occurs when the stigma protrudes beyond the anthers and is usually contacted first, which decreases the likelihood of self-pollination (Robertson and Lloyd, 1991).

In species that require a vector for pollination to occur, self and cross pollen alike must be deposited via the vector (Holsinger, 1996), so the longer the stigma is exposed prior to anther dehiscence, the greater the chance of outcrossing. Pollination studies carried out previously on the New Zealand endemic *Myosotis colensoi,* a pollinator-requiring species that has a large floral display, found flowers were protogynous and will usually have been adequately pollinated prior to anther dehiscence when weather conditions are good (Robertson, 1992). However, Robertson (1992) estimated as many as 50% of pollinations were geitonogamous. *M. colensoi* flowers spend an initial 12.1% of the time open in the female phase prior to anther dehiscence (Robertson and Lloyd, 1991).

A review of the literature has revealed that stylar precocity has generally been assumed to indicate protogyny (e.g. Solbrig and Cantino, 1975). Hoc *et al.* (1994) investigated the assumption in four *Prosopis* species (Mimosaceae) and found that precocious styles did not indicate protogyny and that stigmas were not receptive until after anthesis and anther dehiscence had begun. The Hoc *et al.* (1994) is the only study of this type that has been carried out. Precocious styles and their stigmas have never been proved to be receptive during the precocious phase or even prior to anthesis.

Myosotis monroi has brush blossom floral morphology (Robertson, 1989; Chapter two) (Fig. 1). Flowers exhibit approach herkogamy, as the stigma protrudes further than the anthers. The stigma presentation in the buds is often precocious, potentially exposing the stigma for pollination before the bud opens (Fig. 1, lower insert). It has been suggested stigmatic precocity in this species has the effect of extending the length of the female phase (Robertson, 1989).

Selfing rates and levels of dichogamy can be compared to determine whether mating system evolution is influenced by selfing rates. High levels of selfing may be expected to be accompanied by low levels of dichogamy.

The aims of this chapter are to find out if pollination occurs at the precocious stage, if so, how much, and how the precocious phase affects the time spent in the female phase in comparison to related *Myosotis* species that do not have precocious buds.

Figure 1: *Myosotis monroi*, Dun Mountain. Photos (main and top insert) Alastair Robertson, (lower insert) Bill Malcolm.

Material and Methods

Myosotis monroi has a restricted, disjunct distribution that is confined to an ultramafic mineral belt that lies east of Nelson, New Zealand. All fieldwork for this study was conducted at Dun Mountain (Fig. 2). A previous study has found that *M. monroi* is fully self-compatible, but requires a pollinator to achieve pollination (Chapter two).

Stigmas were collected as pairs from 42 plants - one a precocious bud and another from an open flower at the end of the male phase on the same inflorescence. Collections were made at the end of the day. Stigmas were then mounted on slides in fuchsin gel for later viewing and scoring. Stigmas were scored for pollen load using a compound microscope.

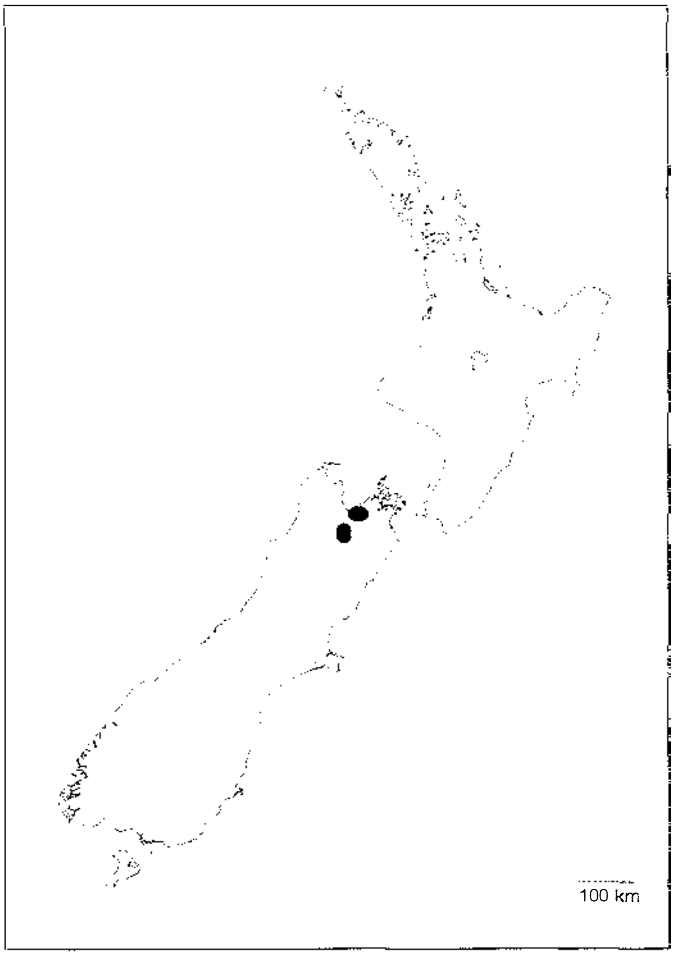

Figure 2: Known distribution of *Myosotis monroi*, Nelson region, South Island, New Zealand.

21 plants were caged to exclude pollinators while inflorescences were in closed bud. As stigmas protruded from the bud and as flowers opened, hand pollinations were carried out. Precocious buds and open flowers on the same inflorescence were hand pollinated to determine whether the stigma was receptive at the precocious phase. Cages were then replaced to avoid any natural pollination occurring after treatments were carried out. Three weeks after treatment, seed set was counted. The developing seeds were easily observed in maturing calyces.

Phenological data were collected on *M. monroi*. 'Static' samples were made to estimate the relative time a flower spends in each sexual stage (*sensu* Robertson and Lloyd, 1991). Static samples are made by recording the sexual stage each flower is at within each inflorescence. If the stigma is protruding from the closed bud, or the corolla is open but none of the anthers have begun dehiscence, then the flower is recorded as in the female phase. When anthers begin to dehisce, flowers are then recorded in the male phase. When all five anthers have dehisced, and pollen is no longer visible, flowers are recorded in the post pollination phase. From these data percentages of flowers at each stage were determined so that the level of within-plant synchrony could be measured and the level of dichogamy could be determined. 'Static' samples were taken of several species of *Myosotis* for comparison. Species were chosen to represent the full range of floral syndromes found in New Zealand *Myosotis*. Samples were made during peak flowering times for all species.

Data Analysis

The seed set data was converted into a proportion of seed set per flower then transformed with an arc-sine square-root transformation. All analyses were performed using *S-PLUS* version 4.5. A one-way ANOVA on the transformed proportion of seed set by flowers pollinated at precocious and fully open stages, was performed. These data are presented as box plots. A poisson analysis of variance was carried out on the number of flowers open per plant, the number of female phase flowers, and the number of inflorescences per plant. A gaussian analysis of variance was performed on the average number of flowers per inflorescence. A binomial analysis of variance was carried out on the proportion of female flowers per inflorescence. Box plots are drawn to examine the data. Following these analyses, *a posteriori* Tukey tests were used to compare the means of each species.

The mean proportions of flowers in each phase, female, male and post-pollination, were determined and expressed as percentages of the total.

Results

Receptivity of precocious buds

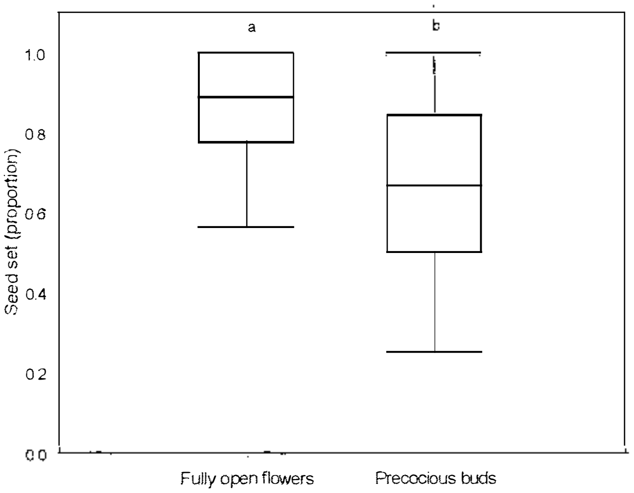

Figure 3: Boxplot of resultant seed set from the pollination treatments carried out at the two phases.

Flowers pollinated during the precocious phase set seed, but there was a significant difference between that and seed set by fully open flowers (p=0.0049; Fig. 3, Table 1). There was no plant effect (p=0.4266, Table 1). Seed set by flowers hand pollinated in the precocious phase averaged 2.7 seeds per flower, while seed set by flowers hand pollinated at the fully open phase averaged 3.5 seeds per flower (Appendix 3).

Table 1 : ANOVA results comparing seed set by hand pollinated precocious buds and fully open flowers

Factor	df	SS	MS	F Value	*P*
Plant	1	0.0884	0.0884	0.6454	0.4266
Fully open flowers or precocious buds	1	1.2213	1.2213	8.9188	0.0049
Residuals	39	5.3404	0.137		

Pollen load

26.19% of the precocious stigmas collected had received pollen, and 11.9 % had received at least 5 pollen grains. 88.1% of the fully open flowers stigmas had received pollen, 75.81% had received at least 5 pollen grains. Therefore some pollination does occur in natural conditions at the precocious bud stage (raw data in Appendix 4).

Phenology

The number of inflorescences per plant varied significantly among the six species (Table 2). *M. monroi* and *M. macrantha* had on average more inflorescences per plant than the other species, and significantly more than *M. brockiei, M. laeta* and *M. tenericaulis* (Fig 4).

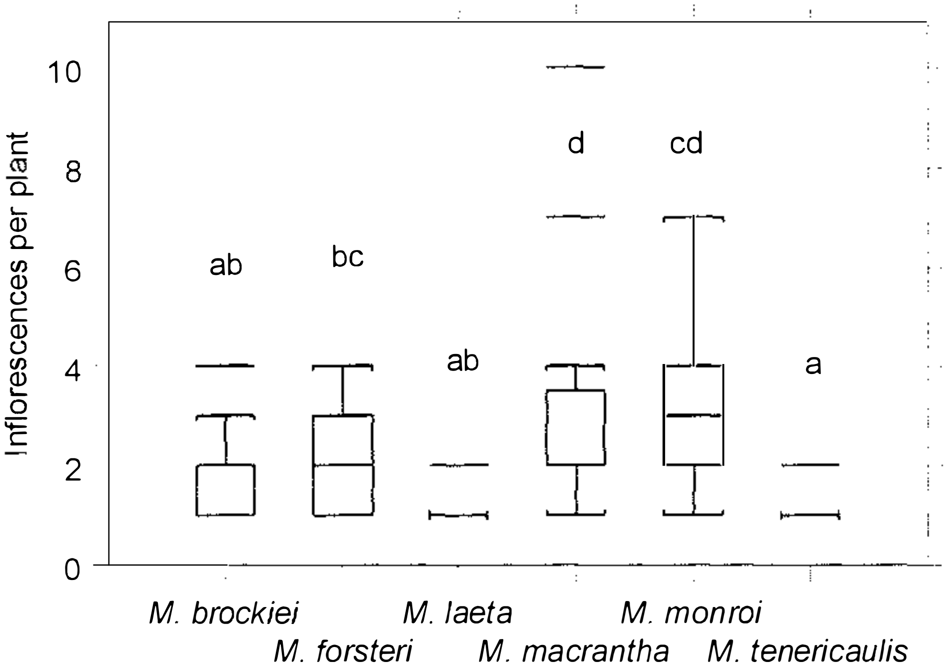

Fig 4: Box plots of the number of inflorescences per plant. Species that share letters above the boxes are not significantly different.

Table 2: Analysis of the number of inflorescences per plant

Model	Df	Deviance	Residual df	Residual deviance	F value	*P* (chi)
Null			119	100.36		
Species	5	40.89	114	59.47	14.00	< 0.00001

The number of flowers open per inflorescence also varied significantly among the six species (Table 3). *M. monroi* and *M. laeta* had the highest average number of open flowers per inflorescence, and significantly more than *M. forsteri* (Fig. 5).

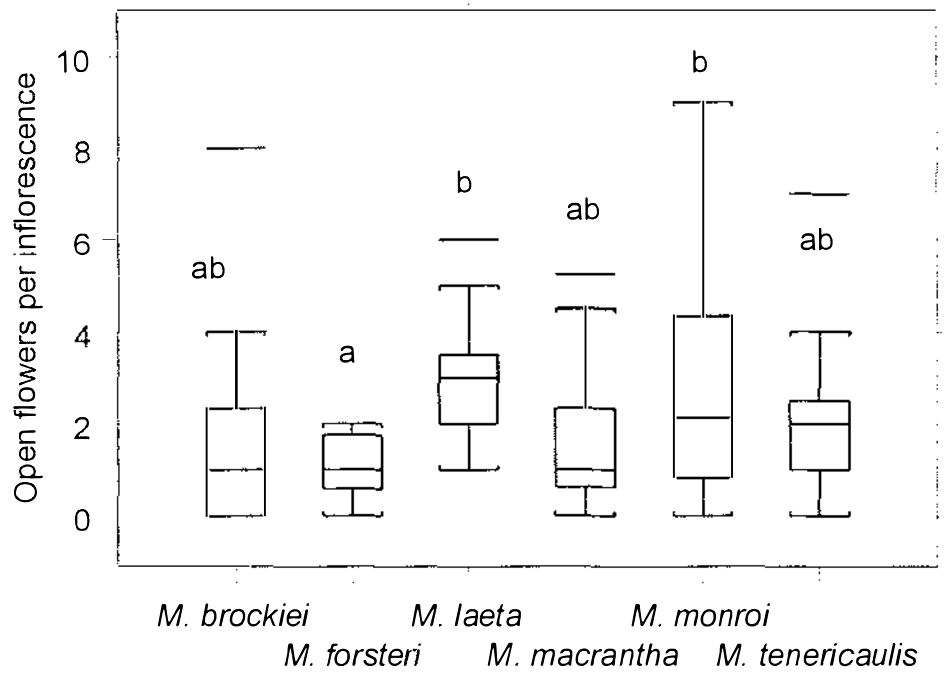

Figure 5: Box plots of the number of flowers open per inflorescence. Species that share letters above the boxes are not significantly different.

Table 3: Analysis of the number of flowers open per inflorescence

Model	df	Deviance	Residual df	Residual deviance	F value	*P* (chi)
Null			119	388.44		
Species	5	51.48	114	336.9546	3.483525	0.0057

Together, these differences meant that the number of open flowers per plant also varied significantly among the species (Table 4). *M. monroi* and *M. macrantha* had the highest average number of flowers open per plant, and *M. monroi* had significantly more than *M. brockiei, M. forsteri, M. laeta* and *M. tenericaulis* (Fig. 6).

Table 4: Analysis of total number of flowers open per plant

Model	df	Deviance	Residual df	Residual deviance	F value	P (chi)
Null			119	334.99		
Species	5	105.12	114	229.88	10.09	< 0.00001

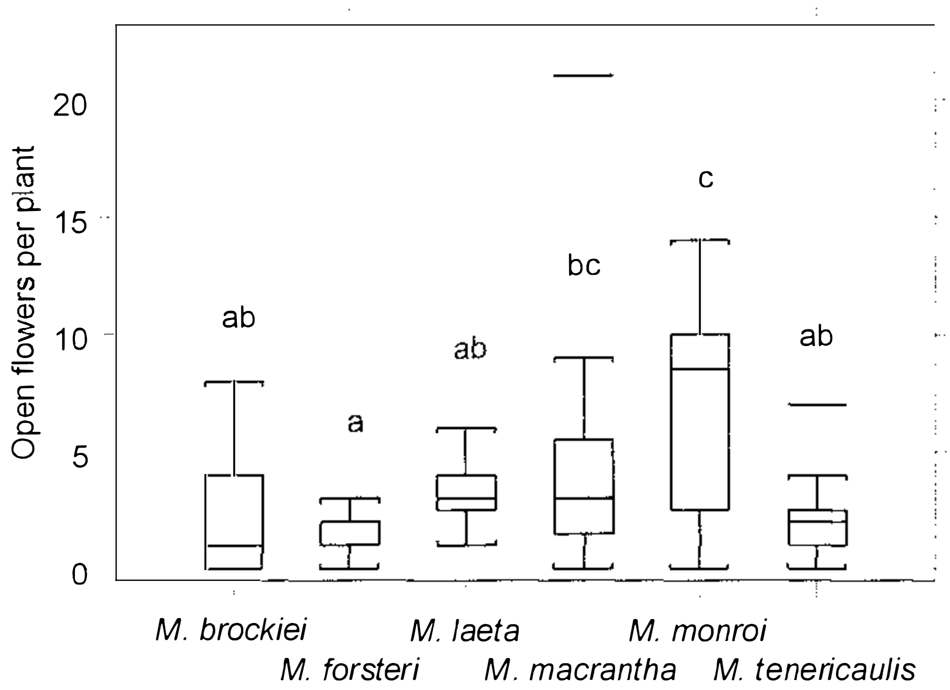

Figure 6: Box plots of the numbers of open flowers per plant. Species that share letters above the boxes are not significantly different.

Finally, the number of flowers in the female phase also varied significantly among the species (Table 5). *M. monroi,* along with *M. macrantha,* had the highest average number of flowers in female phase at any one time, and significantly more than *M. brockiei, M. forsteri, M. macrantha* and *M. tenericaulis* (Fig. 7).

Table 5: Analysis of the number of flowers in the female phase per plant.

Model	df	Deviance	Residual df	Residual deviance	F value	*P* (chi)
Null			119	186.52		
Species	5	64.66	114	121.86	12.02	< 0.00001

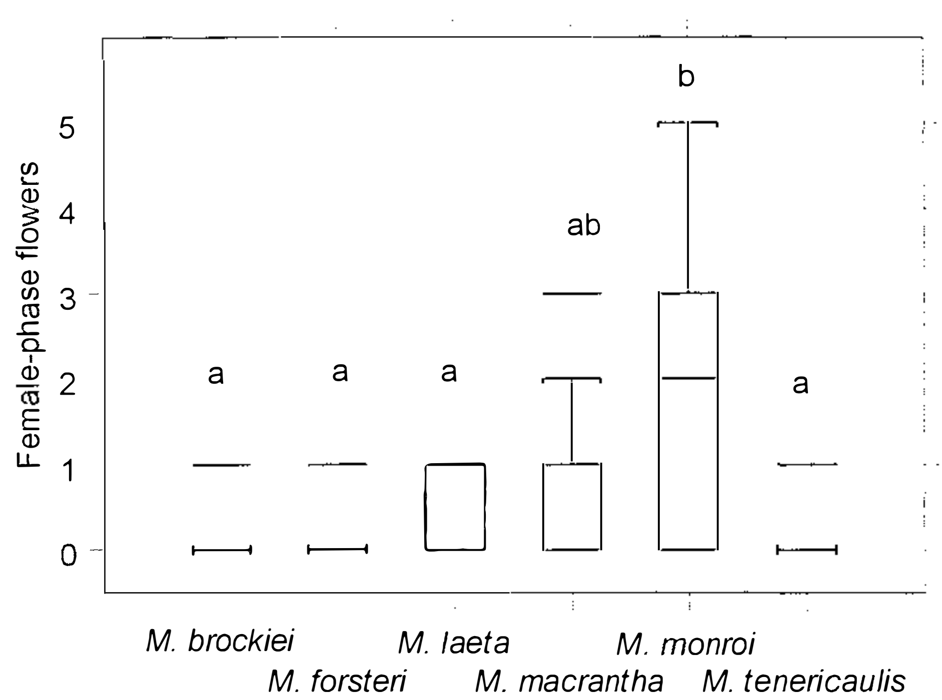

Figure 7: Box plots of numbers of flowers in the female phase per plant. Species that share letters above the boxes are not significantly different.

Dichogamy

On average, *Myosotis monroi* flowers spent nearly 25% of the time in the female phase before the anthers dehisced (Table 6). *M. macrantha* spends longer in this phase than the other species studied, but the variance in the proportions of flowers in each phase was high and so these differences among species were not significant (Binomial ANOVA, $P = 0.225$).

Table 6: Duration of sexual stages (percentage) inferred from the average proportion of flowers in each phase (n=20 for each species)

Species	Female phase	St dev	Male phase	St dev	Post pollination phase	St dev
M. brockiei	10.8	0.4	24.1	0.6	65.1	1.9
M. laeta	8.3	0.4	25.0	0.7	66.7	1.0
M. monroi	23.5	2.1	54.6	3.7	21.8	1.6
M. macrantha	25.6	0.8	47.6	1.2	26.8	1.1
M. forsteri	12.7	0.4	15.5	0.5	71.8	0.8
M. tenericaulis	9.8	0.4	31.7	0.7	58.5	6.0

There appears to be no relationship between dichogamy and selfing rate (Fig. 8). Although the two species with the shortest dichogamy do have very high selfing rates, the pattern does not continue. There are two separate groups of data, with one unusual point (*M. forsteri* (Central Otago) - high selfing rate and long time spent in female phase) and the intermediate one of *M. australis* var. *lytteltonensis*, which has a mixed mating system.

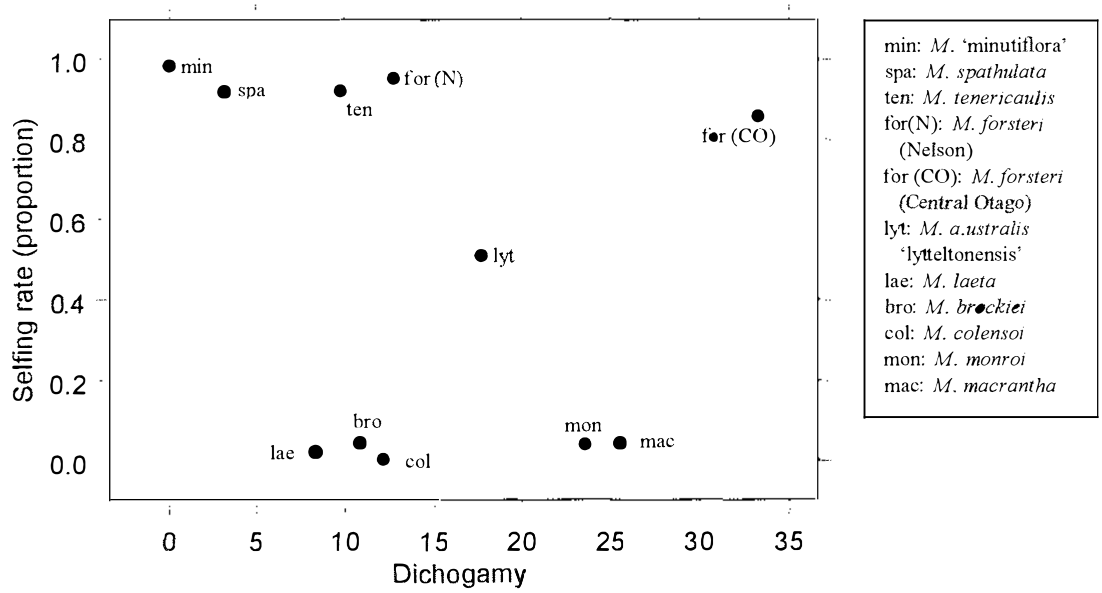

Figure 8: Dichogamy (% time spent in female phase) graphed against the selfing rate (seed set/ovule when pollinators are excluded). Data were extracted from this chapter, chapter two and Robertson and Lloyd, 1991)

Discussion

A small amount of pollination does occur during the precocious phase of *Myosotis monroi*. Although the level of pollination at the precocious phase is low, the small additional amount of cross-pollination this mechanism allows may be a sufficient selective force to maintain this phase. Hand pollinations of precocious buds indicate that the stigma is receptive during this phase although these flowers set significantly less seed than that set by hand-pollinated open flowers. The average seed set by hand pollinated flowers at the precocious stage of 2.7 seeds per flower is 77% of the 3.5 seeds per flower that is set on average by those pollinated at the open phase. Therefore, stigma receptivity at this early stage of the female phase appears to be about three/quarters complete. The precocious style does effectively increase the length of the female phase in *M. monroi*, thereby increasing chances for cross-pollinations.

This study is the first to find precocious styles with receptive stigmas. In this case, stylar precocity does indicate protogyny, but as shown by Hoc *et al.* (1994), stigma receptivity must be proved. I have shown that the stigmas are receptive during the precocious phase and that some pollination is occurring during this phase.

In terms of their phenology, all species were found to differ significantly in the number of inflorescences per plant, the number of open flowers per plant and per inflorescence, and in the number of flowers in the female phase. They did not differ significantly in the duration of the female phase.

Myosotis monroi and *M. macrantha* have the longest relative female phases of the species studied. *M. macrantha,* with funnel blossom flowers, also has a relatively large floral display and sometimes has a precocious phase, but this is not consistent. The prolonged time spent in the female phase by these two species suggests some selective advantage occurs for this mechanism, which may promote outcrossing. It seems likely that the longer the female phase, the more chance there is of being fertilized with pollen from another flower. While *M. monroi* has a more consistent precocious bud phase than *M. macrantha*, *M. macrantha* has the longer relative female phase. By contrast, *M. brockiei* and *M. laeta*, both brush blossom flowered species, have much smaller floral displays and shorter relative female phases. Having a smaller floral display size will reduce the number of potential geitonogamous pollinations. The shorter female phase period may be a mechanism to allow pollination with one visit, rather than two. It is thought that the advantage of herkogamous separation of sexual parts over the dichogamous separation is that both pollen receipt and dispatch can be achieved with one visit to the flower (Webb and Pearson, 1993). Webb and Pearson (1993) considered this system could maintain some degree of outcrossing when the chances for insect pollination are poor. Therefore, under these circumstances, selection would favour a reduction in dichogamy and this is the case for *M. brockiei* and *M. laeta*. This type of separation of phases may help these rare species persist and explain, at least in part, how they manage to exist in relatively small populations. *M. brockiei* and *M. laeta* are the two that are considered most at risk of those taxa included in this study (de Lange *et al.* 1999).

Because of the large floral display of *M. monroi,* there is ample opportunity for both self- and cross-pollination to occur. Incomplete dichogamy and herkogamy are not able to prevent self-pollination occurring in self-compatible plants (de Jong *et al.* 1993). Particular types of dichogamy and herkogamy influence the levels of autonomous and geitonogamous pollinations that may occur. Empirical studies show levels of geitonogamy can be substantial and will increase with floral display size (de Jong *et al.* 1993). Protogyny and approach herkogamy are considered to be more likely to evolve when the avoidance of self-pollination is the selective force (Lloyd and Webb, 1986; Webb and Lloyd, 1986). In species that require a vector to achieve pollination, there will also be selective pressure to increase the size of the floral display to attract pollinators. However, increasing the size of floral display will increase intra- and inter-floral self-pollinations, when the form of dichogamy is incomplete. The relative synchrony of the phases between plants will offer some protection against such self-pollinations. In addition, the ability to increase the relative length of the female phase, will also improve the chances of receiving cross pollen, particularly when there is the synchronous flowering rather than the sequential type operating as in the other brush blossom species studied.

All New Zealand *Myosotis* species studied to date that exhibit dichogamy are protogynous (Robertson, 1989; Chapter two). The known exception is *M.* 'minutiflora' which was found to have no female-only phase at all and self-pollination occurs almost immediately such that there is very little opportunity for outcrossing to occur (Robertson and Lloyd, 1991). There is considerable variation in the length of the female phase across the range of species for which this has been measured, and even among populations of the same species. The relative time *M. forsteri* spent in the female phase contrasts with Robertson's (1989) findings. He found *M. forsteri* (Central Otago), an initially herkogamous species, spent 33% of its time in the female phase, while I found *M. forsteri* (Flora Valley) spent just under 13%. *M. forsteri* is a highly variable species morphologically. Therefore it is not surprising to find such variability in dichogamy. Individuals from different populations will be under different selection pressures and this is represented by the different times spent in different phases.

The generally accepted view of the direction of mating system evolution is that it will usually proceed from outcrossing to selfing (Raven, 1973; Lande and Schemske, 1985). However, as much of the extant flora of New Zealand, including *Myosotis*, is thought to have arrived via recent long distance dispersal (e.g. Winkworth *et al.* 1999), selfing is the more likely ancestral state for these taxa. Outcrossing types are thought to have arisen secondarily in New Zealand during subsequent speciation that has occurred as new environments have emerged (Lloyd, 1980). Within cosexual plants the most effective means of promoting outcrossing is via dichogamy and less so via herkogamy (Webb and Pearson, 1993). Herkogamy, the spatial separation of stigma and anthers, is thought to have evolved from dichogamy to maintain outcrossing, but unlike dichogamy, requires only one visit for pollination (Webb and Lloyd, 1986; Webb and Pearson, 1993). All species in this study exhibit varying levels of incomplete dichogamy, herkogamy and selfing ability. If the ancestral state for the breeding system of New Zealand *Myosotis* was autogamy, then the varying levels of dichogamy and herkogamy found among the taxa may assist in testing the above ideas about the evolution of mating systems. However, although the groups of species separate out into three distinct groups, there is no evidence to suggest a direction for mating system evolution with this data set. These aspects of floral evolution are probably under many selection pressures, with reticulate evolution possibly occurring within populations of many taxa, particularly those with mixed mating systems, but possibly very little gene flow among populations. Within the brush blossom taxa, there are two clearly defined means by which pollination is achieved; large floral displays accompanied by synchronous flowering or small flowering displays accompanied by sequential flowering.

CHAPTER FIVE

Rarity in NZ *Myosotis* L.

Abstract

Rarity is generally considered "the precursor to extinction" (Darwin, 1872). However, there are several different forms of rarity and not all rare species are threatened. I examined the different rarity patterns observed within New Zealand *Myosotis*. New Zealand *Myosotis* species are never common, all are rare in different ways and some species are threatened. Some species are known to occur at one locality where they may be locally common, others may occur in two or more widely disjunct geographic areas, while others may be widespread but never common where they occur. I compared the local abundance, disjunctions and distribution patterns of 33 taxa of *Myosotis* to aspects of their morphologies, including traits related to breeding system, dispersal and life-history. Taxa requiring pollinators to set seed had smaller range sizes and higher local population densities than those that were able to self-pollinate. Apparent adaptations for dispersal and life-history traits were not correlated with range size. The disjunct distributions exhibited by some taxa within the genus were not associated with any of the morphological traits.

Introduction

The word rare has different meanings and is used to describe many different patterns of density and distribution. Gaston (1994) defines rarity 'as the state of having low abundance and/or a small range size'. However, plants can be rare in different ways and several authors have defined different types of rarity (e.g. Rabinowitz, 1981; Fiedler and Ahouse, 1992). Essentially these different forms represent particular range, distribution and abundance patterns within different spatial (e.g. Rabinowitz, 1981; Gaston, 1994) and temporal scales (e.g. Fiedler and Ahouse, 1992). There is generally a positive correlation between geographic range size and local abundance (Brown, 1984; Lawton, 1993; Gaston, 1996). However there is a lot of variation such that individual taxa can be locally abundant but only have a small geographic range and others may be widespread but in consistently low population size (Rabinowitz *et al.* 1986). Rabinowitz (1981) classified seven forms of rarity distinguished by dichotomous differences in local population size (everywhere large-everywhere small), habitat specificity (broad-restricted) and geographic distribution (wide-narrow). However, quite often studies of rare plants fail to address the different types of rarity (but see Kunin and Shmida, 1997). There is little doubt that however rarity is defined, perhaps with the exception of catastrophic events, it will always precede extinction, and it is for this reason that I am interested in this aspect of plant conservation ecology.

Rarity correlates

An important aspect of the study of rare plants is in understanding their reproductive biology. Rare-common congener difference studies have shown rare plants differ from their more common relatives in aspects of their reproductive biology and dispersal traits. Rare plants tend to have lower levels of self-incompatibility, a bias toward asexual reproductive pathways, lower overall reproductive effort and poorer dispersal ability (Kunin and Gaston, 1993). Generally, these studies do not discriminate between different types of rarity (Kunin and Shmida, 1997) and actual tests of the differences are not common (Thompson *et al.* 1999). Plants with different breeding systems are likely to have different population structures and range sizes. Plants growing at low local density are more likely to be able to autonomously self-pollinate (Kunin and Shmida, 1997). Autonomous selfers can potentially grow, reproduce and colonise a new area by the germination of just one seed. They do not require the presence of others to successfully reproduce and so can establish as isolated populations. Therefore, we could expect that their range size will not be constrained by their breeding system and other factors will be important in limiting or improving colonisation successes. Non-autonomous plants require local population densities at levels that ensure an adequate supply of pollen and can attract vectors to achieve pollination. A single seed may disperse into a new area and germinate, but if flowers are never pollinated, and vegetative reproduction is limited, successful colonisation may not occur.

The dispersal abilities of species are expected to have an effect on their distributions. Several researchers have found correlations between dispersal ability, local abundance and range size (e.g. Rabinowitz, 1978; Rabinowitz and Rapp, 1981; Oakwood *et al.*

1993; Quinn *et al.* 1994). However, a reanalysis of the Quinn *et al.* (1994) study by Thompson and Hodgson (1996) using the same taxa, a different classification of dispersal in space (i.e. dispersal distance), and new data on dispersal in time (i.e. seed persistence) showed no relationship between distribution patterns and dispersal. Thompson *et al.* (1999) in a study of the flora of central England, found dispersal ability explained very little of the variation in local range and none of the variation in national range for these species. Further investigation is required as there are other factors involved in determining distributions. There may be different regimes operating on the floras of different countries. Thompson and Hodgson (1996) believe habitat loss is the major determining factor in the distributions of plants in Britain.

Life-history traits of plants may also influence their distributions. Perennial plants and those capable of vegetative reproduction may be able to offset a lack of recruitment during years of poor pollination (Quinn *et al.* 1994) and may be able to spread more effectively than those species that are annual and have no ability to vegetatively reproduce.

New Zealand *Myosotis* L. (Boraginaceae)

A range of rarity types (*sensu* Rabinowitz, 1981) has been observed within the New Zealand taxa in the genus and 33 % are considered threatened in some way (de Lange *et al.* 1999). Some species are point endemics, known to occur at only one locality, while others are found sporadically throughout most of New Zealand. For example, the point

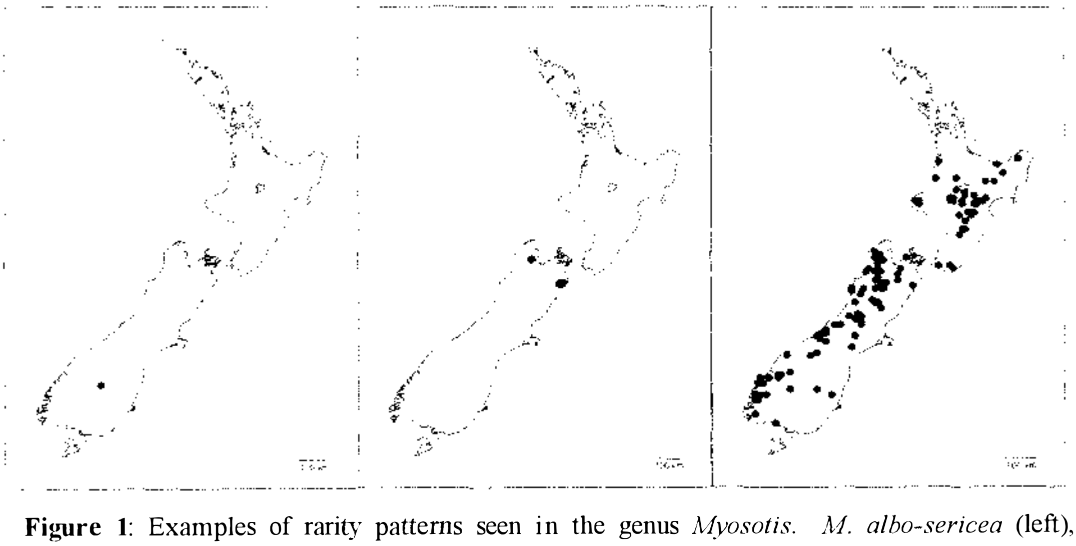

Figure 1: Examples of rarity patterns seen in the genus *Myosotis*. *M. albo-sericea* (left), *M. arnoldii* (center) and *M. forsteri* (right).

endemic *Myosotis albo-sericea* is known from one locality where it is abundant (Fig. 1). *Myosotis arnoldii* is also abundant where it occurs, but has a disjunct distribution, with one major locality in northwest Nelson and the other in Marlborough. *Myosotis forsteri* occurs sporadically throughout New Zealand and although it is the most often recorded species of the New Zealand *Myosotis* group, it is never abundant where it occurs.

All New Zealand *Myosotis* species studied so far have been found to be self-compatible (Chapter two). There is a range of pollinating mechanisms from autogamous selfers through to those that require pollinators to achieve fertilisation (Robertson and Lloyd 1991; Chapter two). Species that require pollinators, have been found to be pollen-limited in low density patches (Chapter three).

The aim of this study is to test whether morphological traits, particularly those related to reproductive biology, have any association with the range of distributions, population disjunctions and local densities that are seen in New Zealand *Myosotis*.

Methodological difficulties

This type of study, in which comparisons are made between rare and common congeners in an effort to understand more about rare species, has been criticised by various authors for several reasons (e.g. Kunin and Shmida, 1997). A problem cited by Kunin and Shmida (1997) is that only a single measure of rarity has typically been used in each study. Plants can be rare in different ways and at different spatial scales. Some studies are of point endemics with dense local populations, while others are of sparse taxa with low densities but wide geographic ranges. Kunin and Shmida (1997) recommend measuring rarity at different spatial scales in an effort to allow for the different types of rarity observed.

The non-independence of the traits of related species is a further problem that has been identified with these types of studies i.e. that suites of traits shared by species may not be independently acquired but inherited from a common ancestor (Brown, 1984; Harvey, 1996; Kunin and Shmida, 1997). It is therefore important to take phylogeny into account when making interspecific comparisons (Harvey, 1996). Unfortunately a resolved phylogeny does not exist for New Zealand *Myosotis*. Molecular work in the genus has so far found little difference among the New Zealand taxa and this is attributed to very recent speciation events within New Zealand (Winkworth *et al.* 1999). In the absence of a resolved phylogeny, it would be tempting to use the taxonomic structure of the genus, as described by Moore in Allan (1961), as a surrogate for phylogeny. However, Moore admitted, "though the position of the anthers in relation to corolla scales is used in the first division of the key below, there is no firm conviction that this leads to the most nearly natural arrangement" (p 807). For this reason (see Chapter 6 for a full discussion of this treatment) I do not consider Moore's classification system appropriate for this type of phylogenetic correction.

Methods

Measurements were made of rarity patterns and various reproductive, life history and dispersal parameters in 33 NZ *Myosotis* taxa (Table 1). Herbarium material (CHR - Landcare Research, Lincoln – see appendix 5 for details of the specimens examined), personal observations, and reports (published and unpublished) provided the data. The results presented are averages from 1-5 separate plants.

Floral Characters: Herkogamy is the spatial separation of anthers and stigma. Species that are always herkogamous require a vector to achieve pollination, while those that are never herkogamous are able to self-pollinate autonomously and do not require a vector to mediate this process (Chapter 3). An intermediate category is required for those taxa that have been found to be initially herkogamous or contain both morphologies within populations.

Growth forms: A woody root system is indicative of a longer-lived species compared to those with a fibrous system. Individuals of some species typically consist of a single rosette (indicative of shorter-lived species), whereas other species form large clumps of several rosettes (indicative of longer lived species).

Seed/calyx characters: *Myosotis* species have dry, shiny nutlets that vary in size from 0.9 mm to 3.5 mm and may have winged edges (Webb and Simpson, 2001). Further, the calyces may split open fully, allowing seed to drop out at maturity, or have short splits, which retain the seeds. In addition, the hairs on the calyces can be strongly, weakly or not hooked.

Table 1: Morphological characters

Character	Units	Explanation	Source
Herkogamy	mm	Spatial separation of anthers and stigma	personal observations, herbarium material
Style length	mm	Length from top of ovary to base of stigma	Herbarium material
Filament length	mm	Length from corolla attachment to anther attachment	Herbarium material
Anther length	mm	Anther length	Herbarium material
Corolla length	mm	Length from base of corolla tube to top of corolla lobe	Herbarium material
Corolla width	mm	Width of flower	Herbarium material
Hooked hairs	0, 0.5, 1	0: none 0.5: weakly hooked 1: strongly hooked	Herbarium material
Root system	0,1	0: fibrous 1: woody	Herbarium material
Rosette number	0, 1	0: single 1: multiple	Herbarium material
Calyx split	mm	Length of split	Herbarium material
Calyx length	mm	Calyx length	Herbarium material
Calyx ratio	proportion	Calyx split/calyx length; the proportion of the calyx that is split	Derived character
Seed length	mm	Seed length	Webb and Simpson, 2001
Winged seeds	0, 0.5, 1	0: none 0.5: slightly winged 1: distinctly winged	Webb and Simpson, 2001
Relative seed length	ratio	Seed length/flower size	Derived character

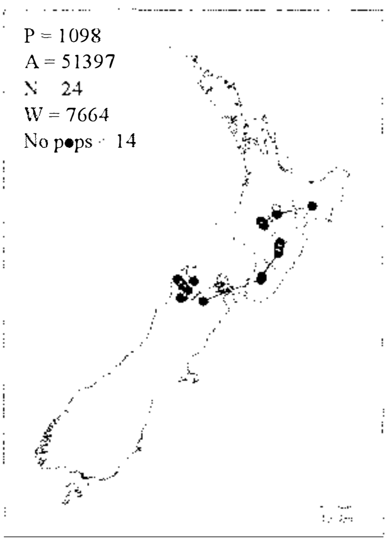

Figure 2: Example of how the geographic traits were quantified for *Myosotis venosa*. P = perimeter, A = area, N = number of discrete collection records, W = the length of the minimum spanning tree, No pops = number of groups of collection records more than a specified distance apart (see Table 2 for more detailed explanations)

Distribution measurements: (Table 2)

Data for the geographic measurements were taken primarily from the Landcare herbarium database (CHIRP). Records were accepted if grid references were supplied by the collector or able to be inferred from the locality description. A mapping program (Amnesia) was developed by Ian Henderson to plot species distributions and calculate the following distribution parameters (see Fig. 2 and table 2).

Table 2: Distribution characters

Character	Units	Explanation	Source
Perimeter	kilometres (log transformed)	Perimeter of the minimal convex polygon (= perimeter around distribution)	CHIRP database/Amnesia
Area	km^2 (log transformed)	Area of minimal convex polygon (= area of distribution)	CHIRP database/Amnesia
N	Number (log transformed)	Number of discrete collection records	CHIRP database/Amnesia
MST	Kilometres (log transformed)	Length of minimum spanning tree	CHIRP database/Amnesia
Number of populations	Number (log transformed)	Number of groups of collection records more than 10 kilometres apart	CHIRP database/Amnesia
Local density	1-4	Subjective assessment of typical population density, 1 being sparse and 4 being locally dense	Andrea Brandon, Alastair Robertson, Bec Stanley, Geoff Walls, Kath Dickinson, Alan Mark
Occupancy	Number of populations/Area (log transformed)	How completely a species fills its range	Derived
Average disjunction	MST/N (log transformed)	Average MST branch length	Amnesia
Disjunction	Kilometres (log transformed)	Longest MST branch	Derived
Relative disjunction	Longest MST length/MST (log transformed)	Measure of relative disjunction	Amnesia
Geological specificity	1-4	Number of different geological substrates known to occur on	Geological map of New Zealand series (1:250000)

Statistics

Some problems have been identified with the methods used in some rare-common congener studies, such as the intercorrelation of traits (Kunin and Gaston, 1993). In an effort to overcome this problem I have chosen to perform separate Principal Components Analyses of morphology and distribution using *PC-●RD* rather than multiple regressions for the data analysis. In addition, a Canonical Correlation Analysis was performed to relate the two datasets using *SYSTAT* version 8.0 (SPSS Inc, 1998) on a subset of the characters. Derived characters (calyx split, seed length, W, No pops) were excluded from the Canonical Correspondence Analysis to avoid the problem of multicolinearity.

Results

PCA of morphology

In the Principal Components Analysis of morphology, 55% of the variance was explained by axis one (table 3 lists the traits and scores from the analysis for the first five axes). Nearly 11 % of the variation is explained by axis two.

Table 3: Pearson correlations between each character and the ordination axes (n = 33). (Correlations greater than 0.400 are shown in bold.)

Axis	1	2	3	4	5
Herkogamy	**-0.609**	-0.008	**0.600**	0.246	0.230
style length	**-0.925**	-0.097	0.180	0.047	0.055
filament length	**-0.901**	-0.075	0.201	0.104	0.049
anther length	**-0.836**	-0.131	0.270	0.273	0.011
corolla width	**-0.844**	0.390	0.175	0.110	0.064
corolla length	**-0.940**	-0.009	0.058	0.208	0.051
hooked hairs	-0.012	0.272	-0.355	**0.803**	0.336
Root system	**-0.643**	-0.156	-0.387	0.193	**0.532**
Rosette number	**-0.694**	**-0.492**	-0.115	0.233	0.198
Calyx split	**-0.796**	0.366	-0.333	0.066	0.250
Calyx length	**-0.791**	-0.195	-0.235	0.244	0.370
Calyx ratio	-0.227	**0.843**	-0.236	0.377	0.048
Seed length	**-0.902**	-0.059	-0.191	0.039	0.001
Winged seeds	**-0.620**	-0.276	**-0.531**	0.316	0.089
Relative seed length	**0.745**	-0.384	-0.265	0.084	0.155
% variance explained	55	10.9	9.6	8.4	4.9

All the floral size measurements have high negative correlation scores on axis one. A number of other characters correlate negatively with the first axis as well: herkogamy, woody root system, multiple rosettes, length of calyx split, calyx length and winged seeds. Relative seed length, which was calculated to take flower size into account, was positively correlated with axis one indicating that taxa with smaller sized flowers tend to have relatively large seeds for their size. Axis two has just two characters with correlation scores greater than 0.400, the calyx ratio with a high positive score and multiple rosettes with a high negative correlation. The calyx ratio is independent of flower size and negatively correlates with multiple rosettes. Axis three, which explains 9.6% of the variance, has herkogamy as well as the winged seeds loading strongly. Herkogamy is correlated with flower size and so also loads on axis one. Axis four, which explains 8.4% of the variance, has the presence of hooked hairs on the calyx loading strongly. Axis five, which explains just 4.9% of the variance, has a positive correlation with woody root system. The woody root system loaded strongly on axis one as well but exhibited a negative correlation.

Essentially, axis one is a measure of flower size (Table 3). The calyx properties, the degree of the split and the presence of hooked hairs, two measures of apparent dispersal ability, are not correlated with each other, and they are not correlated with flower size.

When the geographic measures are overlaid on the ordination of morphology, local density is negatively correlated with flower size (axis 1) and geological specificity is positively correlated with flower size (Fig. 3). Species with smaller flowers have the least dense populations and broadest geological tolerance while large flowered species are locally dense with more specific geological requirements.

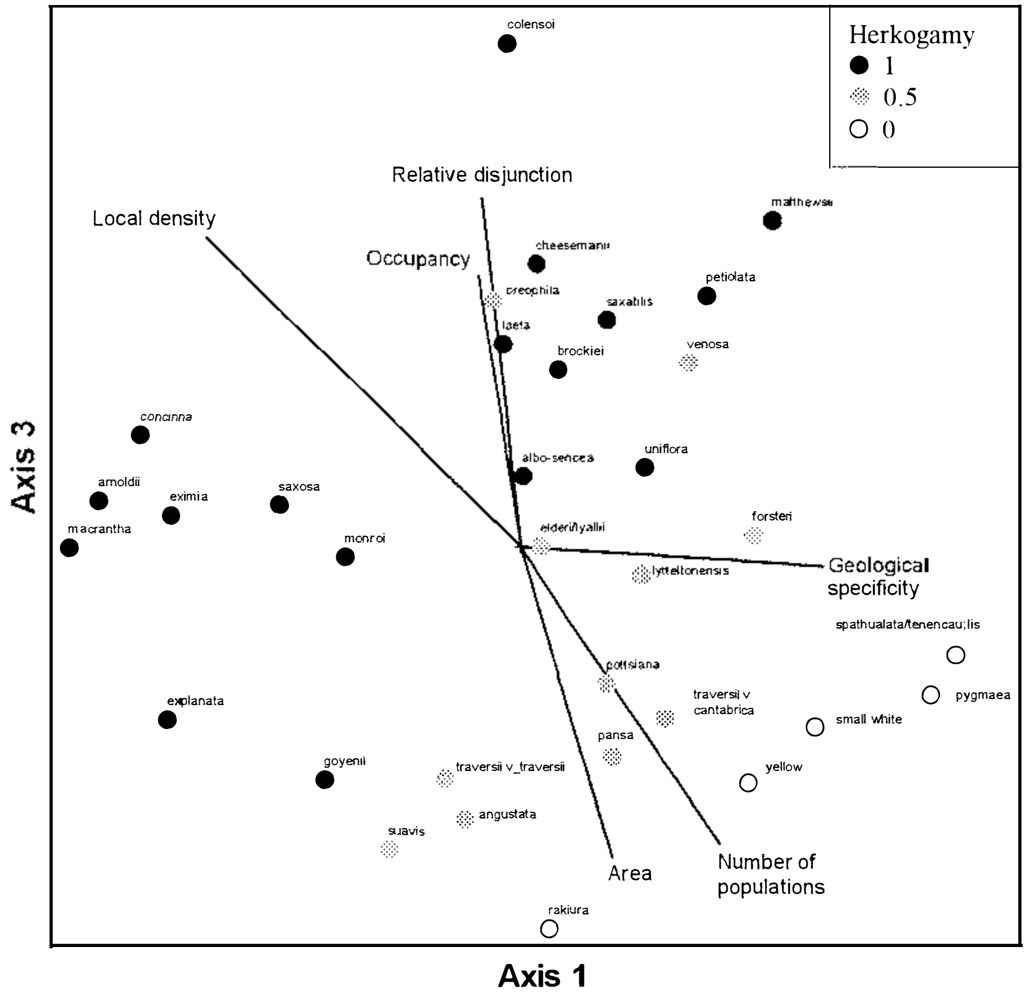

Figure 3: PCA of morphological traits with correlation coefficients of distribution traits overlaid (graphed if greater than 0.45). Species symbols represent herkogamy class.

There are no large distributional correlates with axis 2. However axis 3 has several of the distribution measures loading strongly (Fig. 3). Herkogamy for each species is shown overlaid on the ordination diagram of axis one and three. Herkogamy correlates positively with local density and negatively with the number of populations and the area of distribution (Fig 3).

PCA of distribution

Principal Components analysis of the distribution data shows that 72 % of the variation was explained by axis one, with axis two explaining a further 15 %. Table 4 lists the distribution measures along with their scores in the analysis.

Table 4: Pearson correlations with ordination axes (N= 33). Correlations greater than 0.400 are shown in bold.

Axis	1	2	3	4
Perimeter	**0.967**	0.242	0.030	0.006
Area	**0.978**	-0.015	0.163	0.091
N	**0.869**	**-0.418**	0.175	0.052
MST	**0.985**	0.136	0.020	0.007
Number of populations	**0.939**	-0.228	-0.051	0.000
Local density	**-0.667**	0.286	**0.627**	0.259
Occupancy	**-0.926**	-0.097	-0.262	0.132
Average disjunction	**0.663**	**0.707**	-0.167	0.048
Disjunction	**0.795**	**0.591**	0.030	0.032
Relative disjunction	**-0.709**	**0.640**	-0.142	0.142
Geological specificity	**0.762**	-0.209	-0.196	**0.570**
% variance explained	72	15.4	5.5	4

Essentially axis one is a measure of range size (Table 4). The perimeter, area, minimum spanning tree length, number of discrete collection records and number of populations are all measures of range size and are highly correlated with each other. Geological specificity is also highly correlated with range size - the wider range of geological substrates a species is found on, the larger the range size. Geological specificity is also partly independent of range as it also loads highly on axis four. Two measures of disjunction, the average minimum spanning tree (MST) branch length and the longest MST branch are correlated with range size. However, relative disjunction is negatively correlated with range size. Species with high scores for relative disjunction have small range sizes while those with high scores for longest branch length and average branch length have large ranges. Occupancy, a measure of how well a species fills its range, is negatively correlated with range size. Species with smaller range sizes fill their ranges well while those with large ranges do not. Local density also has a negative relationship with range size. Species with small range sizes have high local densities while those with large range sizes have low local densities. The measures of disjunction, while loading quite strongly on axis one are also loading on axis two. Axis two is the best measure of disjunction since the traits that score highly on this axis are those that describe large gaps in the distribution. Axis three has local density loading quite strongly, but it explains less than 6 % of the variance. Geological specificity loads on axis four as well as axis one, so while it is correlated with range size there are unrelated influences, but axis four explains just 4 % of the variation.

The morphological traits were then overlaid on the ordination of distribution characters (Fig. 4).

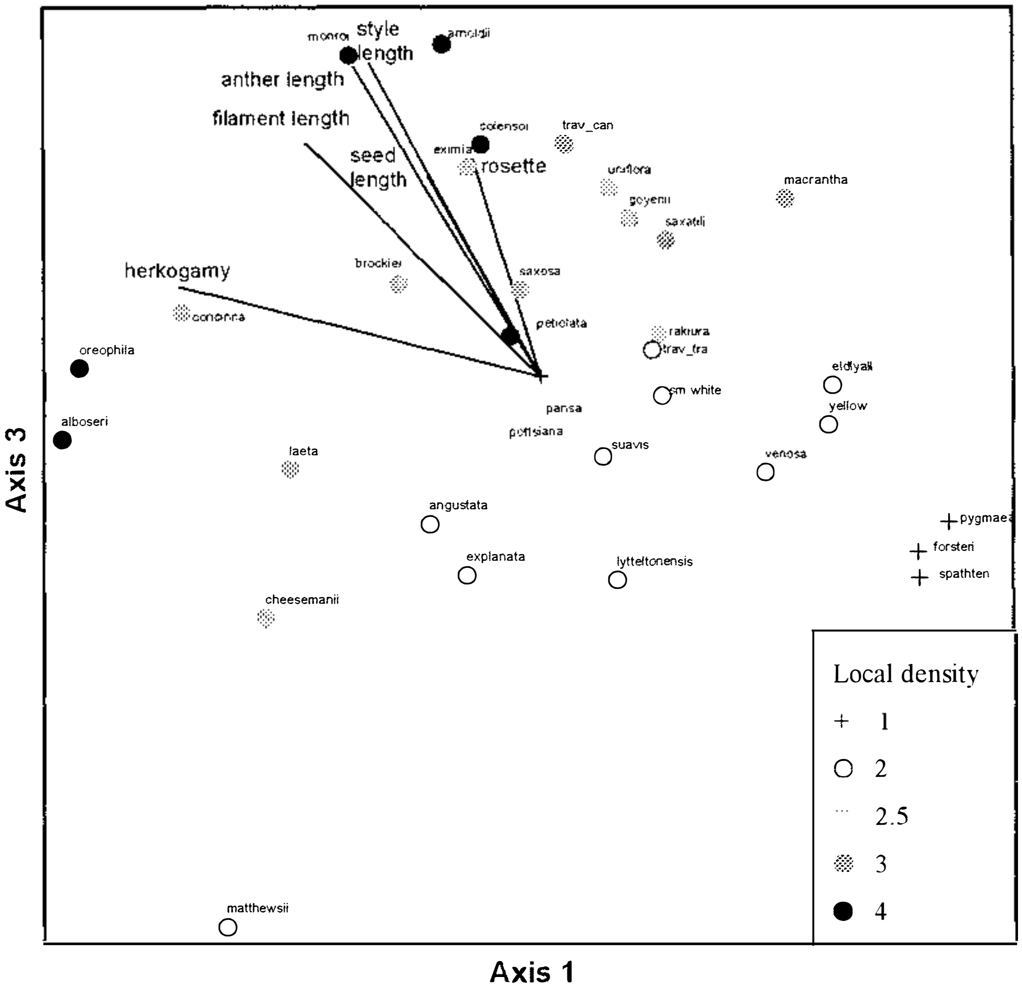

Figure 4: PCA of Distribution with morphology overlaid (graphed if greater than 0.45), species symbols represent local density class.

Small herkogamy is the best predictor of range size (Axis 1), with filament length also showing a strong correlation on the first axis. Species that are always herkogamous have smaller range sizes. Filament length, which is associated with herkogamy, is also a good predictor of range size. Seed length relative to flower size negatively correlates with range size. Plants with wider distributions have longer seeds in relation to their flower size. None of the apparent dispersal attributes correlated with range size. The presence or absence of hooked hairs, the degree that the calyx is split and the winged seed morphology had no relationship to range size. Seed size might also be expected to have some correlation with range size, as small seeds are easier to disperse by wind, but it does not. It correlates with flower size. The life history traits were not correlated with range size. The disjunction measures, which have loaded on axis two in the first PCA, are not correlated with any of the morphological traits included in this study.

CCA of combined data

The canonical correlation analysis for the combined set of traits was significant (p = 0.045) and the Bartlett test of residual correlations found just the first axis was significant (p = 0.015). In the analysis, all floral size characters, herkogamy, life history and relative seed length have high canonical loadings on Axis 1 (Table 5). Only the apparent dispersal traits have low loadings. All distribution measures used in the analysis have high canonical loadings.

This single, significant axis in the CCA closely parallels a combination of the two Axis one's from the separate PCA's.

Table 5: Canonical correlations for the combined dataset (correlations greater than 0.400 are shown in bold).

Morphological Characters		**Canonical loadings**
	Herkogamy	**-0.641**
	Style length	**-0.662**
	Filament length	**-0.683**
	Anther length	**-0.582**
	Corolla width	**-0.577**
	Corolla length	**-0.424**
	Hooked hairs on calyx	0.143
	Root system	**-0.486**
	Rosette number	**-0.402**
	Calyx length	-0.110
	Calyx ratio	-0.040
	Winged seeds	-0.198
	Relative seed length	**0.538**
Distribution Characters	Perimeter	**0.607**
	Area	**0.591**
	N	**0.538**
	Local density	**-0.852**
	Occupancy	**-0.456**
	Average disjunction	**0.482**
	Disjunction	**0.471**
	Relative disjunction	**-0.457**
	Geological specificity	**0.570**

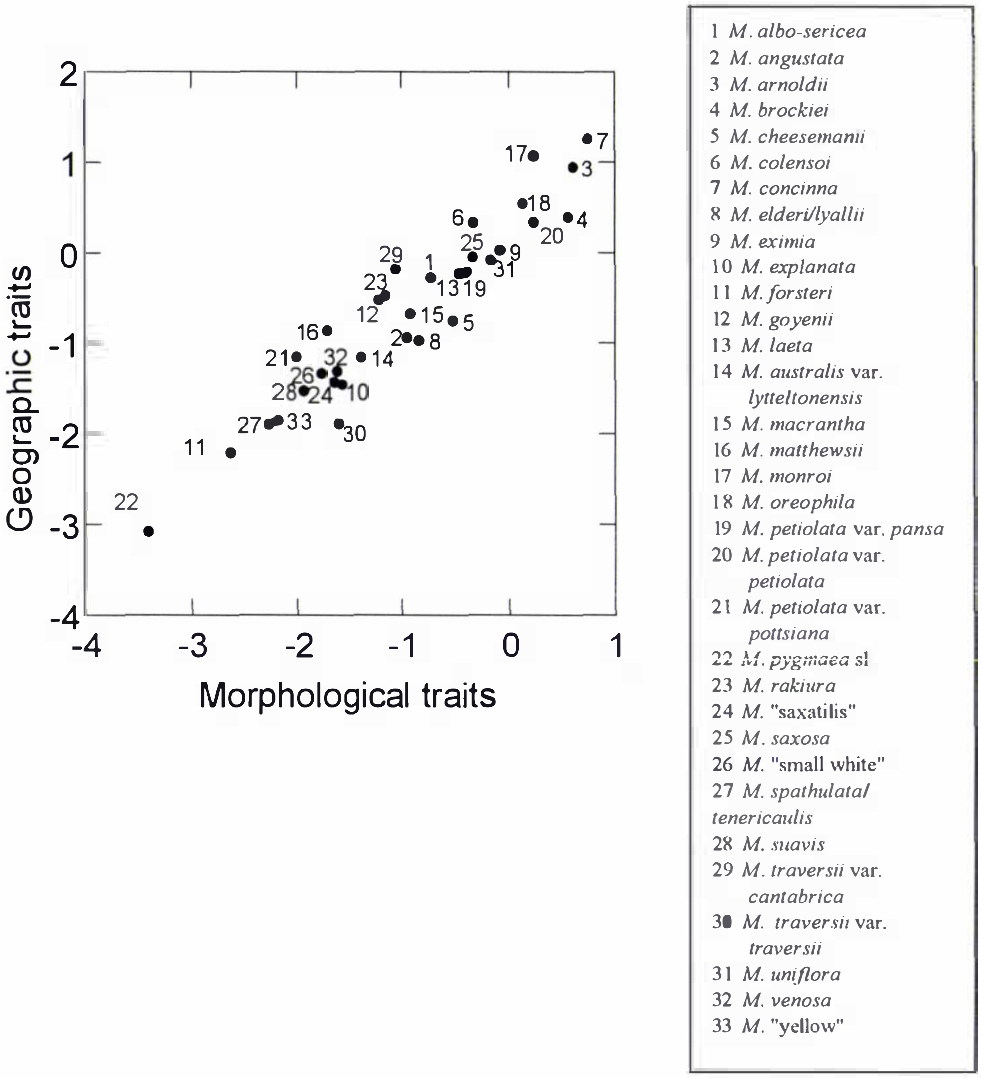

Figure 5: Graph of the first canonical axis of the canonical correlation analysis. It presents the relationship between the scores for the morphological variables with the scores for the geographic data for each species.

Fig. 5 shows the single significant canonical correlation axis separated into morphological and distributional components. Each species position is calculated by the fraction of its total score attributable to morphological traits plotted against the fraction attributed to distributional traits. The species comprise a gradient from selfing, small flowered, locally sparse and widespread taxa at the bottom left to pollinator-requiring, large flowered, locally dense and geographically restricted taxa at the top right. The lack of outliers indicates the predictability of distribution by floral morphology in this combined axis.

Discussion

These results show that breeding system and floral structure together predict accurately the range size of New Zealand *Myosotis* taxa. A key aspect of floral morphology that separates species is herkogamy (spatial separation of anthers and stigmas) and the associated range of flower size. Species with relatively wide distributions have little herkogamy, small flowers and are able to self-pollinate while those with small range sizes have significant herkogamy, larger flowers and are dependent on pollinators for seed set (see Chapter two). The pollination system thus appears to affect range size in New Zealand *Myosotis*. Local density, which is negatively correlated with range size, is also highly correlated with pollination system. As expected, pollinator-requiring taxa tend to occur in locally dense populations while self-pollinating taxa are generally found in low-density populations (as shown in Chapter three). It is not surprising that pollinator-requiring taxa are range-restricted although the link between pollination ecology and range size is not universal (Karron, 1987 c/f Kunin and Shmida, 1997). In fact, range size and abundance are positively correlated in several taxonomic groups (e.g. birds - Gaston, 1999). In New Zealand *Myosotis,* however, range size and local density or abundance are negatively correlated. The pollination system provides a mechanism by which this pattern can be maintained. Individuals of pollinator-requiring taxa that have dispersed too far from the parent population could be selected against as a result of density dependence for pollination.

Geological specificity is also correlated with breeding system. Pollinator-requiring taxa are found on fewer different geological substrates than those that self-pollinate. This may indicate that these taxa are habitat specialists, and that this requirement also restricts their range size. Ecological factors such as soil conditions may play a role in limiting species distributions. Alternatively, this may be an incidental consequence of a restricted distribution.

Occupancy, or the measure of how well a species fills its range, is negatively correlated with all the geographic measures of range size. Species with large range sizes do not fill their ranges as completely as species with small ranges. Occupancy is also correlated with local density, as species that are locally dense fill their ranges more completely that those that are locally sparse. However, occupancy, as I have defined it, may simply be related to range size i.e. point endemics will have an occupancy score of 100%.

A surprising result is that the apparent seed dispersal aids (calyx properties, seed size and winged seed morphology) are unrelated to the distribution patterns of New Zealand *Myosotis*. It may be difficult to test for any relationship between present-day dispersal aids and range size, since dispersal ability has been found to respond rapidly to changes in selection pressure (Cody and Overton, 1996). McGlone *et al*. (2001) conclude that current range patterns for plants found in New Zealand have arisen through Pleistocene extinctions, speciation and dispersal. It is possible that the dispersal aids that influenced range sizes are no longer present within this group, or perhaps they no longer function because of changes in climate or the loss or gain of animal vectors, but I found no evidence to support the concept that dispersal ability explains present day distributions. During the last 10 000 years (since the last glacial), all available habitats may have been filled. Therefore it could be that the most influential aid to dispersal during much of that time has since been eliminated through selection. Range size was negatively correlated with seed size, but positively correlated with relative seed size (adjusted for flower size) (Fig. 4). Small seeds can be expected to be more easily dispersed (Eriksson and Jakobsson, 1999) but having a larger nutritional store gives seeds a competitive advantage over those with smaller reserves in seedling establishment (Gross, 1984). It appears that while selfers do have smaller seeds, they maintain a relatively larger investment in each, possibly reflecting a trade-off between dispersal rate and establishment success. The selfers also produce constantly high proportions of seed per flower (Chapter 3), so in addition to making a relatively large investment per seed, they are also producing more seed per flower than vector-requiring taxa.

There are two processes that can give rise to population disjunctions. Local extinctions of populations that previously connected the now disjunct populations could result in present day distribution disjunctions. Alternatively, long distance dispersal and subsequent successful colonisations could also result in such population disjunctions. These two processes might be expected to relate to different aspects of morphology; long distance dispersal with dispersal aids and local extinctions with breeding system. Neither pattern is supported here. Population disjunctions (axis two of the distribution PCA) were not correlated with any of the morphological traits.

Distribution patterns were independent of the life history traits (rosettes usually single or multiple - used as a measure of vegetative reproduction, and root system woody or fibrous - used as a measure of plant longevity). These traits do not appear to be important determinants of distribution in this genus.

Stanley *et al.* (1998) have argued that environmental factors, such as wind exposure and snow melt, play an important role in governing the demography of *Myosotis oreophila*, an alpine, point endemic. It may be that ecological factors play a greater role in determining *Myosotis* species distributions than my results have indicated. The putatively outcrossing taxa were generally restricted to specialised substrates, which suggests that specific soil conditions may play an equally important role in controlling distributions.

Conservation

Distinguishing between rare species that are threatened from those that are not, is an important goal of conservation prioritisation and the sensible allocation of limited resources. My results can assist threat assessment and conservation management for New Zealand *Myosotis*. Locally dense, geographically restricted taxa are pollinator requiring while sparse, widespread taxa are selfing. Cases that break this general rule may be used to identify taxa at risk. For example *Myosotis australis* var. *lytteltonensis* is now known from just one location where it is not locally abundant. It has declined seriously in recent times (Pender, 1999). This species is at greater risk of extinction than one that is known from just one locality but is locally abundant, such as *M. albo-sericea*. Similarly, *M. petiolata* var. *petiolata* is known to survive certainly from only one of two recorded disjunct localities. This population of around 200 individuals occurs within a very small area and requires pollinators to set seed. This species is therefore more threatened than *M. arnoldii*, a species with a similarly disjunct distribution, but which is abundant at both localities.

This knowledge can also be applied to managing populations both *in situ* and *ex situ*. Hand pollinations may be required to assist seed production for species that have dropped to low numbers, or are being grown in unnatural conditions, such as a glasshouse. When designing translocations, those species that require pollinators should be established at population densities that ensure adequate pollination.

CHAPTER SIX

Species limits in the *Myosotis forsteri* Lehm./*venosa* Col. complex

Abstract

"Though the position of the anthers in relation to corolla scales is used in the first division of the key below, there is no firm conviction that this leads to the most nearly natural arrangement"

Moore (in Allan, 1961)

In the most recent taxonomic treatment, New Zealand *Myosotis* is initially split into two major clades depending the degree of anther exertion exhibited by the flowers. Anther exertion depends largely on the length of the filament. This has led to the recognition of species that cannot be distinguished in any other way and often results in morphologically similar taxa being grouped with morphologically less similar taxa. I examined species limits in *Myosotis forsteri* and *M. venosa,* a pair of vegetatively similar species. Filament length determines the degree of self-pollination that can occur and whether or not reproduction is assured. The use of filament length as a taxonomic character is not considered appropriate to make the initial division in this group of plants.

Introduction

New Zealand is the southern hemisphere centre of diversity for the genus *Myosotis* L. (Boraginaceae). Traditionally, the genus has been divided into sections based on corolla scale morphology and anther position in relation to corolla scales (Robertson, 1989). Lucy Moore carried out the last treatment of New Zealand *Myosotis* (in Allan, 1961). In this treatment, Moore accepted two sections, Myosotis and Exharrhena, for the New Zealand taxa. Moore admitted, "though the position of the anthers in relation to corolla scales is used in the first division of the key below, there is no firm conviction that this leads to the most nearly natural arrangement" (Allan, 1961; p 807). It is thought that this method of classification has led to the recognition of species on the basis of relative anther exertions that could not be differentiated any other way (Robertson, 1989).

The major influence anther exertion has in *Myosotis* is whether or not autonomous self-pollination can occur. Studies have been conducted on the breeding systems of several species of New Zealand *Myosotis* with a range of anther exertions (Chapter two, Robertson, 1989). All species studied to date are fully self-compatible. However, some species are able to self autonomously while others require a vector to achieve pollination depending on the degree of herkogamy and dichogamy exhibited by the flowers (Box 1). Some species have mating types that comprise a mixture of the two, and these species are considered intermediates along an out-crossing-selfing continuum (Robertson and Lloyd, 1991). Robertson and Lloyd (1991) found selfing ability was accompanied by a reduction in style length, pollen allocation and corolla size.

Box 1

Always herkogamous: where the stigma is initially exserted beyond the anthers and remains so.

Initially herkogamous: where the stigma initially protrudes but corolla extension lifts the anthers above the stigma during anthesis.

Never herkogamous: where the anthers and stigma are in close contact throughout.

Robertson and Lloyd (1991)

Cheeseman (1925) made the comment that several species of *Myosotis* "greatly resemble one another in habit and foliage, although widely different in the flowers". Two species that are very difficult to distinguish in the absence of flowering material are *Myosotis forsteri* and *Myosotis venosa*. In the case of *M. forsteri* Moore notes of "plants ... collected ... on many North Id. mountains, (the) relative lengths of filaments, of anthers and of styles vary...tending to bridge the gap between this species and *M. venosa*" (Allan, 1961; p 822). Of *M. venosa* Moore notes, "this species ... more closely resembles *M. forsteri*. Without the corolla and stamens specimens can be determined only tentatively by the slightly finer and more spreading hairs and the long style with clavate stigma" (Allan, 1961; p 823).

One of the study taxa used in the Robertson and Lloyd (1991) paper was *M. forsteri*. They showed this species is initially herkogamous (see Box 1), protogynous and has a "delayed" selfing mode of fertilisation (*sensu* Lloyd, 1979). In this species there is a chance for cross-pollination to occur during an initial female-only phase when the stigma is receptive prior to anthesis. By the end of this initial female only phase, due mainly to continued growth of the corolla, the anthers contact the stigma at anthesis, and any unfertilised ovules are spontaneously fertilized (Robertson and Lloyd, 1991). Seed production in this species is never pollen limited (Chapter two, Robertson and Lloyd, 1991).

The aim of this study is to determine whether relative anther exertion is an appropriate taxonomic character on which to initially segregate New Zealand *Myosotis* taxa, using two species that are very similar vegetatively: *M. forsteri* and *M. venosa*. Taxonomic characters such as floral and vegetative traits that are used to distinguish the two taxa are compared. The pollination requirements of the two species are compared and discussed in relation to mating system evolution and speciation.

Materials and methods

Study taxa

M. forsteri and *M. venosa* are short lived, forest herbs. Details of the floral differences can be seen in the photos of their flowers (Fig. 1). *M. forsteri*, on the left, has anthers borne on short filaments that are positioned within the corolla tube with their tips at the level of the corolla scales. The stigma is in contact with the anthers. In contrast, the anthers of *M. venosa* are borne on filaments such that they are held well above the corolla scales and are spatially separated from the stigma.

M. venosa occurs within the range of *M. forsteri* (Fig. 2). *M. forsteri*, has a much wider geographic distribution than *M. venosa*. Chapter five shows that pollination requirements are the best predictor of range size in New Zealand *Myosotis*.

Figure 1: Details of the floral differences can be seen in these photos. The anthers of *M. forsteri*, on the left, sit within the corolla tube and are in contact with the stigma. The anthers of *M. venosa* are exserted beyond the corolla tube and are not in contact with the stigma, which is held well above the level of the scales on a long style. Photos, Alastair Robertson (left); Bruce Sunnex (right).

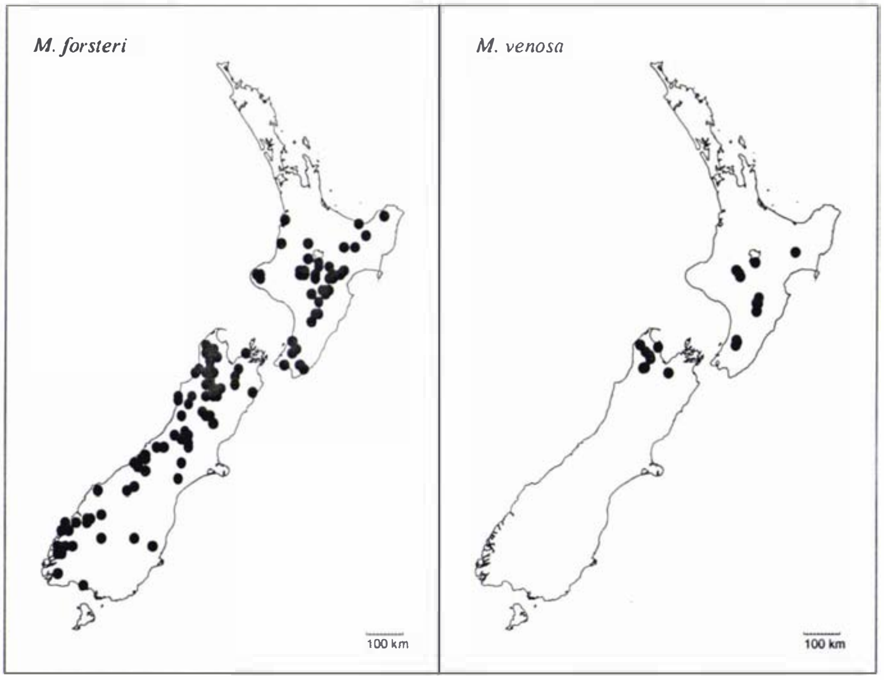

Figure 2: Distributions of *Myosotis forsteri* (left) and *M. venosa* (right) based on collection records held at Landcare herbarium at Lincoln.

Morphological measurements

Herbarium material was used for this study. Specimens were chosen to ensure that the full geographic range of the collections was sampled. Flowers were soaked in glycerine to re-hydrate them. Measurements were taken, under a dissecting microscope, of the following quantitative traits:

Leaf lamina length and width, petiole length, degree the calyx is split, peduncle length, style length, filament length, anther length, and corolla length and width.

Additional observations were made on two qualitative traits; stigma shape and filament attachment position. For each of the above specimens, whether stigmas were clavate or capitate and where the filament was attached in relation to the corolla scales were recorded.

The specimens examined and the raw scores for all traits are listed in appendix 6.

Data analysis

For the multivariate statistical analysis I used *SYSTAT* version 8.0. A step-wise linear discriminant analysis was performed on the quantitative morphological variables, in order to determine which variables are most useful for discriminating between the two species.

Autofertility experiments

Plants were caged to exclude pollinators to determine the pollination requirements at one population of each species in the Cobb Valley, Nelson, New Zealand. Seed set by plants in pollinator-excluded conditions were compared to that set by plants in unmanipulated conditions. Herkogamy was measured for several plants in each of these populations.

Geographic variation

Measurements of corolla length and width and lamina length and width were mapped against geographical position to determine whether clinal variation exists in vegetative traits and whether it is shared between the two species.

Results

Morphology

Stepwise discriminant analysis of the morphological variables stopped at the last variable. The final model found by backward stepping contains just one variable: filament length (Table 1) with an F-to-remove value of 249.04 (i.e. greater than 3.9, the critical F value). This data set provided just one quantitative morphological measurement that can be used to separate the two species.

Table 1: Results of stepwise discriminant analysis

Variable	F-to-remove	Tolerance	Variable	F-to-enter	Tolerance
Filament length	249.04	1.000000	Lamina length	0.84	0.973391
			Lamina width	0.36	0.977962
			Petiole length	0.01	0.995018
			Degree calyx is split	0.84	0.998467
			Peduncle length	0.04	0.992291
			Style length	0.13	0.768336
			Anther length	0.04	0.880618
			Corolla width	0.11	0.900049
			Corolla length	0.46	0.930807

98 % of the specimens are classified correctly using the single variant discriminant function (Table 2). One individual of *M. forsteri* was incorrectly classified as *M. venosa*. This illustrates that there is some degree of overlap between the two species.

Table 2: Jack-knifed classification matrix.

	***M. forsteri* (actual)**	***M. venosa* (actual)**	**% correct**
***M. forsteri* (predicted)**	53	1	98
***M. venosa* (predicted)**	0	10	100
	53	11	

Boxplots were graphed to examine the distributions of the quantitative traits that were used in the discriminant analysis (Fig. 3). The distributions of the filament lengths do not overlap for the two species, but the longest *M. forsteri* filament is close to the shortest *M. venosa* filament (Fig. 3). All the other distribution ranges overlap when comparing the two species (Fig. 3). *M. venosa* always has higher medians within the floral measurements and lower medians within the leaf size measurements than *M. forsteri* (Fig. 3). These results show generally continuous distributions for those quantitative traits measured for this study.

Stigma type and filament attachment position

Both clavate and capitate stigma types were observed on specimens of both species (Appendix 6).

Filaments were generally attached below the scales in *M. forsteri* except for two cases in which filament attachment was at the scales. The filaments of *M. venosa* were more usually attached at the level of the scales, and less often below the scales (Appendix 6).

Autofertility experiments: seed set results for caged and open pollinated plants.

M. forsteri sets consistently high levels of seed whether or not pollinators are excluded. These results show that *M. forsteri* does not depend on pollinators for seed production and is never pollen limited. *M. venosa* sets very low levels of seed when pollinators are excluded, and sets considerably lower seed in unmanipulated conditions than *M. forsteri*. These results show *M. venosa* depends on pollinators for seed production and is sensitive to pollen limitation (Table 3).

Geographic variation

Figures 4 and 5 show leaf and flower sizes as they vary geographically. Note that leaf and flower sizes vary considerable at different sites, but generally they vary independently of each other (Fig. 4 and Fig. 5). However, adjacent populations of *M. venosa* and *M. forsteri* tend to share leaf size (note the large leaves in the southern North Island, and small sizes in Nelson).

Table 3: Mean seed set/flower (maximum 4) in open conditions (control) and with pollinators excluded. The range of herkogamy measurements with means is presented.

Species	Control	Pollinator excluded	Herkogamy (mm)
Myosotis forsteri	3.84	3.8	range -0.8 - 0 (0)
Myosotis venosa	1.4	0.5	range 0.3 - 1.3 (0.7)

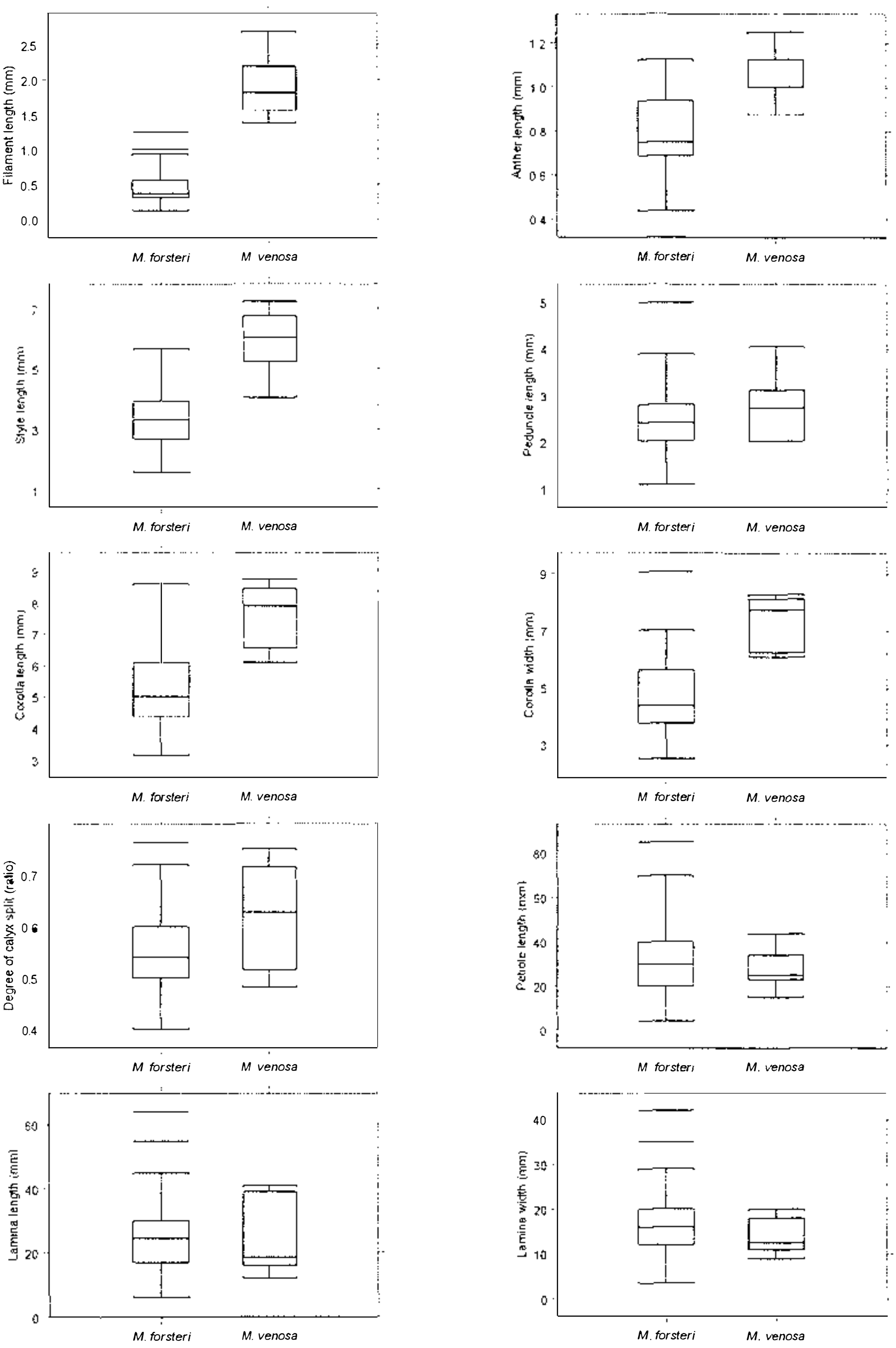

Figure 3: Character distribution and variation for each of the ten morphological traits graphed for each species.

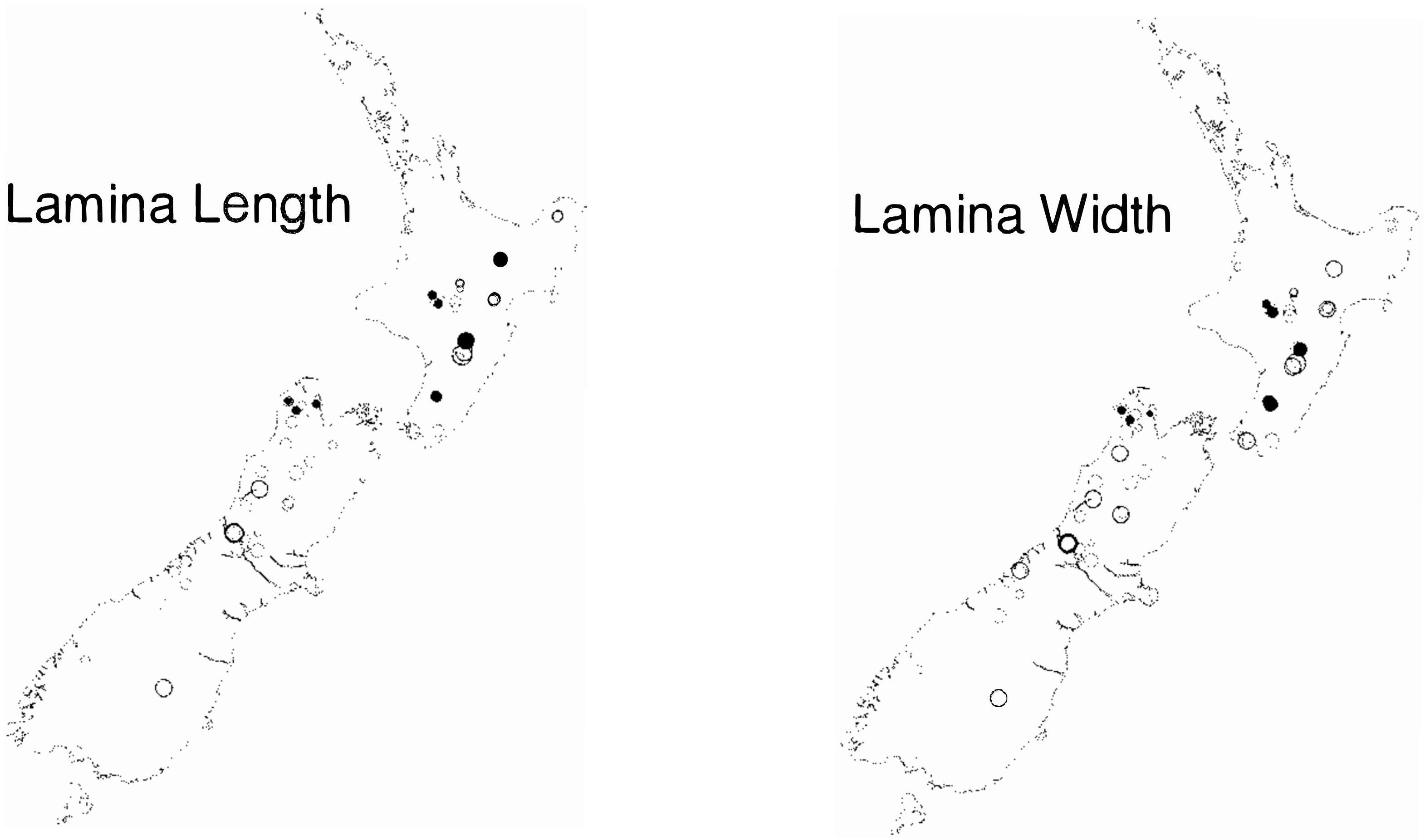

Figure 4: Lamina length and width mapped for *M. forsteri* (open circles) and *M venosa* (closed circles). The size of the symbol represents the size of the leaves.

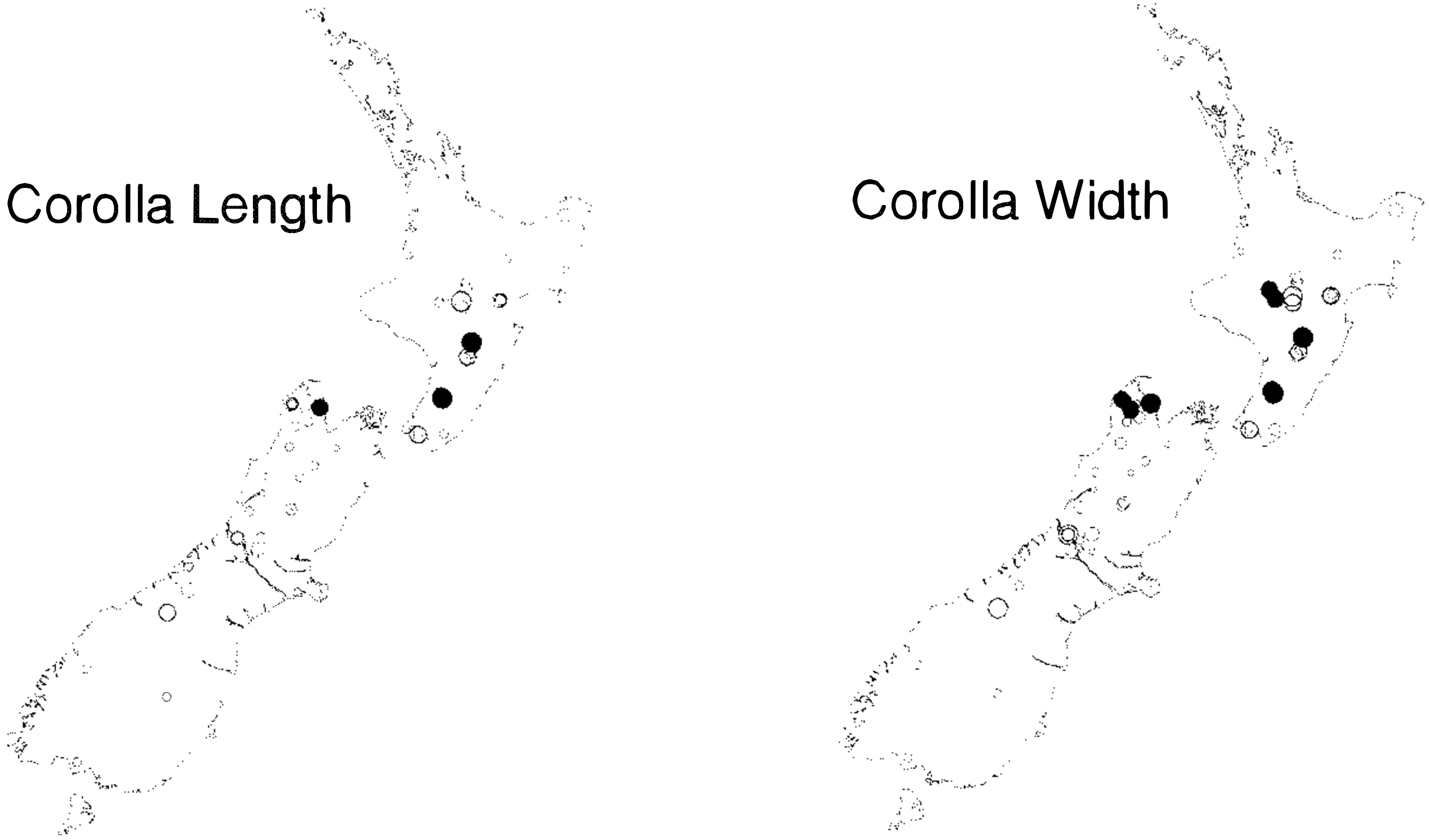

Figure 5: Corolla length and widths mapped for the two species, *M. forsteri* (open circles) and *M. venosa* (closed circles).

Discussion

These results are consistent with the initial view of Robertson (1989) that these species are vegetatively indistinguishable. With the exception of filament length, none of the floral characters measured have distinct dimensions with which to classify these species. Even filament length was unable to discriminate 100% of the cases correctly.

However the effect that the filament length has on the pollination requirements of these two species is considerable, in that it determines what degree of self-pollination may occur and whether or not reproduction is assured. This study showed the plants in the Cobb Valley population of *M. forsteri* have the same delayed selfing mode of fertilisation as Robertson and Lloyd (1991) described. These plants reproduce by a mixture of cross and self-pollinations. Plants are able to self-pollinate autonomously after all chances for cross-pollination have lapsed and seed production is constantly high regardless of pollinator activity. The results for *M. venosa* provided evidence for quite a different mating type. *M. venosa* is always herkogamous, requires a vector to achieve good seed set, and is vulnerable to pollen limitation in open conditions. This is inferred from the data collected on other species presented in Chapter two. In this situation a vector must mediate fertilization, whether by cross- or self-pollination. When pollinators are scarce, *M. venosa* will be at a disadvantage in being unable to produce seed, while *M. forsteri* will be able to produce a consistently high number of seeds. When pollinators are common, *M. venosa* may be able to produce up to the same level of seed as *M. forsteri* in the same conditions, but the seed produced by *M. venosa* may have been done so by a greater level of cross-pollination.

Much has been written regarding the superior quality of outcrossed over selfed progeny (see Holsinger, 1996). In general, selfing should provide reproductive benefits whenever the pollination environment (availability of pollinators and mates) is poor (Lloyd, 1979). Levels of inbreeding depression will affect this. However, the high and consistent seed production of self-fertile plants could allow for considerable selection in the offspring without affecting demography at all (Luitjen *et al.* 1998). Populations of plants with mixed mating systems such as *M. forsteri* will be at an advantage in changeable environments. When pollinators are absent, individuals with selfing ability will be the only ones contributing genes to the next generation. When pollinators are present, the ability to delay selfing and receive cross-pollen will improve seed quality if inbreeding depression is occurring. Wright (1939) considered such a situation to be advantageous to populations in which reproduction is predominantly selfing. He considered occasional crossing could allow for effective selection by genotypes in a "continuously restored field of variability" (Wright, 1939). The mixed mating strategy therefore allows individuals to reproduce in difficult circumstances but also take advantage of more favourable conditions should they occur. It appears that *M. forsteri* has the advantage of being able to set seed in all conditions.

Theoretical models suggest that pollination biology may play an important role in mating system evolution (Holsinger, 1991). The increased quantity of seed production that selfing assures and the increased quality of the seed production that crossing potentially delivers are opposing forces which act at different levels on individuals and populations in different ecological circumstances (Lloyd, 1979). These circumstances will be unique to each individual and population. In particular, pollinator abundance is expected to have considerable influence over selection for self-pollination when some level of reproduction is required for population persistence. Pollinators were found to be less abundant and less dependable when self-pollinating populations were compared with out-crossing populations of *Clarkia xantiana* (Fausto *et al.* 2001).

An important outcome of the evolution of autogamy is the associated development of reproductive isolation. When pollinators become scarce, individuals with selfing ability will become reproductively isolated from others within their own populations and from other populations. This process splits the population into non-interbreeding groups and over time leads to the production of groups of essentially identical individuals. The switch to selfing would also switch selection from individuals to genotypes, with less adaptive combinations being displaced by the most adaptive ones until just one clone per ecological niche exists (Wright, 1939). If we consider *M. forsteri*, with the delayed selfing syndrome, pollinator activity will determine what degree of reproductive isolation will occur and whether individuals or genotypes are under selection. When we consider the effects on *M. venosa*, pollinator activity will ultimately determine whether or not a population will persist and selection will act at the population level.

The generally accepted view of the direction of mating system evolution is that it will usually proceed from outcrossing to selfing (Raven, 1973; Lande and Schemske, 1985).

However, as much of the extant flora of New Zealand, including *Myosotis*, is thought to have arrived via recent long distance dispersal (e.g. Winkworth *et al.* 1999; McGlone *et al.* 2001), selfing is the more likely ancestral state for these taxa. Outcrossing types are thought to have arisen secondarily in New Zealand during subsequent speciation that has occurred as new environments have emerged (Lloyd, 1980) though Lande and Schemske (1985) suggest that it is difficult to evolve in this direction. Distributions may provide some insight into the direction of mating system evolution within this group. Geographic range will be affected by pollination biology. Self-pollinating populations are more likely to establish than outcrossing populations (Baker, 1955, Pannell and Barrett, 1998). Self-pollinating individuals are potentially able to colonize new sites by the germination of just one seed. All they require for successful colonisation to occur, is to disperse into an environmentally suitable, unoccupied habitat. They do not require the presence of pollinators or conspecifics to successfully reproduce. Self-pollinating individuals are therefore expected to be superior colonizers of pollinator- and conspecific-poor environments. Therefore, self-pollinating populations would be expected to occupy a wider range of sites and this will affect the geographic distribution of populations. This pattern has been observed within New Zealand *Myosotis* generally. Species with selfing mating types have larger range sizes than species with pollinator-requiring types (Chapter five). *M. forsteri* and *M. venosa* also follow this pattern. That selfing species have wider geographic ranges than pollinator-requiring species adds some weight to the argument that selfing is the ancestral state. If they are an older lineage, they will have had more time to expand their range. However, it could be argued that as selfing species are superior colonizers, they will become more widespread than their pollinator-requiring ancestor, for which successful colonization hinges on more variables. This requires further investigation.

Whatever the direction of evolution, self-pollinating individuals are expected to put less resources into their pollen production and flower size (Lloyd, 1987). Although corolla size, anther length and style length are not useful characters to distinguish these two taxa, the vector-requiring *M. venosa* tends to have larger values for these traits than *M. forsteri*. This trend was also shown for selfing versus non-selfing taxa in Robertson and Lloyd (1991).

Stigma type did not divide the species at all. Further study of this character is required, however, preliminary observations suggest the shape of the stigma may be an artefact of the drying and pressing process associated with the preparation of herbarium specimens.

The continuous variation observed in filament, style and anther lengths and corolla sizes shows that there is a lot of phenotypic variation within each taxon. Finding geographic clines in vegetative traits that are independent of breeding system along with the continuous variation observed in floral characters, lends some strength to the proposition that this species pair may be switching between the two breeding systems. This will be primarily influenced by pollinator abundance at specific sites. In areas where pollinators are scarce the individuals with the ability to self-fertilize will be the only ones producing offspring and could quickly become the dominant morphological type in the population. It is unusual to see these two taxa growing together but it is not unknown. There are collections of *M. venosa* that were found "growing with *M. forsteri*" (CHR 190975; CHR 219277).

It would be interesting to assess how much of the observed phenotypic variation in floral traits has a heritable basis that may respond to natural selection. Future studies could examine this in detail.

The only morphological character that distinguishes this pair of species is filament length, which in turn determines the level of autonomous selfing that can occur. Such variability in autofertility has not been considered enough of a distinction to support species status in other taxa. Luijten *et al.* (1999) found such variation in *Gentianella germanica* in three populations studied. They also found that the average herkogamy exhibited by flowers in one of their study populations had changed from being positive in 1992 to being negative in 1998 (Luijten *et al.* 1999). In *Arenaria uniflora* there are large-flowered protandrous populations and small-flowered autonomously selfing populations (Fishman and Wyatt, 1999). On the other hand, outcrossing populations of *Clarkia xantiana* have been awarded subspecies status (subsp. *xantiana*), as they are considered distinct from autonomously selfing populations (subsp. *parviflora*).

The fact that these two taxa are indistinguishable without flowers, leads me to believe that they are more closely related to each other than any other species of *Myosotis*. Therefore, I conclude that the degree of anther exertion in relation to the scales is not an appropriate character for the initial division of the New Zealand taxa, as it separates those species that are most closely related to each other and groups together those that are less closely related.

CHAPTER SEVEN

Conclusion

The preservation of biological diversity depends on maintaining self-sustaining, viable populations of all those species that remain on this planet. The focus for achieving this goal is at those species that are threatened with extinction. A general rule of thumb predicts that rarity will precede extinction. While there has been a recent increase in rarity studies our knowledge of rare plant biology and population dynamics is still inadequate (Gaston, 1994). There are several different types of rarity and not all rare species are threatened. The scale of the problem is such that conservation management of rare plant populations must invariably proceed on less that ideal knowledge of the species in question. Therefore, any improvement in our knowledge of specific rare plant biology will assist in the preservation of our biodiversity.

The results of these studies have increased our knowledge of specific taxa of New Zealand *Myosotis*. Recurrent patterns have emerged such that they can be extrapolated to infer similar conclusions for other biologically similar species within the genus. This increase in knowledge regarding the biology of specific rare taxa improves our general knowledge of rarity in *Myosotis* and improves our overall understanding of rare plants generally.

This thesis has improved the level of pollination biology knowledge in the genus by expanding on the work of Robertson (1989). In particular, we now know for certain that pollinators are required for the brush blossom taxa (Chapter two). A simple measurement of herkogamy in the field can assist in determining the pollination requirements for other species in this genus.

Pollinator -requiring taxa typically occur in larger, denser populations than non-pollinator-requiring taxa. Examining the level of variation in seed set under natural conditions for those taxa that required pollinators, found much of this variation was due to local flower density (Chapter three). These plants were pollen limited in low-density patches. With as many as 33% of the New Zealand taxa considered threatened in some way (de Lange *et al.* 1999) this has implications for conservation management.

Pollinator behaviour will be influencing this process. Population management will be enhanced by knowledge of the pollination requirements of the species. Threat assessments will be aided by knowledge of pollination requirements, as the risks associated with low-density, small population sizes are not there for self-pollinating taxa. Hand pollinations may be required to get seed production in *ex-situ* collections if the species cannot autonomously self-pollinate. If management is *in situ*, population enhancements may be required to improve seed production, to maintain self-sustaining populations. We need to understand pollinator behaviour in order to advance our knowledge of Allee effects.

The precocious bud pollination found in Dun Mountain populations of *M. monroi* is an interesting and previously unstudied phenomenon. This characteristic effectively lengthens the initial female-only phase for this species thereby increasing chances for cross-pollination to occur in extreme conditions where the flowering period is very short. This type of stigma presentation is otherwise not known in the genus and has not been reported for any other species worldwide.

The New Zealand-wide study into how rarity relates to species characteristics makes the connection that, typically, the species with different pollination requirements have different forms of rarity. Locally dense, geographically restricted taxa are pollinator requiring while sparse, widespread taxa are selfing. Cases that break this general rule may be used to identify species at risk.

The species complex of *M. forsteri* and *M. venosa* study is useful in that it identifies a major problem with the current taxonomic treatment of the New Zealand species of the genus. The initial division using relative anther exertion splits the most closely related species apart and groups them with less closely related taxa. It also suggests that breeding system may be unstable and subject to flux.

In conclusion, the increase in knowledge regarding specific taxa has provided a much-needed boost to our understanding of the population dynamics of these rare species, which ultimately can be used in conservation management for those taxa considered at risk of extinction. It can assist in the protection of populations that are not threatened in such a way that they do not become threatened and it can direct efforts towards the more pressing problem situations. It has also highlighted one of the significant problems with the current classification system.

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Appendix 1

Nelson *Myosotis*

A report for: The Conservator

Department of Conservation
Nelson/Marlborough Conservancy
Nelson

Attention: Technical Support Officer (Nelson Threatened Plants)

Under Contract: NELCO-12554

June, 2000

Andrea Brandon

Ecology Group
Institute of Natural Resources
Massey University
Private Bag 11-222
Palmerston North
06 356 9099
A.M.Brandon@massey.ac.nz

Abstract

The current taxonomic status of all native *Myosotis* in the Nelson region is presented. There are 18 distinctive taxa in the region. Factors that threaten population viability of all species are assessed. Species limits in the *Myosotis forsteri* / *venosa* complex are delineated. These species differ only in one respect; that of filament length. Species limits in the *M. brockiei* complex are discussed. *M.* 'gorge creek' falls within the species description of *M. brockiei*. *M.* 'otuhie' falls just outside the limits of *M. brockiei* in that it has different shaped corolla lobes and a wider corolla tube. Pollination syndromes of selected species were studied. These species fall into either one of two pollination syndromes based on the degree of herkogamy (spatial separation of stigma and anther presentation) exhibited by the flowers. Results of pollination experiments showed all species are self-compatible but differ in their ability to set seed autonomously. Those that could not set seed without a vector (non-autonomous) were all found to set variable amounts of seed in natural conditions. For these plants local density had strong effects on seed set and plants were always pollen limited in low-density patches. Those that could set seed without vectors of any sort (autonomous) were found to set consistently high levels of seed, local density had no effect on seed set and plants were never pollen limited. These results have implications for the conservation of threatened *Myosotis* species as knowledge of pollination syndrome and density dependent reproduction can be used to determine suitable management strategies. Rarity patterns in the genus are linked to reproductive biology, as density dependent reproduction is likely to influence both population structure and size of existing populations, while eliminating those populations too small to attract pollinators.

1. INTRODUCTION

1.1 The genus *Myosotis*

The forget-me-not *Myosotis* is a cosmopolitan genus of the Boraginaceae and has a worldwide temperate distribution. Current references for the total number of species are inaccurate. Mabberly (1987) puts the worldwide distribution at 50 with 41 of these occurring in Europe. However, there are 34 New Zealand species described in the Flora of New Zealand (Allan, 1961), although Webb *et al.* (1990) put the number of New Zealand species at 47 and the worldwide total at about 100. The New Zealand total may still increase as the numerous tag named taxa are given taxonomic treatment. Druce (1993) puts the New Zealand total at about 60 species, making *Myosotis* one of this country's largest plant genera. New Zealand is the Southern Hemisphere centre of diversity for a distinct austral group of species (Grau and Schwab, 1985; Winkworth *et al.* 1999). In addition to the New

Zealand taxa the austral group contains four other species; two in Australia, one in South America and one in New Guinea.

1.2 Rarity and the New Zealand taxa

Within the New Zealand group there are a large number of extremely localised taxa, which are often restricted to a single mountain or range, and the genus contains some very rare species. By contrast, other species have very sparse distributions and although they are relatively widespread, they are never common where they occur. Several different abundance and distribution patterns or rarity patterns are evident within the New Zealand taxa. For example *Myosotis forsteri* is found within about three quarters of New Zealand's land area south of latitude 38° S (Landcare Research Herbarium, Lincoln, hereafter referred to as CHR; Allan, 1961). While having a widespread distribution, it is often found as a single individual or as two or three plants in a population and has only occasionally been seen in populations as large as 30. Although never common where it occurs, it is not considered threatened in any way. By contrast, *M. concinna*, an endemic of the Marino Mountains in north-west Nelson, occurs in a very restricted geographic area. Although confined to specific habitats within the alpine zone, it is quite common where it occurs.

Species can be rare in different ways and for many reasons. Aspects of reproductive biology have been linked to rarity in several studies (e.g. Rabinowitz, 1978; Oakwood *et al.* 1993, Quinn *et al.* 1994; Kunin and Shmida, 1997). Breeding system, pollination ecology, dispersal ability and vegetative reproduction are important factors in determining plant distributions. For example, self-pollinators can grow, reproduce and colonize a new area by the germination of just one seed. They can reproduce in isolation and do not require the presence of others to persist. Therefore small population size is not necessarily a sign that a population of such a species is threatened. Obligate outcrossers or self-incompatible plants require local population densities at levels that attract vectors and ensure a supply of cross pollen to achieve pollination. Therefore, to be successful, they may need population sizes that are considerably larger than those required for selfing species and a small population size could be a threat to viability. Other factors, such as environmental constraints, and the evolutionary and recent history of populations will affect abundance and place limits on distributions. Identifying the factors involved in maintaining the distribution patterns observed will assist in understanding the vulnerability of small populations.

The state of rarity can be used to describe several different patterns of density and distribution. Rarity is defined simply by Gaston (1994) 'as the state of having low abundance and/or a small range size'. Some authors have attempted to define rarity by categorising it, as a temporal and/or spatial phenomenon (e.g. Rabinowitz, 1981; Fiedler and Ahouse, 1992). Others have focused on evolutionary history (relictual versus incipient e.g. Prober *et al.* 1990), genetics (e.g. Hamrick *et al.*

1991), or conservation priority (e.g. Dopson *et al.* 1999). de Lange and Norton (1998) addressed the inconsistency in both the meaning and usage of the term rarity as it relates to our threatened plants and proposed revised risk categories that took this into account. In particular, the new system, which has now been adopted by the New Zealand threatened plant committee and used for the recent reappraisal (de Lange *et al.* 1999), attempts to separate rarity from threat.

1.3 Threatened *Myosotis*

A large proportion of the New Zealand taxa in the genus are considered threatened, with 33% featuring on the latest list (de Lange *et al.* 1999), but are often not well enough known in the field or are of uncertain taxonomic status (Molloy and Davis, 1994; Cameron *et al.* 1995). Priority setting and management decisions for their conservation are impeded by this lack of knowledge. Six of the Nelson taxa are currently listed as threatened in some way. *M. laeta* has been classified as Vulnerable (Cameron *et al.* 1995) but the reappraisal to the newly erected Range Restricted category (de Lange *et al.* 1999) acknowledges recent survey results; *M. brockiei* has been classified as Rare (Cameron *et al.* 1995), I (Insufficiently known) (Dopson *et al.* 1999) and is now classified as Range Restricted (de Lange *et al.* 1999); *M. angustata*, *M. arnoldii* and *M. concinna* were on the local plant list (Cameron *et al.* 1995) and are now classified as Range Restricted (de Lange *et al.* 1999). *M. petiolata* var. *petiolata* has been classified as Insufficiently Known since 1993 (Cameron *et al.* 1993; Molloy and Davis, 1994; Cameron *et al.* 1995; de Lange *et al.* 1999).

1.4 Taxonomic problems

Numerous tag-named taxa have arisen over the years and there is little doubt that a full revision of the austral group of the genus is overdue. Their rarity has contributed to the taxonomic difficulties as they are seldom seen in the field and are simply not that well known. New Zealand *Myosotis* is now thought to be of very recent speciation history (Winkworth *et al.* 1999). This may be a causal factor as speciation may be so recent that incipient taxa have not yet experienced full population expansion nor dispersed into all potential habitats. The species is an evolutionary concept (Harvey, 1996) but at any point in time we are looking at a 'snapshot' of the evolutionary process, where for some taxa, boundaries may be vague and inconsistent across the range of forms while for others species limits may be clearly evident.

A lot of the taxonomic difficulties have arisen within selfing taxa. For each individual in a selfing population, reproduction is usually uniparental, therefore there is little chance for any gene flow from within their own population much less another population. Each plant is essentially reproductively isolated from any other. Under these circumstances even when chance occurrences for pollinator-mediated pollen transfer between local flowers does take place, the close familial relationship between plants in the same population will be unlikely to confer any new genetic material to their progeny (i.e. inbreeding limits the effectiveness of meiosis). This results in races being formed, where in a single population, all individuals may be genetically identical, and the lack of any recombination between unrelated individuals results in reduced variability within that particular population.

2. NELSON TAXA

The genus is well represented in the Nelson region by rare but well described species as well as taxa whose affinities in the genus are not well understood. The Nelson area contains 20 named taxa (Allan, 1961; Moore and Simpson, 1973; CHR), with an additional 7 tag-named taxa (Druce, 1993; Courtney, pers. comm.). Of these, *Myosotis* 'gorge creek', *M.* 'otuhie', *M.* 'paynes ford', *M.* 'cundy creek' and *M.* 'flora' were unusual forms that were difficult to place into species and required taxonomic interpretation. The other tag-named taxa are in the *M. australis* aggregation; *M. australis* 'small white', *M australis* 'calcareous white' and *M. australis* 'yellow' (Druce, 1993).

2.1 *Myosotis forsteri / venosa* complex

The highly variable *M. forsteri* aggregation is a self-pollinating taxon with many morphological forms. Small differences are often found among populations of this species. *M. forsteri* has an initial stage when stigmas are receptive and pollination can occur prior to anther dehiscence, allowing some chance for outcrossing (Robertson and Lloyd, 1991). This is variable among populations, which is reflected in the morphological variation seen in the field.

The first key in the current treatment by Moore (in Allan, 1961) follows the traditional pattern of sub-generic classification of the genus by separating species on the basis of degree of anther exertion into two sections. This trait has proved to be difficult to use to identify taxa as there are intermediate forms that make division arbitrary. Moore admitted, "though the position of the anthers in relation to corolla scales is used in the first division of the key below, there is no firm conviction that this leads to the most nearly natural arrangement" (p 807: Allan, 1961). It is thought that this method of classification led to recognition of species on the basis of relative anther exertions that could not be differentiated any other way (Robertson, 1989). In the case of *Myosotis forsteri* Moore notes that "plants ... collected ... on many North Id. mountains, (the) relative lengths of filaments, of anthers and of styles vary...tending to bridge the gap between this species and *M. venosa*" (p 822). Of *M. venosa* Moore notes, "This species ... more closely resembles *M. forsteri.* Without the corolla and stamens specimens can be determined only tentatively by the slightly finer and more spreading hairs and the long style with clavate stigma" (p 823: Allan, 1961).

In order to gain a little more insight into how such differences affect species limits, a detailed study of *M. forsteri* and *M. venosa* was carried out. The difference in leaf hair and stigma type varied within the specimens studied in a manner that reflects regional rather than specific variation. These species cannot be distinguished without flowers being present on specimens. These two taxa differ in only one dimension; that of filament length, which determines the extent of anther exertion. The major difference anther exertion makes to an individual is whether or not they are able to self-pollinate. As there are several of these species complexes in the New Zealand group, taxonomic decisions regarding this situation will have to be made when the genus is revised as a whole. For now, these taxa will remain treated as separate entities.

2.2 *Myosotis brockiei* complex

Populations of *M. brockiei* vary considerably in habit, leaf shape and leaf hairiness. These differences occur among populations at the Cobb Reservoir, all growing on the same geological substrate but also occur between populations of those plants and those on the limestone outcrops. The old Magnesite Quarry at the Cobb Reservoir has a population of plants that have a relatively sparse covering of more or less appressed hairs on their rosette leaves. These plants have a narrow petiole that tapers gradually from an elongated, elliptic lamina and plants are often growing as single rosettes. More typical plants, which can be found nearby in the Cobb Gorge below the quarry, have crowded, spreading leaf hairs that give the leaves a velutinous texture. Plants found on the limestone outcrops have leaves with similar texture, but they are larger, more robust leaves, with less well defines petioles than the Cobb reservoir ones. They usually consist of several rosettes.

Plants were collected and grown on to determine whether tag-named entities *M.* 'gorge creek' and *M.* 'otuhie' were distinctive from *M. brockiei*. *M.* 'gorge creek' was found to fall within the species description of *M. brockiei* (Moore and Simpson, 1973) which widens that species known geographic and altitudinal range.

M. 'otuhie' on the other hand has a couple of distinctive traits that place it just outside the description of *M. brockiei* (in Moore and Simpson, 1973). Differences are in the shape of the corolla lobes, which are rounded rather than ovate and have a wider corolla tube (~2mm diameter). The general habit is unusual for *M. brockiei*, however, in that plants form very large mats with many more rosettes than typically seen. The habitat in which these plants are found has been modified for sheep and dry stock farming. The largest matted plants seen are well within browse level and may have been unnaturally "cropped" into a more vegetatively reproducing type of plant. Other plants observed are hanging from limestone cliffs above the level of browse but these may be atypical forms that were able to survive in refugia. The multicipital habit is in Moore and Simpson's (1973) description.

2.3 Other problem taxa

The *M. pygmaea* complex is a self-pollinating group in which there are four named varieties and additional tag-named taxa, such as the threatened *M.* 'volcanic plateau'. *M. p.* var. *pygmaea* and *M. p.* var. *drucei* and have been given variety status in the previous treatment but Druce (1993) considered them sufficiently distinct to be species. An attempt to separate *M. p.* var. *pygmaea* from *M. p.* var. *drucei* was made at the CHR herbarium. Traits that distinguish the two taxa convincingly in the Nelson region using the descriptions by Moore (in Allan, 1961) could not be used for specimens of other localities, where traits were unsystematically shared among those specimens seen (Robertson, pers. comm.).

Myosotis tenericaulis and *spathulata* are difficult to separate on the basis of vegetative characters alone for the same reason. They have both been studied and were found to self-pollinate (Brandon and Robertson, in prep (b); Robertson and Lloyd, 1991). They can be discriminated, using the treatment by Moore (in Allan, 1961), on the degree of anther exertion. In *M. tenericaulis,* anthers are completely included inside the corolla tube, in *M. spathulata,* they are exerted at least partly above corolla scales and can be carried as far as halfway up the corolla lobes. Plants whose anthers are exerted beyond the halfway point then become *M. matthewsii*. However, this is a continuum, which does not enable clear-cut boundaries to delineate the limit of each species. In addition, in the Nelson region, the vegetative features of one species can be found with the flower type of the other. As these taxa cannot be reliably distinguished they are treated together in this report.

A set of collections that are curious are those of *M. matthewsii* which otherwise is known only from Northland. These collections were examined at the CHR herbarium, and were found to be misidentified *M. spathulata / tenericaulis.*

M. elderi is thought to be synonymous with M. *lyallii* var. *lyallii* as is *M. lyallii* var. *townsonii* (Robertson, 1989; Druce, 1993). Filament length distinguishes *M. lyallii* from *M. elderi.* Unlike the *forsteri / venosa* complex, style length is variable in *M. elderi* which provides this taxon with a mixed pollination system without filament length variability. As the filament length variation does not separate a selfing from a non-selfing taxon there is not such a dramatic dividing line that can be used to delineate individuals on the basis of pollination syndrome. M. *lyallii* var. *lyallii* and var. *townsonii* were considered distinct varieties by Moore in Allan, (1961) due to differences in the calyx hairs and lobes. At that time, they were only known from disjunct locations in the South Island. However, more collections have since been made, and their distributions are now much wider with both taxa occurring in the Nelson mountains. It seems unlikely that these two taxa are distinct enough to be considered varieties.

These types of problem do not arise as frequently in the vector-requiring species that occur in the area. For example, *M. monroi* is quite uniform morphologically throughout its range of populations at Dun Mountain. Plants at the Red Hills Ridge and Porters Ridge populations are also uniform at those sites. There are, however, small differences in rosette leaf morphology between the two disjunct sites. *M. arnoldii* plants differ between Hoary Head and Chalk Range populations in one respect, the hooked hairs on the calyces of Marlborough plants are absent on Nelson plants. In taxa that require vectors to reproduce, gene flow is occurring to some extent between individuals, and due to a far greater degree of shared genetic material, individuals are much more uniform within their populations. As soon as a barrier to gene flow is present, which can be due to self-pollination or geographic separation, differences start to appear between populations.

2.4 Summary

The table below lists the taxa known to occur in the Nelson area, including the tag-named entities along with their perceived distinctiveness in the region.

Table 1: Summary of Nelson taxa including all tag-named entities with their taxonomic status

Taxon	**Taxonomic status**
1. *M. elderi* L. B. Moore	Synonymy with *M. lyallii*
2. *M. tenericaulis* Petrie	Difficult to distinguish from *M. spathulata*
3. *M. pygmaea* Col. var. *pygmaea*	Distinctive taxon in N.W. Nelson
4. *M. pygmaea* Col. var. *drucei* L. B. Moore	Distinctive taxon in N. W. Nelson
5. *M. traversii* Hook. f. var. *traversii*	Species
6. *M. forsteri* Lehm.	Species
7. *M. venosa* Col.	Species
8. *M. laeta* Cheesem.	Species
9. *M. petiolata* Hook. f. var. *petiolata*	Species
10. *M. monroi* Cheesem.	Species
11. *M. concinna* Cheesem.	Species
12. *M. macrantha* (Hook. f.) Benth. Et Hook. f.	Species
13. *M. arnoldii* L. B. Moore	Species
14. *M. angustata* Cheesem.	Species
15. *M. lyallii* Hook. f. var. *lyallii*	Synonymy with *M. elderi*
16. *M. lyallii* Hook. f. var. *townsonii*	Synonymy with *M. lyallii*
17. *M. spathulata* Forst. f. var. *spathulata*	Difficult to distinguish from *M. tenericaulis*
18. *M. matthewsii* L. B. Moore	These specimens have been mis-identified, they are in the *M. spathulata / tenericaulis* complex
19. *M. brockiei*	Species
20. *M.* 'otuhie'	Distinctive taxon; var. of *M. brockiei*
21. *M.* 'gorge creek'	*M. brockiei*
22. *M.* 'cundy creek'	One form of the variable *M. forsteri* aggregation.
23. *M.* 'flora'	No such entity
24. *M.* 'paynes ford'	*M. spathulata/tenericaulis* – spathulata leaves, tenericaulis flo
25. *M. australis* R. Br. 'yellow'	Distinct taxon
26. *M. a.* 'small white'	Distinct taxon
27. *M. a.* 'calcareous white'	Synonymy with 'small white'

2.5 Checklist of Nelson taxa

The table below lists the distinctive taxa in the region, their current conservation status and recommendations where considered appropriate.

Table 2: Distinctive Nelson taxa

Taxon	**Current conservation status**	**Recommendations**
M. monroi	Category I, removal recommended (Dopson *et al.* 1999)	Ok
M. macrantha	None	Ok
M. laeta	Range restricted (de Lange *et al.* 1999) Category B recommended (Dopson *et al.* 1999)	**Vulnerable**; small range size, small number of populations known, habitat invasion by manuka into large population site
M. brockiei	Range Restricted (de Lange *et al.* 1999) Category I, medium priority (Dopson *et al.* 1999)	Range Restricted
M. 'otuhie'	None	**Vulnerable**; small geographic range, small number of plants known, modified habitat, lack of legal land protection
M. tenericaulis/spathulata	None	Ok
M. forsteri	None	Ok
M. venosa	None	Ok
M. petiolata var. *petiolata*	Insufficiently Known (de Lange *et al.* 1999) Category I, high priority (Dopson *et al.* 1999)	**Endangered**, small population sizes, only 2 populations known, tiny range size
M. arnoldii	Range Restricted (de Lange *et al.* 1999)	Range restricted
M. angustata	Range Restricted (de Lange *et al.* 1999)	**Vulnerable**, small populations, not many populations known, small range size
M. concinna	Range restricted (de Lange *et al.* 1999)	Range restricted
M. lyallii/elderi	None	Ok
M. traversii var. *traversii*	None	Ok
M. pygmaea var. *drucei*	None	Ok
M. pygmaea var. *pygmaea*	None	Ok
M. australis 'yellow'	None	Ok
M. australis 'small white'	None	Ok

3. Reproductive biology in New Zealand *Myosotis*

3.1 Glossary of terms

Dichogamy: temporal separation of stigma and anther presentation

Protogyny: the form of dichogamy in which stigma presentation precedes anther presentation (opposite of protandry)

Herkogamy: spatial separation of stigma and anther presentation

Allee effects: Inverse density-dependent effects that occur when a component of individual fitness (e.g. rate of reproduction) decreases disproportionately in response to a decrease in population density or size (Allee, 1951)

Tube blossom: These flowers usually have a narrow corolla tube of variable length. The tube is framed by conspicuous corolla scales, with flat, free corolla lobes (fig 1). Filaments are more or less short carrying anthers near the top of the tube, but the degree of inclusion/exertion varies.

Funnel blossom: These flowers have a wider corolla tube that is more or less funnel shaped. The corolla lobes are spreading rather than flat and often fused above the scales giving the flower a funnel shape (fig 2).

Brush blossom: These flowers have a narrow corolla tube, with a rim of conspicuous scales and free flat corolla lobes, but differ from the tube blossom type in having anthers and stigma on long exerted filaments and styles (fig 3).

Fig 1. *M. traversii* var. *traversii* (BS)

Fig 2. M. arnoldii (BM)

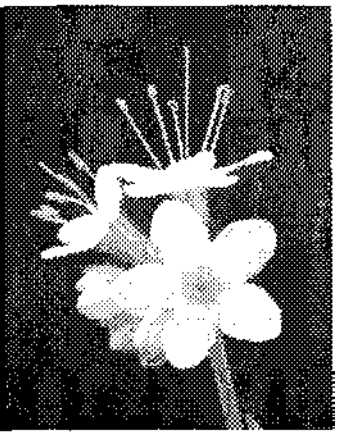

Fig 3. M. brockiei (AWR)

3.2 The general condition

New Zealand *Myosotis* species studied in detail have been found to have different pollination syndromes that depend, in part, upon the degree of anther exertion displayed and length of the style in relation to the position of the anthers (Robertson and Lloyd, 1991). Moore (in Allan, 1961) states that "...exserted anthers are little known in the genus outside NZ". The general character of the genus here differs in many respects from the Northern Hemisphere relatives. Several aspects of the reproductive biology of selected taxa have been studied. All species studied so far have been found to be fully self-compatible, but differ in their ability to set seed autonomously (Robertson, 1989; Brandon, unpubl.). With the exception of *M. pygmaea* var. *minutiflora*, which was not dichogamous, all species studied to date have incomplete dichogamy in the form of protogyny (Robertson and Lloyd, 1991; Brandon and Robertson, in prep (a)). Allee effects in reproductive effort were found in four vector-requiring species of *Myosotis* (Brandon and Robertson, in prep (b)). Some species have precocious buds (*sensu* Robertson, 1989), in which stigmas are exerted before the bud opens. Pollination of precocious buds occurs in *Myosotis monroi* (Brandon, in prep.). Herkogamy measurements indicate some species employ mixed pollination syndromes within populations.

3.3 Summary of manuscripts in preparation

3.3.1 Pollination syndrome, herkogamy and rarity in six species of New Zealand *Myosotis*

The reproductive biology of six species of *Myosotis* was studied. New Zealand *Myosotis* species studied to date fall into either one of two pollination syndromes based on the degree of herkogamy exhibited by the flowers. Details of the pollination syndromes operating in six species of *Myosotis* are presented. Hand pollination tests showed all six species are fully self-compatible. The four species with brush or funnel blossom flowers, *Myosotis monroi, M. brockiei, M laeta* and *M. macrantha,* require pollinators to transfer pollen and set seed. The two tube blossom species, *M. forsteri* and *M. spathulata / tenericaulis* are able to set seed without pollinators and are therefore autonomous. These pollination syndromes are directly related to the degree of herkogamy displayed. Pollen limitation was found to occur in the four pollinator-requiring species but not in the two autonomous species. Not all tube blossom species are autonomous. Robertson (1989) found two tube blossom species, *M. colensoi* and *M. uniflora,* require pollinators. The association between pollination syndromes and rarity patterns are discussed. The breakdown in dichogamy and herkogamy are linked to the evolution of autogamy.

3.3.2 Allee effects in plant reproductive performance: Local density, population size, rarity and reproductive success in natural populations of *Myosotis* L. (Boraginaceae)

Reproductive failure due to Allee effects is a possible consequence of low population density and small population size for plants. Data were collected from natural populations of five species of *Myosotis* L. (Boraginaceae) with different pollination requirements and rarity patterns in the Nelson region of New Zealand. Seed set per flower was measured in populations of varying density and size. For pollinator-requiring species, *Myosotis monroi*, *M. macrantha*, *M. laeta* and *M. brockiei*, local population density had strong effects on seed set, while population size had no effect. These plants were always pollen limited in low density patches. For a self-fertile species, *M. forsteri*, seed set was always high and unaffected by either local density or population size and pollen was never limiting. These results indicate reproductive success in pollinator-requiring species of *Myosotis* is subject to Allee effects and these effects occur at a very local scale. This density-dependent process can explain how the different patterns of density and distribution observed are maintained for each species. These results have implications for the assessment and management of threatened *Myosotis* species in particular and rare plants generally. It is essential to know the pollination requirements and levels of density dependence for reproduction both for the assessment of threat and for determining management strategies for rare plants. Species that are able to self-pollinate are not affected by the presence of conspecifics and will not necessarily be at risk if found at low density. Species that suffer Allee effects will be more susceptible to local population extinction when densities fall below a critical threshold. Identifying density dependence and determining critical thresholds of density below which reproductive success is affected should be an important component of rare plant conservation.

3.3.3 Precocious bud pollination: Maximizing chances for cross pollination in the ultramafic endemic *Myosotis monroi*

M. monroi has brush blossom floral morphology (Robertson, 1989; Brandon, unpubl.). In *M. monroi* the buds open almost simultaneously and the stigma presentation in the buds is precocious, exposing the stigma for pollination before the bud opens, which has the effect of extending the relative length of the female phase (Robertson, 1989). In species that require a vector for pollination to occur, self and cross pollen alike must be deposited via the vector (Holsinger, 1996), so the longer the stigma is exposed prior to anther dehiscence, the greater the chance of outcrossing. Stigmas were collected from precocious and fully open flowers to determine whether pollination was taking place at the precocious phase. 25% of the precocious stigmata collected had received pollen, and 11.6% had received more than 5 pollen grains. Of the brush blossom species studied so far, *Myosotis monroi* is one of the more successful taxa, which although it has been considered at risk in the past, is no longer regarded as threatened. Rarity patterns within the genus have been found to be affected by the pollination syndromes of the taxa studied (Brandon and Robertson, in prep (b)). The evolutionary consequences for species that employ pollination syndromes that increase the chance of outcrossing are discussed.

3.4 Reproduction and rarity

Species that exhibit density dependent reproductive effort, such as *M. monroi*, typically have clumped local densities and restricted distributions. Such density dependent processes are likely to influence the population dynamics of these species. Low seed production in low density patches will limit population growth and local extinction may eventually result at those sites. Species that do not exhibit density dependence in their reproductive effort, such as *M. pygmaea*, typically have sparse but widespread distributions. The population dynamics of these species are not affected at all by the presence of conspecifics. These populations do not appear to be limited by seed production, which is always high. Population growth for these species is more likely limited by microsite availability. These typical patterns are not always observed in the field. For instance, *M. angustata* has typically small to moderate sized populations but also a limited geographic distribution. This species was found to have both types of pollination syndrome operating within a single population. The presence of self-pollinating plants will mask any density dependent interactions that may be occurring.

3.5 Summary

The table below summarizes the results from the studies conducted on reproductive biology in *Myosotis*. Inferences are made for taxa on which detailed studies were not carried out based on the degree of herkogamy exhibited by flowers.

Table 3: Summary of the reproductive biology of the Nelson taxa

Species	**Herkogamy (mm)**	**Selfing rate[1]**	**Pollination syndrome**	**Evidence of density-dependence**	**Evidence of pollen limitation**
M. monroi	0.7 – 2.8	0.039	Non-autonomous	Yes	Yes
M. macrantha	2.8 – 5.2	0.042	Non-autonomous	Yes	Yes
M. laeta	0.9 – 1.8	0.02	Non-autonomous	Yes	Yes
M. brockiei	2.7 – 4.9	0.042	Non-autonomous	Yes	Yes
M. 'otuhie'	1.5 – 3.4	-	Non-autonomous[2]	-	-
M. spathulata / tenericaulis	0	0.92	Autonomous	No	No
M. forsteri	0	0.95	Autonomous	No	No
M. venosa	0.5 – 0.9	0.04	Non-autonomous	-	Yes
M. petiolata	2 – 5.1	0.0275	Non-autonomous	-	Yes
M. arnoldii	2.2 – 7.1	-	Non-autonomous[2]	-	-
M. angustata	0-1.5	-	Both[2]	-	-
M. concinna	3.6 – 6.2	-	Non-autonomous[2]	-	-
M. lyallii / elderi	0 – 1.4	-	Both[2]	-	-
M. traversii	0-1.2	-	Both[2]	-	-
M. p. var. *pygmaea*	0	-	Autonomous[2]	-	-
M.p. var. *drucei*	0	-	Autonomous[2]	-	-
M. australis 'yellow'	0	-	Autonomous[2]	-	-
M. a. 'small white'	0	-	Autonomous[2]	-	-

[1] = proportion of ovules that mature into seeds per flower when pollinators are excluded; [2] = inferred from herkogamy measurements

4. Acknowledgments

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Bill Malcolm has kindly granted permission for reproduction of his slides of *Myosotis forsteri, M. arnoldii* and *M. monroi.*

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Rabinowitz, D. 1981. Seven forms of rarity pp. 205-217 *In:* H. Synge (ed) *The biological aspects of rare plant Conservation,* John Wiley & Sons, New York.

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6. Appendices

6.1 Explanation of fields in species profiles

6.1.1 Abbreviations

P	pollinator required
S	able to self pollinate
DD	density dependent seed production

6.1.2 Reproduction and recruitment

All observations and results presented in this section are largely based on as yet unpublished data collected by the author during 1996-2000 while conducting research for a PhD.

Herkogamy	spatial separation of stigma and anthers measured in the field in millimeters
Selfing rate	seed set by plants when pollinators were excluded
Dichogamy	proportion of time each flower spends in the female phase (i.e. receptive stigma) prior to anther dehiscence
Average seed set	seed set/flower in open pollinated plants under natural conditions

6.2 Distribution Maps

The maps were produced using CHR collection records as well as the author's observations over the period of research. Herbarium records are italicised where grid references were not supplied by the collector/s. In these cases, approximate grid references have been estimated based on the locality description on the sheets.

6.3 Photographs

AWR	Alastair Robertson
BM	Bill Malcolm
BS	Bruce Sunnex
AMB	Andrea Brandon

6.4 Species profiles

Myosotis monroi Cheesem.

Ranking: *M. monroi* was considered threatened in previous threatened plant lists (category I in Molloy and Davis (1994) and the Botanical Society Local Plant list in Cameron et al (1995)). *M. monroi* is now known to occur in very large populations within its range and is no longer considered threatened (Courtney, pers. comm.) which has prompted its removal from the threatened plant lists (de Lange et al, 1999).

Abundance and Distribution: *M. monroi* has a limited geographic distribution, restricted to the serpentine mineral belt which appears sporadically along the ranges east of Nelson, from D'Urville Island (records in the National Museum (WELT)) in the north through to the Red Hills in the south. This species has a clumped distribution, with some plants occurring in dense patches, while other sites, which appear to be suitable, contain no plants at all. Population sizes for this species are typically large.

Habitat: non-forest, ultramafic, rocky pavement/fell-field, above 800 m.

Threats: None.

Distinguishing features: Plants usually consist of several rosettes, the leaves are often bronze, with short appressed hairs, which are absent underneath. The inflorescence remains compact throughout flowering, which along with synchronous opening of flowers gives it an almost capitate appearance. Each plant can have several inflorescences, which along with the capitate cymes produce a large floral display. Flowers have a white corolla with yellow scales, are scented and of the brush blossom type.

Plants are similar to *M. laeta*, but distinguishable by the absence of hairs on the underside of rosette leaves in the latter and inflorescence type, which in *M. laeta* elongates over the reproductive season and flowers open sequentially rather than synchronously. Plants are distinguishable from *M. macrantha* in that the rosette leaves are greener in the latter, but the main difference is in flowers, which in *M. macrantha* are variably coloured but never white. Plants can be distinguished from *M. angustata* by the rosette leaves which in the latter are very narrow, green/blue in colour and when flowering material is present, by the length of the filaments, which in *M. angustata* are short.

Reproduction and recruitment: Study site: Dun Mountain. Results: herkogamy: 0.7 – 2.8 mm; selfing rate: 0.039; dichogamy: 23.5%; average seed set: 2.21; vector required to achieve pollination, fertilization and seed set; open pollinated plants set highly variable amounts of seed under natural conditions; plants in low density patches are pollen limited but the size of population does not influence pollination success; 25% of precocious stigmata receive pollen.

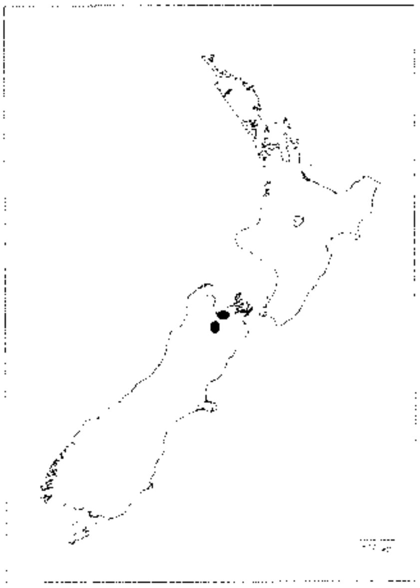
CHR, 193141, Northwest Nelson, South-west of Dun Mountain, Windy Point, on Dun tramline, 25385, 59844, Given DR, 1963; CHR, 212652, Dun Mountain track, half a mile before Roding-Maitai Saddle, 253--, 598--, Given DR, 1960; CHR, 24229, Dun Mountain, Mineral Belt, 2541-, 5984-, Moore LB, 1939; CHR, 273147, Wairau Valley, Red Hills, 2512-, 5944-, Druce AP, 1974; *CHR, 295240, Dun Mountain, 2541-, 5984-, Wall A, 1921; CHR, 295244, Dun Mountain, 2541-, 5984-, Gibbs FG; CHR, 295245, Dun Mountain, 2541-, 5984-, Gibbs FG, 1904; CHR, 295249, Dun Mountain, 2541-, 5984-, Gibbs FG; CHR, 295251, Dun Mountain, 2541-, 5984-, Gibb FG; CHR, 295252, Dun Mountain, 2541-, 5984-, Sainsbury GOK, 1922; CHR, 29837, Dun Mountain, 2541-, 5984-, Mason R, 1941; CHR, 301561, Dun Mountain, 2541-, 5984-, Talbot H*; CHR, 387382, Bryant Range, Dun Mountain, 2540-, 5984-, Druce AP, 1981; CHR, 387447, Wairau Valley, Red Hills, Upper Motueka River, 2514-, 5952-, Druce AP, 1980; CHR, 401655, Motueka Valley, (left branch), Gordon Range, 2512-, 5956-, Druce AP, 1985; CHR, 405021, Nelson, Dun Mountain, Windy Point, 25385, 59844, Park GN, 1975; *CHR, 60319, Wairau Mountains, Dun Mountain, 254--, 598–, McKay W; CHR, 97282, Dun Mountain, 254--, 598--, Allan HH; Gibbs FG;* **Authors records:** Red Hills Ridge, 25132, 59453; Red Hills Ridge, 25146, 59514; Red Hills Ridge, 25145, 59508; Red Hills Ridge, 25135, 59536; Porters Ridge, 25123, 59532; Porters Ridge, 25119, 59518; Porters Ridge, 25132, 59530; Coppermine Saddle, 25399, 59845; Dun Saddle, 25407, 59841; Dun Saddle, 25406, 59843; Dun Saddle, 25409, 59843

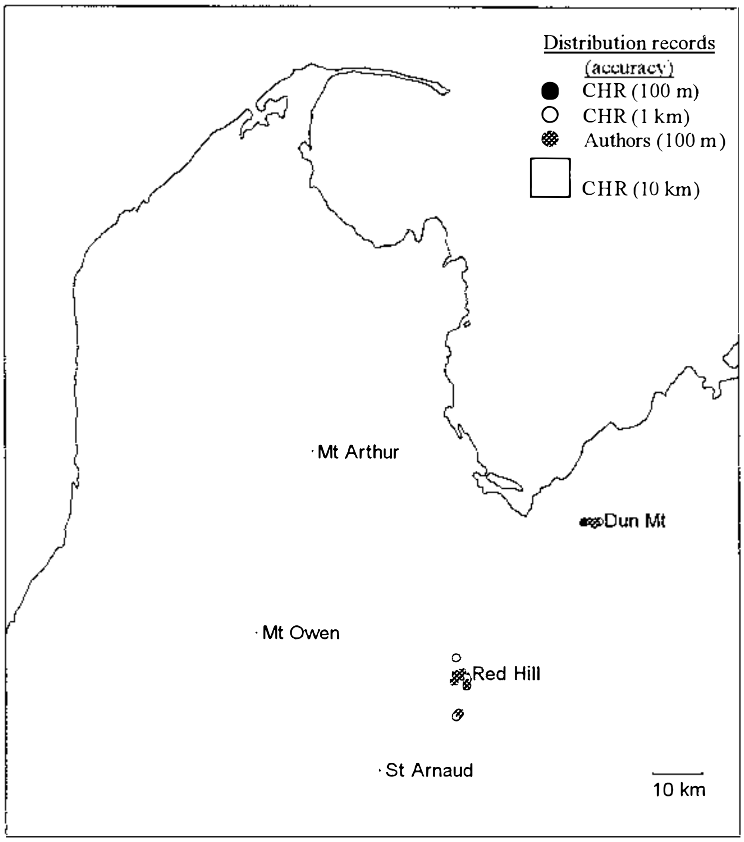
Endemicity: Mineral belt, E. Nelson, S. Is.

Reproduction: P; DD

Typical population size: L

Threat status: None





Main and top inset: AWR; Lower inset: BM

Myosotis macrantha (Hook. f.) Benth. et Hook. f.

Ranking: None

Abundance and Distribution: This species occurs throughout the South Island, mainly to the west of the Divide. Populations of this species are typically moderate to large.

Habitat: Alpine, on or at the edges of rock outcrops, usually on the south facing aspect.

Threats: None

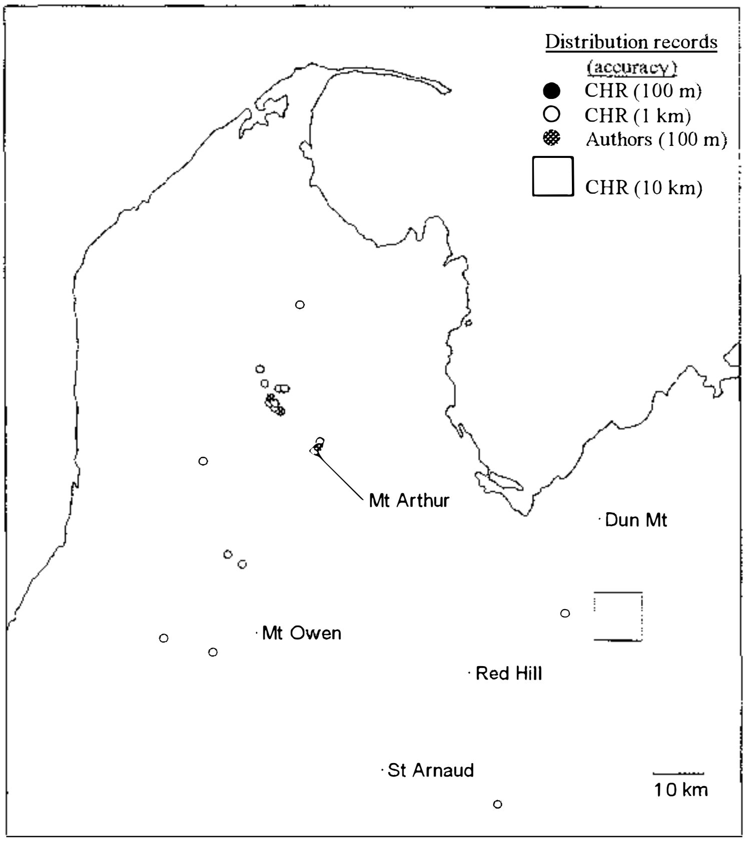
Distinguishing features: Plants usually consist of several rosettes, the leaves of which are variable throughout the South Island. The rosette leaves of the Nelson forms are usually glabrous underneath, except for some hairs on the midrib. The variably coloured flowers distinguish this species from others. The flowers can be dark-purple-brown, bronze or yellow in colour, have yellow scales, are scented and of the funnel blossom type. The inflorescence is compact throughout flowering; each plant can have many inflorescences, which, along with synchronous opening of flowers, all contribute to producing a large floral display.

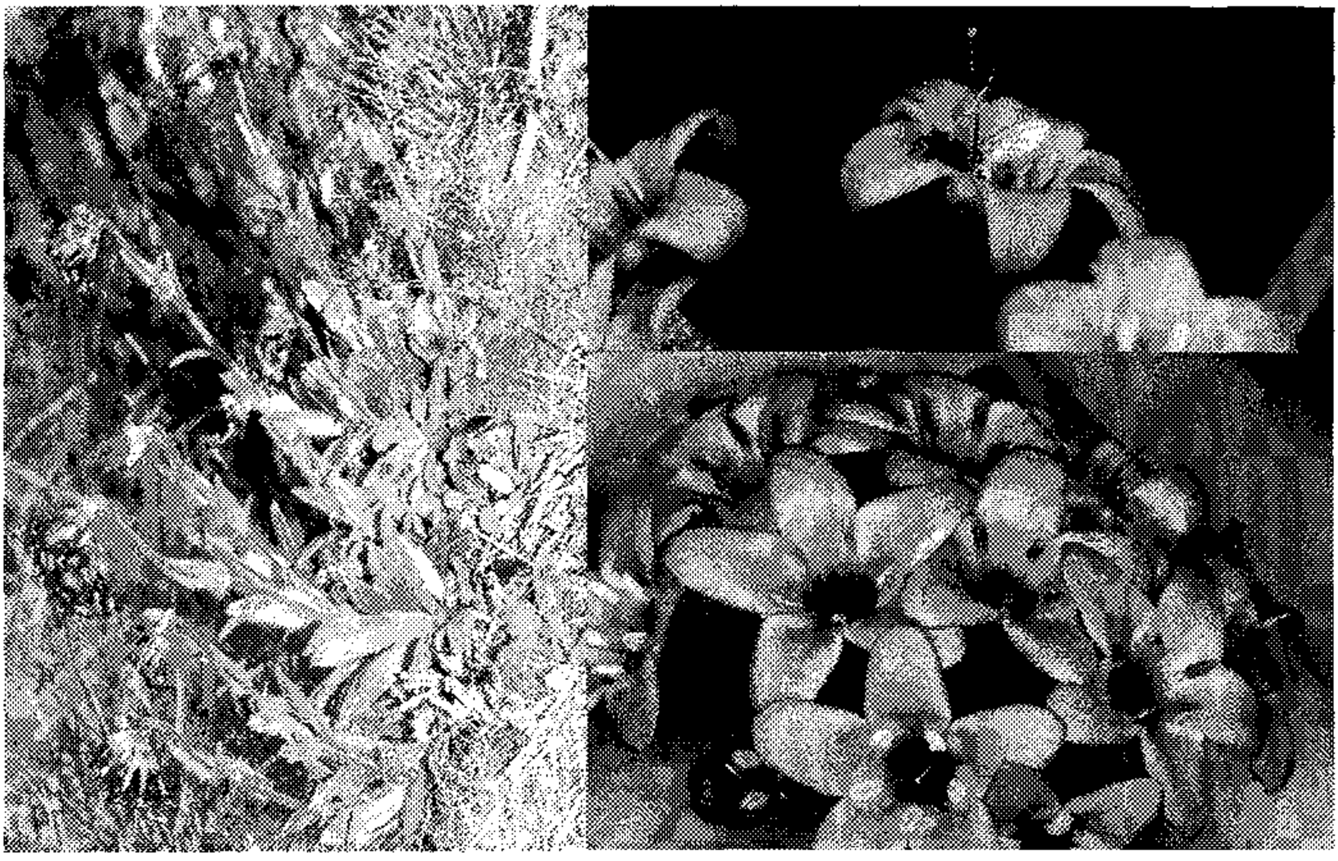
Plants can be distinguished from *M. monroi* by the green rather than bronze rosette leaves, but more reliably by the coloured flowers. Plants are similar vegetatively to those in the *lyallii/elderi* complex but have larger, more robust leaf size and very different flowers. The flowers are most like those of *M. arnoldii*, but plants are very easily distinguished from *M. arnoldii* by green rather than silver leaves.

Reproduction and recruitment: Study sites: Mt. Mytton and Lake Peel. Results: herkogamy: 2.8 – 5.2 mm; selfing rate: 0.042; dichogamy: 25.6%; average seed set: 2.9; vector required to achieve pollination, fertilization and seed set; open pollinated plants set highly variable amounts of seed under natural conditions; plants in low density patches are pollen limited but the size of the population does not influence pollination success.

CHR, 24228, Mount Arthur, 2483-, 5998-, Moore LB, 1939; CHR, 36067, Iron Hill, 2477-, 6011-, Mason R, 1942; CHR, 51505, Tasman Mountains, Iron Hill, 2476-, 6011-, Mason R, 1942; CHR, 76066, Mount Arthur, 2483-, 5998-, Hay JA, 1950; CHR, 76999, Mount Arthur, 2483-, 5998-, Hay JA, 1952; CHR, 151028, Cobb Valley, Iron Hill, 2477-. 6011-, Moore LB. 1964: CHR. 191793. Mount Arthur, basin at head of Horseshoe Creek, 2484-, 6000-, Ritchie IM, 1969; CHR, 191797, Mount Arthur, basin at head of Horseshoe Creek, 2484-, 6000-, Ritchie IM, 1969; CHR, 237862, Mount Arthur, 2483-, 5998-, Simpson C, 1970; CHR, 252254, Northwest Nelson, Cobb Valley, Thorns' Creek, 2474-, 6008-, Druce AP, 1970; CHR, 273650, Northwest Nelson, Mount Patriarch, 2468-, 5975-, Druce AP, 1974; *CHR, 277622, Northwest Nelson, Mount Arthur, 2484-, 6000-, Druce AP, 1975*; CHR, 279021, Northwest Nelson, Cobb Valley, 2473-, 6012-, Druce AP, 1970; *CHR, 295242, Mount Peel, 2475-, 6007-, Wall A, 1921; CHR, 326763, Mount Peel, 2475-, 6007-, Talbot H,* ; CHR, 355183, Northwest Nelson, Matiri Range, Head of Larrikin Creek, 2452-, 5960-, Druce AP, 1979; CHR, 358515, Northwest Nelson, Garibaldi Ridge, 2460-, 5996-, Druce AP, 1980; CHR, 365449, Northwest Nelson, North West of Mount Benson, 2472-, 6015-, Druce AP, 1980; *CHR, 371665, Northwest Nelson, Cobb Valley, Upper Cobb River, Thorns' Creek, 2475-, 6008-, Ritchie IM, 1970*; CHR, 387041, Richmond Range, West of Old Man, 2534-, 5965-, Druce AP, 1981; CHR, 389033, Northwest Nelson, South Arthur Range, Near Luna Lake, 2465-, 5977-, Druce AP, 1982; CHR, 395640, Northwest Nelson, Bald Knob Ridge, 2462-, 5957-, Courtney S, 1989; CHR, 401060, Northwest Nelson, Anatoki Range, 2480-, 6028-, Druce AP, 1984; **Authors records:** Mt Mytton, 24748, 60097; Mt Mytton, 24748, 60097; Lake Peel, 24767, 60069; Horseshoe Basin, 24843, 59993

Endemicity: S. Is.
Reproduction: P; DD
Typical population size: L
Threat status: None





All photos: AWR

Myosotis laeta Cheesem.

Ranking: *M. laeta* was listed as Vulnerable in the previous Botanical Society lists (Cameron et al, 1995) and is now classified as Range Restricted (de Lange et al, 1999).

Abundance and Distribution: *M. laeta* has a very limited geographic distribution, restricted to the serpentine mineral belt east of Nelson. There are two known populations, one on the Red Hills Plateau and the other on the lower flanks of Mt. Starveall. The Red Hills Plateau population is large (10's of thousands of individuals) although it occurs in one small area (2x1km). The extent of the Mt. Starveall population is not known.

Habitat: Ultramafic, non-forest, tussock-manuka shrubland, 900-1200m

Threats: manuka regeneration and recolonisation into the Red Hills Plateau population (Dickinson, pers. comm.).

Distinguishing features: Plants usually consist of a single rosette, the leaves of which have bronze margins, with short, fine hairs, longer at margins and retrorse underneath. The inflorescence, usually one per plant, elongates over the flowering period with flowers opening sequentially. Flowers are unscented, of the funnel blossom type but with long filaments and extended anthers. The corolla is white-cream coloured with yellow scales.

Plants can be distinguished from *M. monroi* by their very different inflorescence type and flowers, but in the absence of flowering material, can be distinguished by the absence of hairs on the underside of the rosette leaves. Plants are similar to members of the *M. australis* aggregation, but can be reliably distinguished with flowering specimens by the colour and shape of the corolla, which is tube form with short filaments in the latter. The rosette leaves offer further differences, which in *M. laeta* have that bronze margin; the hairs are short, less crowded and appressed. Plants can be distinguished from *M. macrantha* in the absence of flowering specimens by the absence of hairs on the undersides of the rosette leaves in the latter.

Reproduction and recruitment: Study site: Red Hills. Results: herkogamy: 0.9 – 1.8 mm; selfing rate: 0.02; dichogamy: 8.3%; average seed set: 1.8; vector required to achieve pollination, fertilization and seed set; open pollinated plants set highly variable amounts of seed under natural conditions; plants in low density patches are pollen limited.

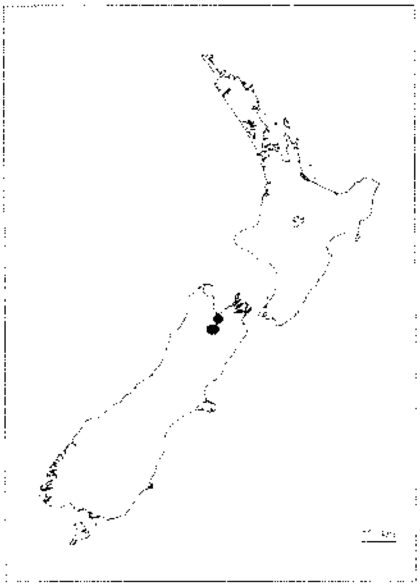
CHR, 387376, Richmond Range, Mount Starveall, 2528-, 5971-, Druce AP, 1981; CHR, 387462, Wairau Valley, Red Hills, 2509-, 5941-, Druce AP, 1980; *CHR, 489540, Wairau, Red Hills, 2509-, 5941-, Cheeseman TF, 1852*; **Authors records**: Red Hills, 25107, 59432; Red Hills, 25094, 59425; Red Hills, 25095, 59419; Red Hills Plateau, 25114, 59436; Starveall, 25293, 59715

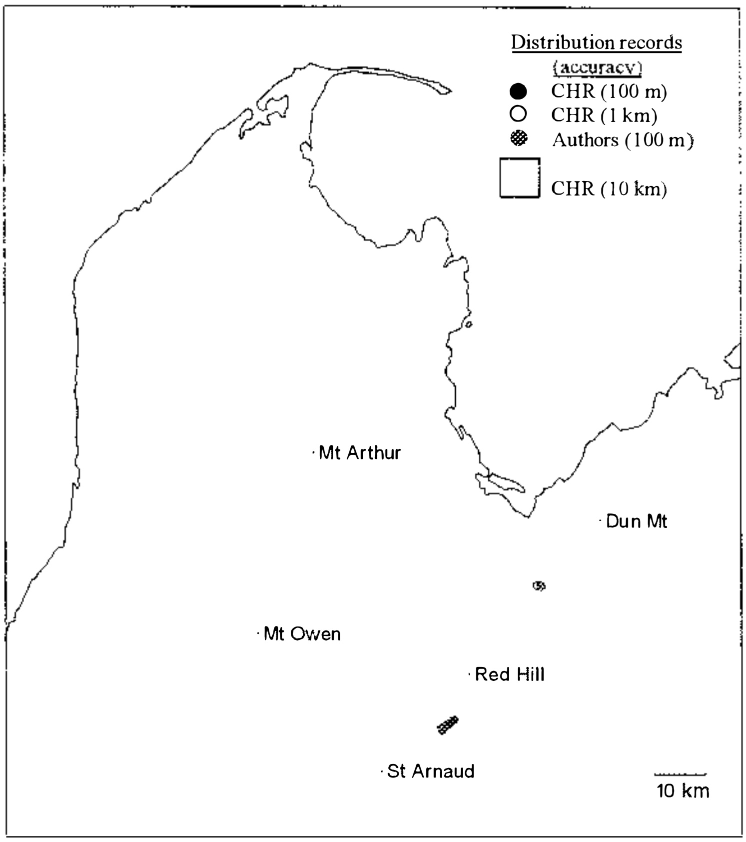
Endemicity: Mineral Belt, E. Nelson, S. Is.

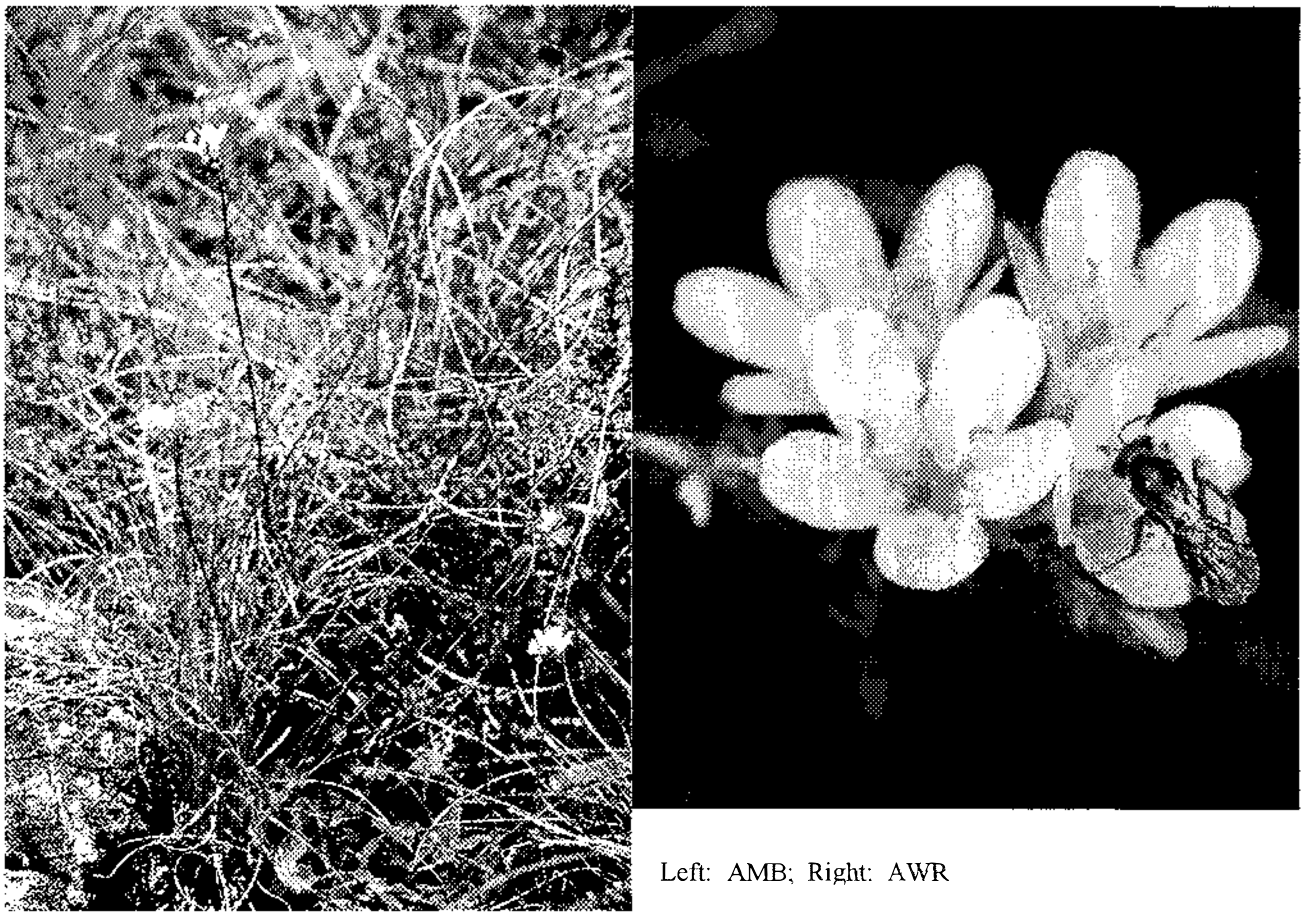
Reproduction: P; DD

Typical population size: L & S

Threat status: RR.







Left: AMB; Right: AWR

Myosotis brockiei Moore et Simpson

Ranking: *M. brockiei* has appeared in past lists in Category I, (Molloy and Davis, 1994), and as Rare, (Cameron et al, 1995). It is now considered Range Restricted (de Lange et al, 1999).

Abundance and Distribution: *M. brockiei* has a restricted distribution, which is centered, around the Cobb Valley, within which there are several small, often isolated populations. This species is confined to two types of geological substrate; an ultramafic belt, the Cobb Igneous Complex, which has a magnesite-talc composition, and on various limestone outcrops.

Habitat: Basicole, limestone and ultramafic outcrops, often growing in very little soil, within forest, or at forest margins, sea level - tree-line.

Threats: Small population sizes that are typical of this species coupled with restricted geographic distribution; goats damaging habitat (Flora Valley limestone remnant populations; Gorge Creek), goat browse (Gorge Creek); *Hieracium* invasion of habitat (Gridiron Creek).

Distinguishing features: This species has white, unscented, brush blossom type flowers with very long, narrow filaments and a very narrow and short corolla tube (~1x1 mm) with bright yellow scales. Flowers open sequentially, and the inflorescence elongates over the flowering period. Plants can have one to several rosettes, the leaves of which are quite variable in shape, size and hairs. The Magnesite Quarry plants have a relatively sparse covering of hairs that are more or less appressed. These plants can only be distinguished from *M. petiolata* ss by their leaf shape, as their flowers are very similar. *M. petiolata* ss have a much longer petiole with a shorter, spathulate lamina. The Magnesite Quarry plants have a less well defined petiole that tapers gradually from a more elongated, elliptic lamina. More typical plants in the species have larger, more robust leaves with crowded leaf hairs that are not appressed which give the leaves a velutinous texture. These plants bear much more resemblance to *M. concinna* in the vegetative form but can be distinguished by the appressed hairs on the rosette leaves, and more reliably by the very different flowers, which in *M. concinna* are yellow with a longer corolla tube.

Reproduction and recruitment: Study sites: Magnesite Quarry, Cobb Gorge, Flora Valley. Results: herkogamy: 2.7 – 4.9 mm; selfing rate: 0.042; dichogamy: 10.8%; average seed set: 1.4; vector required to achieve pollination, fertilization and seed set; open pollinated plants set highly variable amounts of seed under natural conditions; plants in low density patches are pollen limited but the size of the population does not influence pollination success.

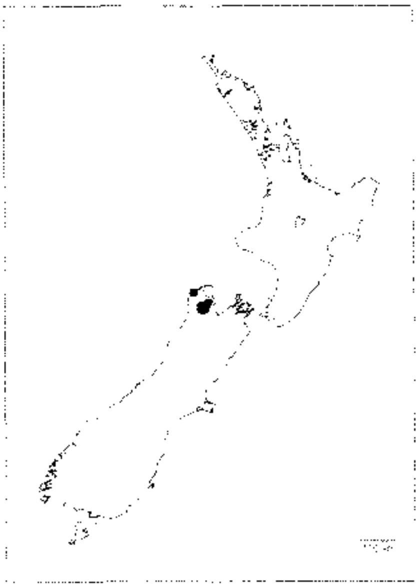
CHR, 121708, Richmond, ex garden, 2483-, 6012-, Brockie WB, 1968; CHR, 171679, Richmond, garden, 2483-, 6012-, Brockie WB, 1966; CHR, 188084, Richmond, garden of Mr W Brockie, 2483-, 6011-, Brockie WB (received), 1968; CHR, 208218, Mount Arthur Tableland, Salisbury Open, 2480-, 6002-, Brockie WB, 1969; CHR, 227877, Graham Valley, Mount Arthur, 248--, 600--, Brereton J, 1974; CHR, 233860, Northwest Nelson, Cobb Valley, 248--, 601--, Brockie WB, 1965; CHR, 271212, Cobb Valley, 248--, 601–, Talbot H, 1964; CHR, 277574, Northwest Nelson, Mount Arthur Tableland, near Salisbury Hut, 2480-, 6001-, Druce AP, 1975; CHR, 311717, Northwest Nelson, Cobb Valley, Magnesite Quarry, 2483-, 6012-, Druce AP, 1977; CHR, 311718, Northwest Nelson, Cobb Valley, Magnesite Quarry, 2483-, 6012-, Druce AP, 1977; CHR, 311722, Northwest Nelson, Takaka Valley, 2483-, 6005-, Druce AP, 1977; *CHR, 335754, Mount Arthur Plateau, 248--, 600–, Wall A, 1930*; CHR, 365422, Northwest Nelson, Northwest of Mount Benson, 2472-, 6015-, Druce AP, 1980; CHR, 365506, Northwest Nelson, Cobb Valley, 2483-, 6012-, Druce AP, 1979; CHR, 401375, North of Cobb Dam, Magnesite Quarry, 24834, 60124, Macmillan BH 88/35; Fife AJ, 1988; **Authors records:** Cobb Ridge, 24834, 60104; Gridiron Ck, 24843, 60038; Salisbury Open, 24808, 60020; Dry Rock, 24814, 60033; Flora limestone, 24837, 60050; Asbestos, 24841, 60085; Mt Mytton, 24751, 60092; Cobb Gorge, 24838, 60122; Cobb Gorge, 24837, 60124; Cobb Gorge, 24837, 60122; Mg Quarry, 24834, 60124; Gorge Ck, 24973, 60288; Gorge Ck, 24976, 60290

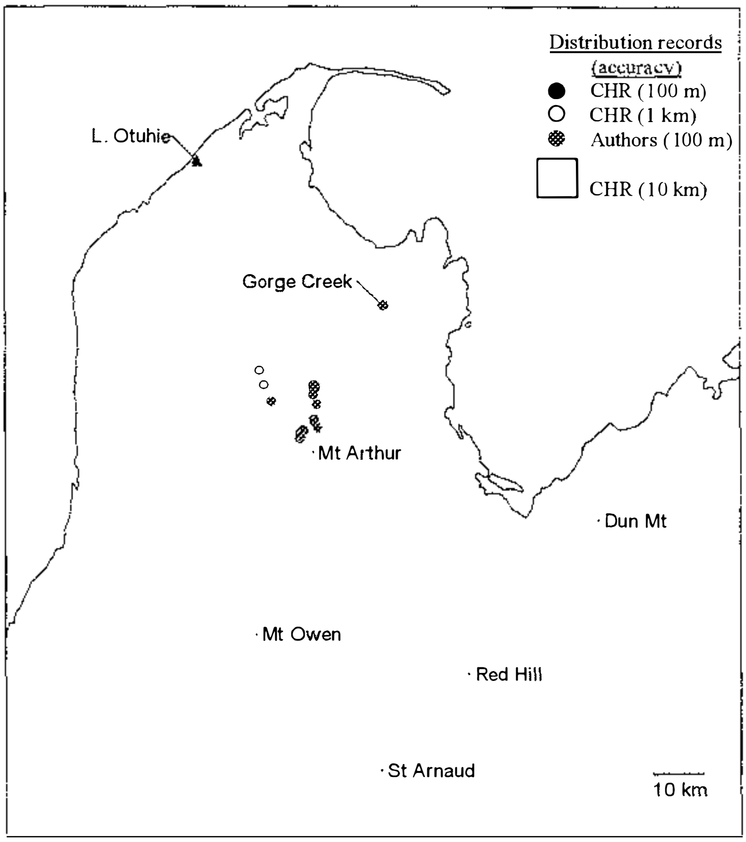
Endemicity: N.W. Nelson, S. Is.

Reproduction: P; DD

Typical population size: S

Threat status: RR





Main photo: AMB; Left and right insets: BS

Myosotis brockiei Moore et Simpson 'otuhie'

Ranking: None

Abundance and distribution: Very limited geographic range, only a few plants known hanging from limestone cliffs

Habitat: Coastal, calcicole, limestone cliffs, growing directly out of limestone crevices, no soil

Threats: Recruitment failure, habitat modification, grazing by sheep and cows, small number of plants known in a very small geographic area

Distinguishing features: Plants usually consist of many rosettes matted together, usually hanging from vertical cliffs above browse height. Inflorescences elongate throughout flowering and flowers open sequentially. Flowers are white, unscented brush blossom types with overlapping, rounded corolla lobes.

Plants can be distinguished from *M. brockiei* by their overlapping corolla lobes, corolla tube (~2 mm) is not as narrow and very different habit.

Reproduction and recruitment: Study site: Lake Otuhie. Results: herkogamy: 1.5 – 3.4 mm.

Herkogamy measurements suggest a vector is required to achieve pollination, fertilization and seed set. The population density patterns expected from species with this type of pollination syndrome are not seen. Seed set counts from one plant were very low, indicating pollination failure. Vegetative reproduction appears to be maintaining this population at Lake Otuhie.

CHR, 497375, Lake Otuhie, 2459-, 6058-, Metcalf LJ; Heenan P 84/94, 1993, **Authors records:** Lake Otuhie, 24596, 60584; Lake Otuhie, 24595, 60587; Lake Otuhie, 24594, 60587

Gorge Creek plants (left) and Lake Otuhie plants (below). Note purple edged corollas

Top photos: AMB; Lower photos: BS

Myosotis spathulata Forst. F. /*M. tenericaulis* Petrie

Ranking: None

Abundance and Distribution: Widespread but sparse throughout New Zealand including the Chathams.

Habitat: basicole, fertile, dry substrates, shady cave entrances, overhangs, forest, sea level - treeline

Threats: None

Distinguishing features: Plants are small, generally prostrate and usually consist of a single rosette but, as they can form roots from decumbent lateral branches, they can form a mat of rosettes. Leaves are small and spathulate with short, sparse, appressed hairs, similar on the underside. The inflorescence is prostrate and elongates over the flowering period as the bracteate flowers open sequentially. There will be a maximum of three flowers out at any one time on each inflorescence. Flowers are small, white, tube-blossom type with yellow scales. Corolla lobes vary from flat to spreading as anther exertion increases.

Plants can be distinguished from *M. pygmaea* by their erect vegetative habit, their rosette leaves which are spathulate with a well defined petiole and appressed hairs on both sides of the leaves. Plants are very similar vegetatively to *M. petiolata* ss. and can only reliably be distinguished by their very different flowers.

Reproduction and recruitment: Study site: Flora Valley; Results: herkogamy: 0 mm; selfing rate: 0.92; dichogamy: 9.8%; average seed set: 3.7; vector not required to achieve pollination, fertilization and seed set; open pollinated plants set unvarying high levels of seed under natural conditions; plants in low density patches are never pollen limited. The consistently high levels of seed set under natural conditions suggests population growth is probably not seed limited.

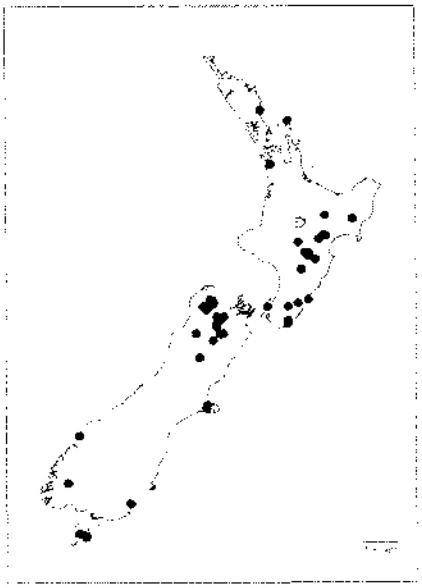
CHR, 129115, Brightwater, Snowden's Bush, 2518-, 5981-, Talbot H, 1962; CHR, 225961, Snowdon's Bush, 2518-, 5981-, Simpson MJA 6957, 1972; CHR, 269155, Brightwater, Snowdons Bush, 2518-, 5981-, Talbot H, 1972; CHR, 269156, Brightwater, Snowdons Bush, 2518-, 5981-, Talbot H; CHR, 311719, Northwest Nelson, Takaka Valley, 2483-, 6006-, Druce AP, 1977; CHR, 311720, Northwest Nelson, Takaka Valley, 2483-, 6005-, Druce AP, 1977; CHR, 311941, Northwest Nelson, Pikikiruna Ranges, Canaan, 2500-, 6027-, Druce AP, 1976; *CHR, 208219, Roding river, 253--, 598--, Brockie WB, 29, 1969; CHR, 208220, Cobb River Basin, Lake Sylvester, 2478-, 6011-, Brockie WB, 1969; CHR, 269157, Cobb river, between Rockett and Peel ranges, 247--, 601--, Brockie WB, 1969; CHR, 269158, Cobb Valley, between Rockett and Peel ranges, 247--, 601--, Brockie WB, 1969; CHR, 269159, Cobb Valley, between Rockett and Peel ranges, 247--, 601--, Kelly GC, 1971; CHR, 269160, Between Richmond and Brightwater, Aniseed Valley, 25246, 59797, Elder NL 236 37, 1943*; CHR, 396052, Northwest Nelson, Cobb Valley, 2470-, 6015-, Druce AP, 1983; CHR, 401639, Motueka river (left branch), Gordon range, 2510-, 5956-, Druce AP, 1985; **Authors records:** Mg Q Rd, 24835, 60118; Old U. Tak. Track, 24844, 60066; Old U. Tak. Track, 24849, 60067; Paynes Ford, 24941, 60351; Flora limestone, 24837, 60049; ridge to Hoary Head, 24927, 60102; Takaka Hill, 24983, 60193; Starveall, 25292, 59714

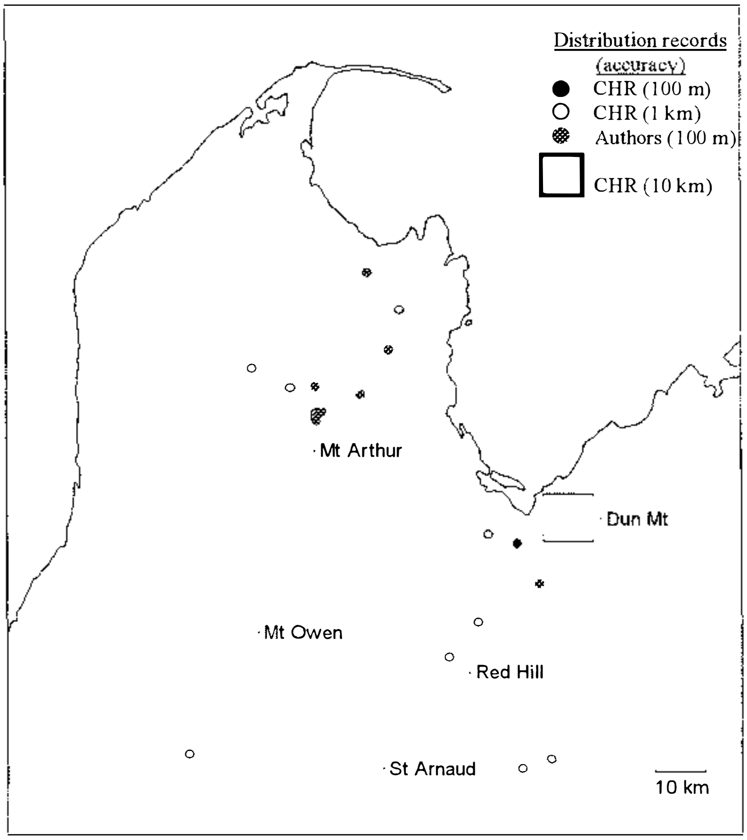
Endemicity: N.Z.

Reproduction: S

Typical population size: S

Threat status: None





All photos: BS

Myosotis forsteri Lehm.

Ranking: None

Abundance and Distribution: *M. forsteri* has a widespread distribution, and is found from 38 ^{0} South. Population sizes are typically small and it is never common where it occurs.

Habitat: forest, streamsides, damp seepages, track edges

Threats: None

Distinguishing features: These plants usually consist of a single rosette, the rosette leaves of which are spathulate often with red gland dots on underside. The hairs are short, stiff, sparse, can be appressed, and are not retrorse underneath. The usually single inflorescence elongates during the flowering period as the flowers open sequentially. There will be a maximum of three flowers out at any one time on each inflorescence. The tube type flowers are white, have yellow scales and are usually small but can vary from 2.5 – 7 mm across. The anthers are always held on filaments that vary in length, but keep the anthers within range of the stigma such that self-pollination can occur.

This species can only be distinguished from *M. venosa* when flowering, by the long filaments, which carry anthers away from the stigma, preventing selfing from occurring in the latter. This species can have quite similar flowers to *M. tenericaulis/spathulata* but is distinguished by its overall size, being a much larger specimen with spreading leaf hairs on the rosette leaves, that are not usually appressed and by their ebracteate, erect inflorescence. Plants can be distinguished from members of the *australis* complex by their fine, crowded, spreading leaf hairs that are retrorse underneath in the latter.

Reproduction and recruitment: Main study site: Myttons Creek. Results: herkogamy: 0 mm; selfing rate 0.975; dichogamy: 12.7%; average seed set: 3.9; vector not required to achieve pollination, fertilization and seed set; open pollinated plants set unvarying high levels of seed under natural conditions; plants in low density patches are never pollen limited and the size of the population does not influence pollination success.

CHR, 132052, Lake Cobb, 2569-, 6016-, Scott VM, 1963; CHR, 140690, Mount Arthur Range, track from Flora Saddle to Mount Arthur, 248--, 600--, Macmillan BH, 1963; CHR, 191798, Upper Takaka River, between Dew Drop Inn and Flora Hut, 248--, 600--, Ritchie IM, 1969; CHR, 191799, Upper Takaka River, between Broken Bridge and Dew Drop Inn, 248--, 600--, Ritchie IM, 1969; CHR, 235573, Matiri scenic reserve (Owen River), 2464-, 5946-, Kelly D; Kelly GC, 1973; *CHR, 24227, Flora Saddle, 2488-, 6001-, Moore LB, 1939*; CHR, 277555, Northwest Nelson, Tableland, Mount Arthur, Rock Shelter, 2481-, 6003-, Druce AP, 1975; CHR, 277593, Northwest Nelson, Mount Arthur Tableland, Cundy Creek, 2478-, 6002-, Druce AP, 1975; CHR, 277742, Flora Stream, north of Mount Arthur, 2485-, 6003-, Druce AP, 1975; *CHR, 278287, Big River, Gouland Downs, 245--, 603--, Simpson MJA, 1973*; CHR, 310592, Northwest Nelson, Above Lake Aorere, 2453-, 6016-, Druce AP, 1977; CHR, 355176, Northwest Nelson, Matiri Range, head of Larrikin Creek, 2452-, 5960-, Druce AP, 1979; CHR, 358511, Northwest Nelson, Garibaldi Ridge, 2462-, 5997-, Druce AP, 1980; CHR, 365738, Richmond Range, Peak West of Mount Richmond, 2541-, 5970-, Druce AP, 1980; CHR, 401005, Northwest Nelson, Anatoki Range, 2478-, 6027-, Druce AP, 1984; CHR, 415341, Nelson, Matakitaki Valley, Peak Creek Track., 24706, 59090, Burke WD 22, 1984; CHR, 468139, Nelson, Anatoki River, 2476-, 6027-, Mason R 13295, 1977; CHR, 476118, Northwest Nelson, Gouland Downs, 2454-, 6035-, Druce AP APD1854, 1992; **Authors records** Myttons Ck, 24752, 60108; Aitkens Crossing, 24833, 60049; Cundy Ck, 24785, 60025; L. Peel track, 24785, 60074; Lake Cobb, 24690, 60167; Lake Cobb, 24694, 60167; Lake Cobb, 24692, 60167; Thorn's Ck, 24765, 60090; Hannah Ck, 24737, 60122; Myttons Ck, 24752, 60108; Mt Arthur track, 24880, 60007; Gouland Downs, 24554, 60350; Gouland Downs, 24553, 60349; Gouland Downs, 24554, 60349; Nuggety Ck, 24706, 59676; Kiwi Saddle, 24678, 59774; Peat Creek, 24823, 60068

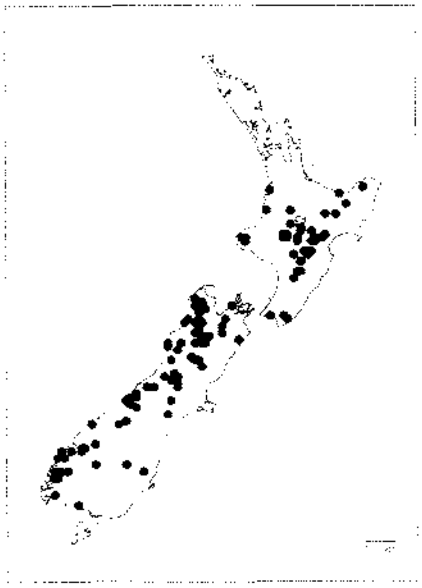
Endemicity: N.Z.

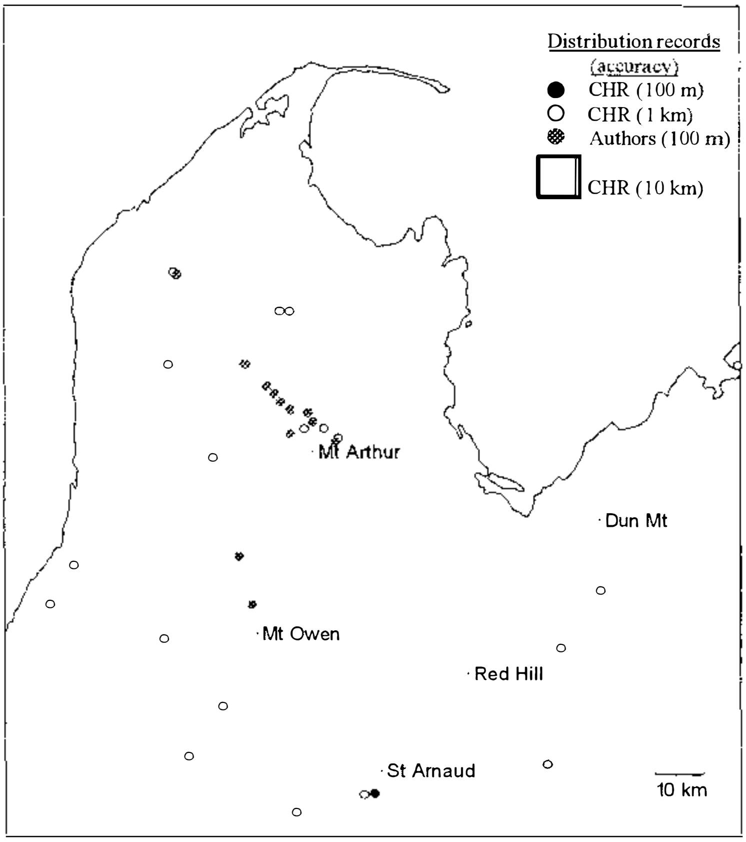
Endemicity: N.Z.

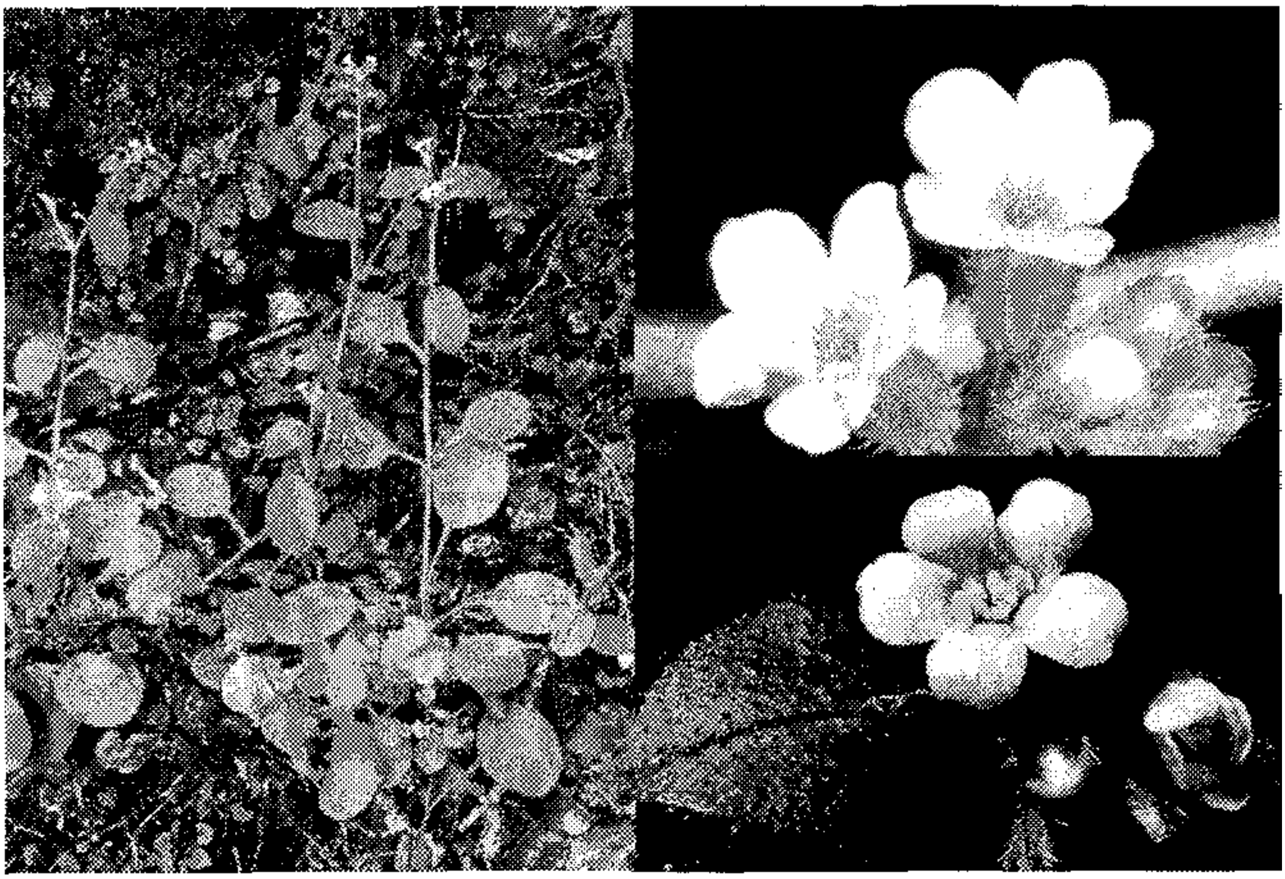
Reproduction: S

Typical population size: S

Threat status: None







Main and top right insert: AWR; Lower right insert: BM

Myosotis venosa Col.

Ranking: None

Abundance and Distribution: *M. venosa* has a central New Zealand distribution that extends from the central North Island to the Nelson region.

Habitat: Damp, mossy, stream sides, damp cave entrances, track sides, occurs within a range of fertilities

Threats: None

Distinguishing features: These plants usually consist of a single rosette, the rosette leaves of which are spathulate often with red gland dots on underside. The hairs are short, stiff, sparse, can be appressed, and are not retrorse underneath. The flowers are white-cream with yellow scales and are unscented. Flowers are of the funnel blossom type but with anthers on long filaments.

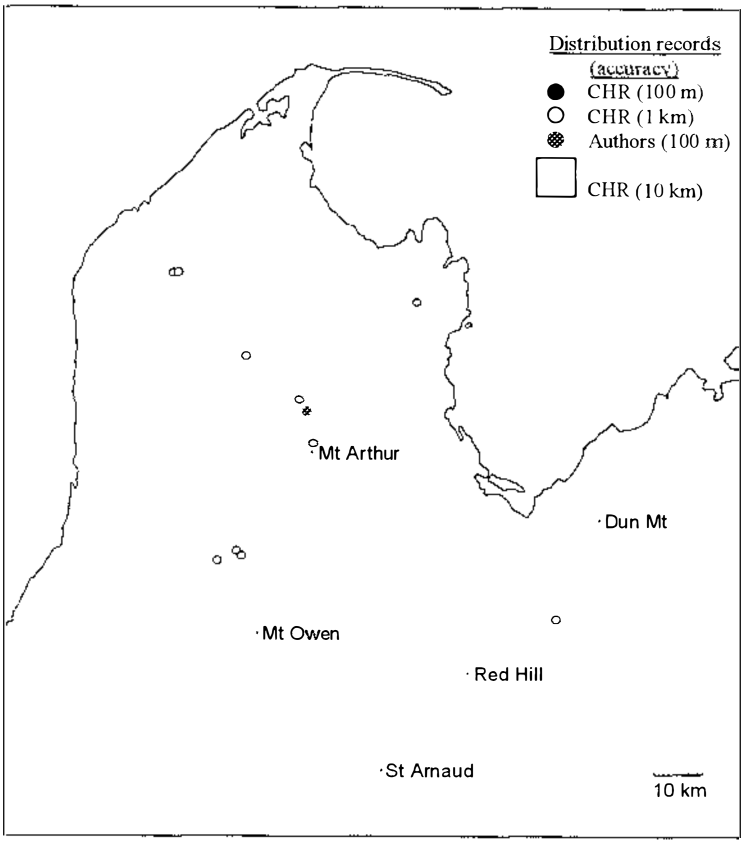
Plants can only be distinguished from *M. forsteri* by filament length, which is long enough in *M. venosa* to prevent the anthers from contacting the stigma, thus preventing self-pollination from occurring. Plants are similar to *M. petiolata* but can be distinguished by the petiole, which in *M. petiolata* is more defined and longer in proportion to the lamina and the leaf hairs are appressed. They have quite different flowers, which are funnel-form with cream coloured spreading corolla lobes in *M. venosa*, as opposed to the brush blossom type with white, flat corolla lobes in *M. petiolata*. Flowers and inflorescences are similar to *M laeta* but can be distinguished by the rosette leaves, which in *M. laeta* have a bronze margin and retrorse hairs underneath.

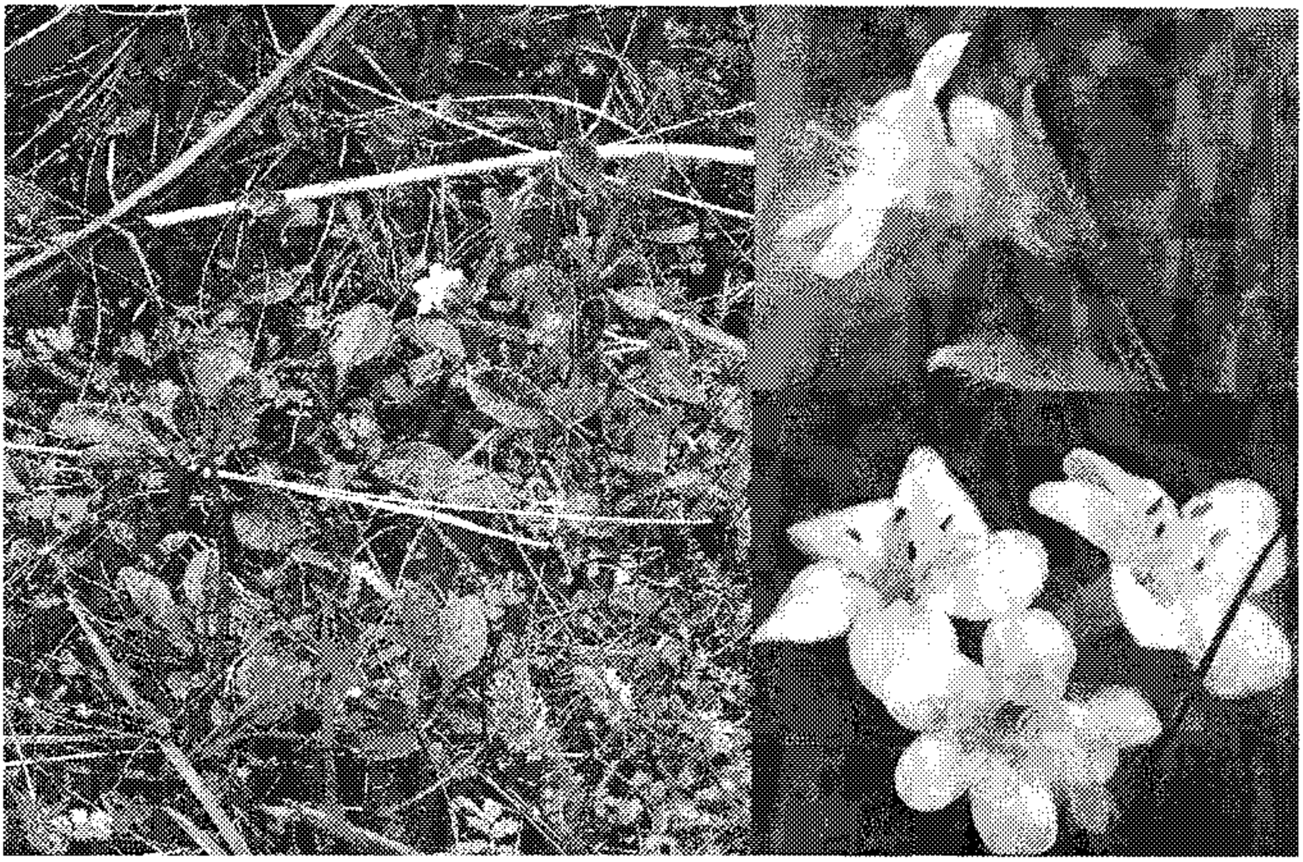
Reproduction and recruitment: Study site: Peat Creek; Results: herkogamy: 0.5 – 0.9 mm; selfing rate: 0.04; average seed/set: 1.4; vector required to achieve pollination, fertilization and seed set.

The herkogamy measurements, selfing rate and seed set by open pollinated plants suggest local density will have an effect on seed set due to pollen limitation.

CHR, 140919, Northwest Nelson, Gouland Downs, 2455-, 6035-, Talbot H; Brockie WB, 1962; CHR, 151030, Cobb Valley, damp slope of Sylvester Stream, 2480-, 6009-, Moore LB, 1964; CHR, 189160, Abel Tasman National Park, Moa Park, 2504-, 6029-, Druce AP, 1969; CHR, 190975, Northwest Nelson, Gouland Downs, 2454-, 6035-, Druce AP, 1969; *CHR, 219277, North branch of Wangapeka river, Stone creek, 2463-, 5976-, Macmillan BH 71/63, 1971*; CHR, 252136, Northwest Nelson, Burgoo stream, 2469-, 6018-, Druce AP, 1970; *CHR, 259065, Northwest Nelson, Gouland Downs, 2455-, 6035-, Talbot H, 1962; CHR, 259066, Northwest Nelson, Gouland Downs, 2455-, 6035-, Talbot H, 1962; CHR, 269164, Arthur Range, Mount Arthur, south of Gordons Pyramid, 2483-, 6000-, Talbot H, 1964; CHR, 273653, Northwest Nelson, north of Mount Patriarch, near Kiwi saddle, 2468-, 5977-, Druce AP, 1974; CHR, 278246, Northwest Nelson, Gouland Downs, 2455-, 6035-, Simpson MJA 7240, 1973; CHR, 278316, Talylor Valley, near Kiwi saddle, 2467-, 5978-, Simpson MJA 7380, 1974; CHR, 28839, Six mile creek, near Mount Lodestone, 248—, 600--, Mason R, 1941; CHR, 387065, Richmond range, near Old Man hut, 2532-, 5964-, Druce AP, 1981; CHR, 87639, Northwest Nelson, Gouland Downs, 2455-, 6035-, Talbot H, 1956; CHR, 87640, Northwest Nelson, Gouland Downs, 2455-, 6035-, Talbot H, 1956*; **Authors records:** Peat Creek, 24820, 60071.

Endemicity: Central N. Is., N.W. S. Is.
Reproduction: P
Typical population size: S
Threat status: None





All photos: BS

Myosotis petiolata Hook. f. var. *petiolata*

Ranking: This species has been considered threatened since 1994 (Category I in Molloy and Davis, 1994; Insufficiently known in Cameron et al, 1995) It is still categorized as Insufficiently Known in the latest list (de Lange et al, 1999). This is the rarest species of *Myosotis* known in the area, but requires survey to determine the true limits of its distribution.

Abundance and Distribution: The historical distribution of this species is a disjunct one, with a few collections having been made in the East Coast and in N. W. Nelson. Currently, this species is only known from two populations near Asbestos Cottage, in the Upper Takaka Valley, one of which contains just three plants, the other a few hundred plants in a tiny area (100x10m). This is very rare and the Nelson populations may be the only extant populations in existence.

Habitat: The two known sites are quite different; one is under an ultramafic rock overhang, and the other is in a damp, tussock-shrub, seepage.

Threats: Small total number of known plants, from two small populations, that occur in a very restricted area, pollen limitation, lack of recruitment; chance event such as a landslide could eliminate most of the known plants in existence.

Distinguishing features: These plants usually consist of a single rosette, the leaves of which consist of a small, more or less orbicular lamina with very long petioles, > 4 x as long as the lamina. There is usually one inflorescence per plant, sometimes branched, that elongates during flowering and flowers open sequentially. Flowers have white, unscented brush-blossom type flowers, with a narrow, short corolla tube with bright yellow scales and flat lobes.

Flowers and inflorescence type can not be distinguished from *M. brockiei*. These two can only be distinguished by the orbicular leaf blade and long narrow well defined petioles on *M. petiolata*. Vegetatively plants can not be distinguished from *M. spathulata / tenericaulis* plants, but they have very different flowers and flowering specimens are required for reliable identification.

Reproduction and recruitment: Study site: Asbestos population. Results: herkogamy: 2 – 5.1 mm; selfing rate: 0.0275; average seed set: 2.6; vector required to achieve pollination, fertilization and seed set; hand pollinations revealed plants were pollen limited.

The reasonable level of seed set by these plants under natural conditions suggests plants are getting adequately pollinated, at least in the flowering season when the study was conducted.

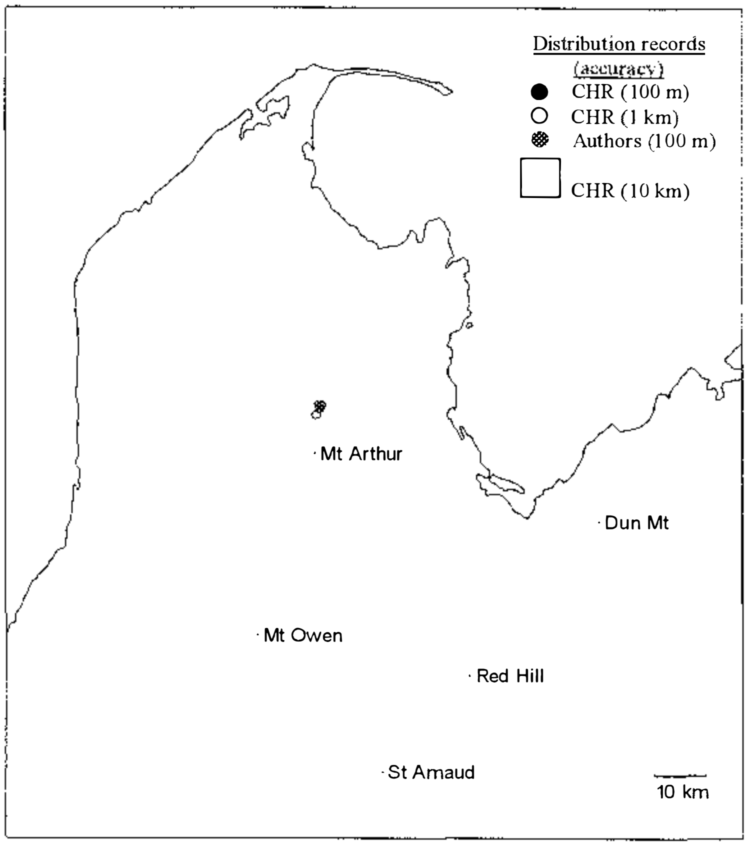
CHR, 191795, Upper Takaka River, near Broken Bridge, 2483-, 6006-, Ritchie IM, 1969; CHR, 401092, Northwest Nelson, Takaka Valley, 2484-, 6008-, Druce AP, 1989; *CHR, 87811, Upper Takaka River, Peat Creek, 248--, 600--, Hay JA, 1950*: **Authors records:** Asbestos, 24840, 60086; Asbestos, 24844, 60077

Endemicity: E. Coast, N. Is & N.W. Nelson, S. Is.

Reproduction: P

Typical population size: S

Threat status: I



All photos: AWR

Myosotis arnoldii L.B. Moore

Ranking: *M. arnoldii* appeared on the Botanical Society Local Plant list (Cameron et al, 1995) and is currently listed as Range Restricted (de Lange et al, 1999).

Abundance and Distribution: *M. arnoldii* has a disjunct distribution. It occurs on a couple of peaks in the Arthur Range (Crusader and Hoary Head), and over on the Chalk Range, in the Kaikouras. It has very restricted geographic distributions, but is reasonably plentiful where it occurs and populations are typically large.

Habitat: Alpine, calcicole, marble and limestone mountains

Threats: None

Distinguishing features: The unique combination of dark purplish-black, scented, funnel blossom flowers with yellow scales and silver leaves along with its highly restricted distribution make identification of this species mistake free. Plants usually consist of several rosettes, the leaves of which are distinctively silver. This species has a compact inflorescence that does not elongate in fruit, there can be many inflorescences per plant, which along with flowers opening synchronously, results in a large floral display. There is a difference between plants of Marlborough and Nelson populations, Marlborough plants have hooked hairs on their calyces, but the Nelson plants do not.

In the absence of flowering specimens, these plants can be distinguished from *M. angustata* by their distinctive silver leaves.

Reproduction and recruitment: Study site: Hoary Head. Results: herkogamy: 2.2 – 7.1 mm; dichogamy: 20%.

Herkogamy measurements suggest a vector is required to achieve pollination, fertilization and seed set.

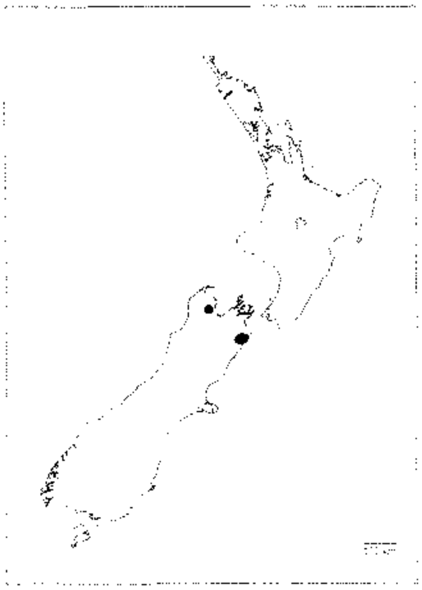
CHR, 132039, 34 Quebec Road, 2493-, 6008-, Given D, 31, 1962; CHR, 208212, Mount Hoaryhead, 2493-, 6008-, Brereton J, 1971; CHR, 243884, Lincoln, Botany Division, 2493-, 6008-, Moore LB, 1974; CHR, 258649, Northwest Nelson, Arthur Range, Hoary Head, 2493-, 6008-, Sneddon BV, 1973; CHR, 258969, Mount Hoary Head, 2493-, 6008-, Sneddon BV, 1973; CHR, 258970, Botany Division Lincoln, cultivated, 2493-, 6008-, Moore LB, 1973; CHR, 269136, Northwest Nelson, Hoary Head, 2493-, 6008-, Brereton J, 1970; CHR, 387709, Northwest Nelson, Mount Hoary Head, 2493-, 6008-, Druce AP, 1980; **Authors records:** Hoary Head, 24936, 60080; Hoary Head, 24935, 60087; Hoary Head, 24937, 60086; Hoary Head, 24935, 60084; Hoary Head, 24936, 60085; Crusader, 24925, 60067.

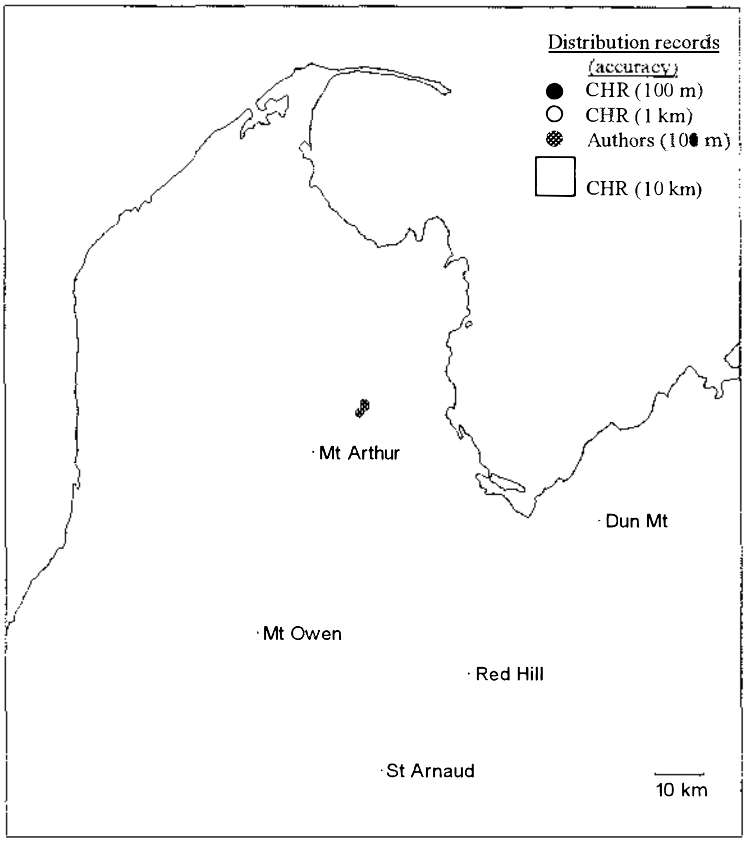
Endemicity: N.W. Nelson & E. Marlb., S. Is.

Reproduction: P

Typical population size: L

Threat status: RR.





Main and top right: AMB; Lower left: BM

Myosotis angustata Cheesem.

Ranking: *M. angustata* appeared on the Botanical Society Local Plant list (Cameron et al, 1995) and is currently listed as Range Restricted (de Lange et al, 1999).

Abundance and Distribution: This species is known to occur over a small geographic range in typically small populations.

Habitat: Alpine, calcicole, marble mountains, steep but stable scree slopes

Threats: small geographic range, small populations, possible over collecting at the Mount Arthur population due to ease of access.

Distinguishing features: These plants usually consist of several rosettes, the leaves of which are narrow and bluish green in colour with leaf hairs that are both short and long, appressed, not retorse underneath. The inflorescence remains compact throughout flowering, which along with synchronous opening of flowers gives it an almost capitate appearance. Each plant can have several inflorescences which along with the capitate cymes produce a large floral display. Flowers are white, unscented, tube-blossom type with yellow scales, short filaments and variable style length.

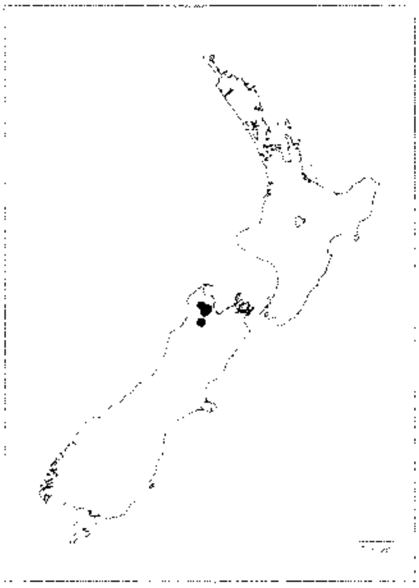
Plants can be distinguished from *M. taversii,* which has similar inflorescence type and flowers, by their leaf hairs which in *M. traversii* are long, overlapping, not appressed, and retrorse underneath. An additional difference is that *M. traversii* has hooked hairs on its calyces. Plants can be distinguished from *M. macrantha* by their very different flowers, but in absence of flowers, in *M. macrantha* usually leaf hairs are absent on the under sides of the rosette leaves.

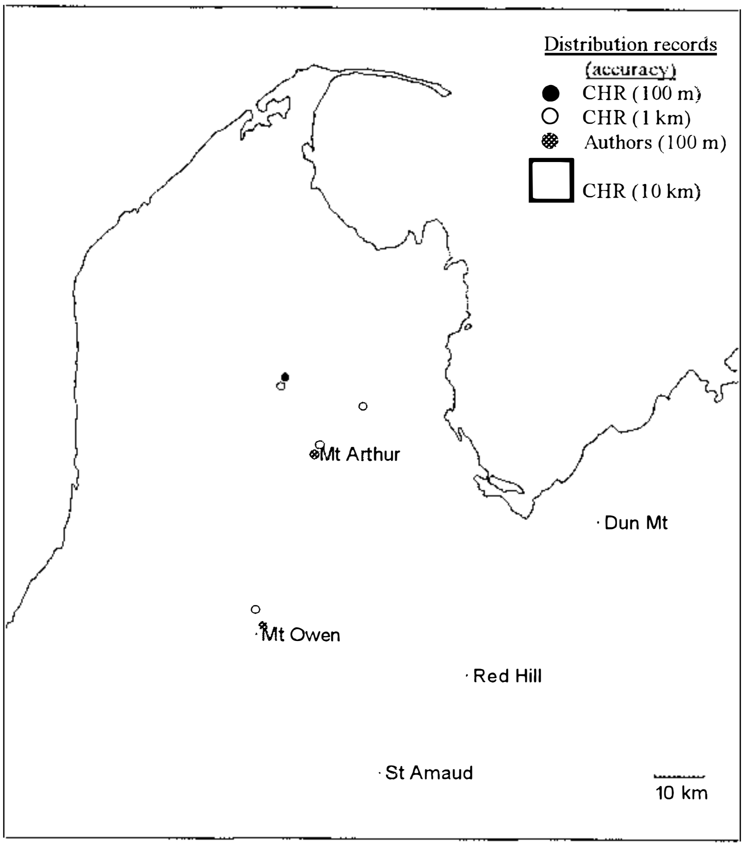
Reproduction and recruitment: Study site: Mount Arthur. Results: herkogamy: 0 – 1.5 mm.

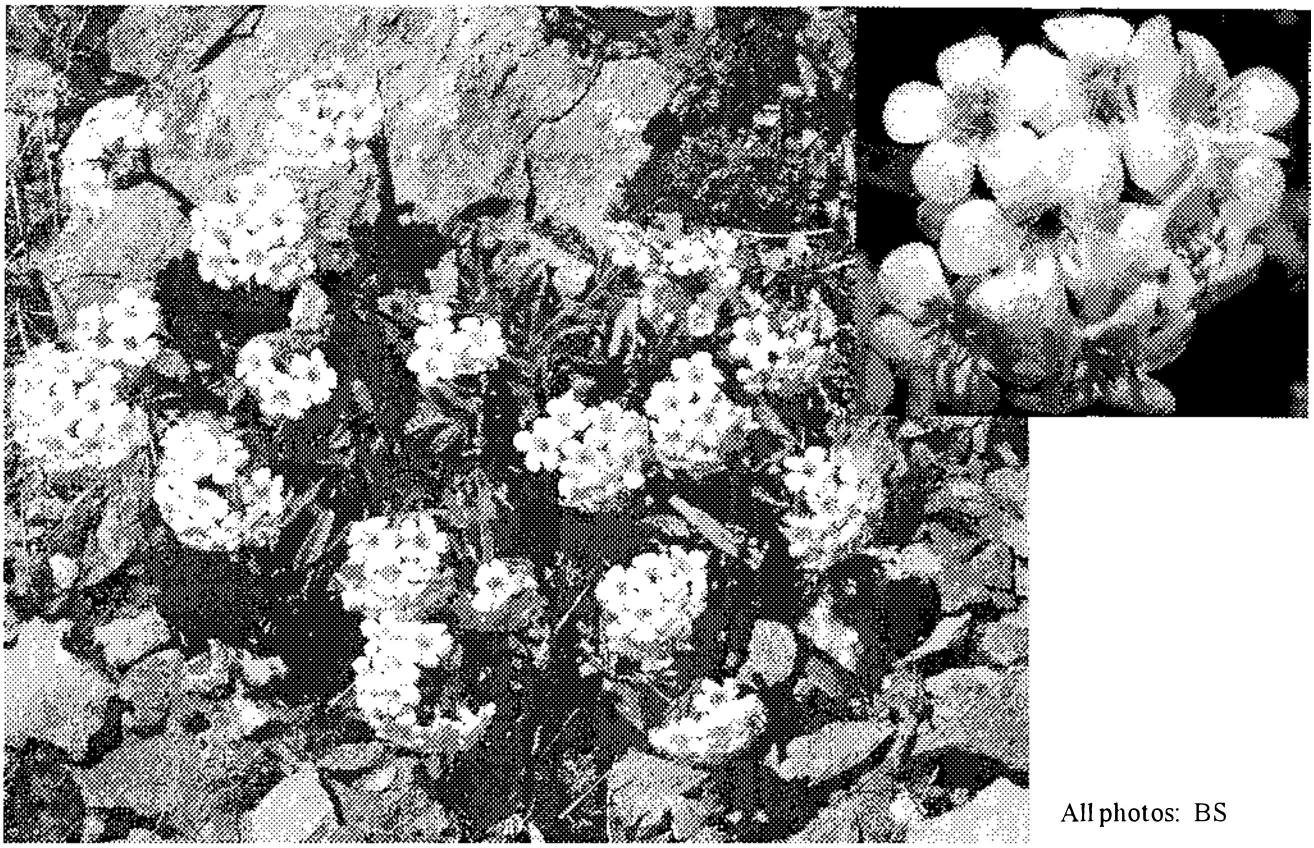
The herkogamy measurements suggest this species has both selfing and non-selfing type flowers on different plants within the population. This was due to variation in the length of the style, carrying the stigma either above or at the level of the anthers. The herkogamy measured suggests some of the plants in the population will not be affected by density in their reproductive effort.

CHR, 187715, Northwest Nelson, Top of Mount Arthur, 2483-, 5998-, Ritchie MA; Ritchie IM, 1970; CHR, 187735, Northwest Nelson, Top of Mount Arthur, 2483-, 5998-, Ritchie IM, 1970; *CHR, 191794, Northwest Nelson, Mount Arthur, basin at head of Horseshoe Creek, 2484-, 6000-, Ritchie IM, 1969; CHR, 208217, Northwest Nelson, Mount Hoaryhead, 2493-, 6008-, Brereton JA, 1971;* CHR, 277613, Northwest Nelson, Mount Arthur, 2483-, 5998-, Druce AP, 1975; *CHR, 295297, Northwest Nelson, Mount Arthur, 2483-, 5998-, Wall A, 1921;* CHR, 387628, Northwest Nelson, Mount Arthur, 2483-, 5998-, Druce AP, 1982; CHR, 387640, Northwest Nelson, Iron Hill, 2476-, 6012-, Druce AP, 1982; CHR, 393696, Northwest Nelson, Owen Range, Culliford Hill, 2471-, 5966-, Druce AP, 1983; *CHR, 97309, Mount Arthur, 2483-, 5998-, Gibbs FG, 1895; CHR, 97310, Northwest Nelson, Cobb Valley, Mount Lockett, 24774, 60144, Gibbs FG;* **Authors records:** Mt Arthur, 24836, 59986; Mt Arthur, 24833, 59986; Mt Arthur, 24834, 59985; Mt Bell, 24732, 59633

Endemicity: N.W. Nelson
Reproduction: P&S
Typical population size: S
Threat status: RR.







All photos: BS

Myosotis concinna Cheesem.

Ranking: *M. concinna* appeared on the Botanical Society Local Plant list (Cameron et al, 1995) and is currently listed as Range Restricted (de Lange et al, 1999).

Abundance and Distribution: *M. concinna* is endemic to the Mount Owen marble massif. It has a very restricted geographic distribution but is quite common where it occurs. Population sizes are typically large.

Habitat: Calcicole, south facing marble bluffs and rock ledges, or close to rock ledges, stable marble screes, from about 1300 - 1870 m a.s.l.

Threats: none

Distinguishing features: Plants usually consist of several rosettes, the leaves of which have a crowded covering of hairs that are fine, silky and appressed. The inflorescence elongates during flowering with flowers opening sequentially. The flowers are yellow in colour, scented, and of the brush blossom type with very long filaments.

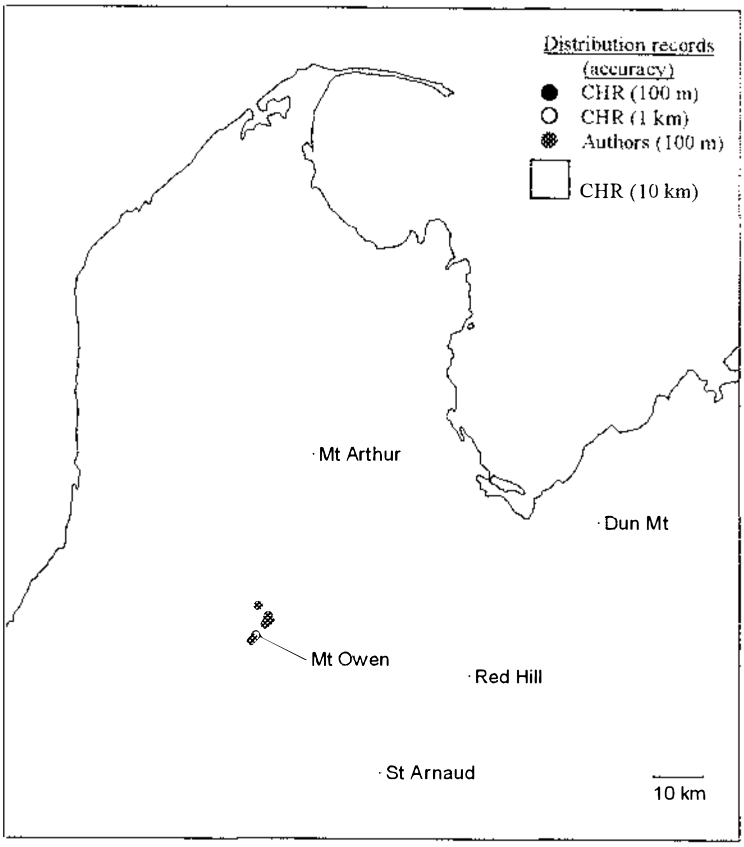
Plants are difficult to distinguish from *M. brockiei* using leaf characters alone as both have fine overlapping hairs on their rosette leaves, but in *M. concinna* they are silky and appressed, in *M. brockiei* they are arctuate. Although their habitats overlap, *M. concinna* is the only one found in the high alpine zone, and never below tree line. They can reliably be distinguished by their flowers, which in *M. concinna* are yellow with long corolla tube, in *M. brockiei* flowers are white with a very short tube. *M. concinna* can be distinguished from *M. macrantha* in the absence of flowers by leaf hairs, which are usually absent on the undersides of the rosette leaves in *M. macrantha.* Plants can be distinguished from *M. angustata* by their rosette leaves which, in *M. angustata*, are very narrow, with short and long leaf hairs, that are not retrorse underneath. Plants can be distinguished from *M a.*. 'small white' apart from the general size of the plant, *M. concinna* being a much larger plant, by rosette leaf hairs which in *M. concinna* are appressed, in 'small white' are not.

Reproduction and recruitment: Study site: Granity Pass. Results: herkogamy: 3.6 – 6.2 mm.

The herkogamy measurements suggest this species requires a vector to achieve pollination, fertilization and seed set.

CHR, 225936, Mount Owen, 247--, 596--, Moss JB, 1971; CHR, 227638, Mount Owen, 2471-, 5961-, Simpson MJA 6673, 1972; CHR, 227638, Mount Owen, 2471-, 5961-, Bultin M, 1972; CHR, 227660, Mount Owen, Granity Pass, 2473-, 5964-, Simpson MJA 6695, 1972; CHR, 227661, Mount Owen, Granity Pass, 2473-, 5964-, Simpson MJA 6696, 1972; CHR, 233848, Mount Owen, 2471-, 5961-, Wellington Botanical Society, 1972; CHR, 249718, Owen Range, Granity Pass, 2473-, 5963-, Druce AP, 1972; CHR, 335755, Mount Owen, 2471-, 5961-, Townson W; CHR, 335756, Mount Owen, 2471-, 5961-, Cheeseman TF, 1883; AK, 7549, Mount Owen, 247--, 596--, Cheeseman TF; **Authors records:** Granity Pass, 24744, 59646; Granity Pass, 24741, 59645; Granity Pass, 24737, 59646; Granity Pass, 24738, 59645; Billies Knob, 24739, 59650; Billies Knob, 24741, 59655; Mt Bell, 24735, 59638; Castle Basin, 24710, 59609; Castle Basin, 24706, 59604; Culliford Ridge, 24722, 59676.

Endemicity: N.W. Nelson, S. Is.
Reproduction: P
Typical population size: L
Threat status: None



All photos: BS

Myosotis lyallii Hook. F. / *M. elderi* L.B. Moore

Ranking: None

Abundance and Distribution: lower North Island and South Island. Known to occur in small populations.

Habitat: sub-alpine - alpine, rocky sites

Threats: None

Distinguishing features: This species complex has little separating individuals other than filament length. Plants usually consist of several rosettes, the leaves of which have an obovate lamina which is longer than the winged petiole, with long hairs which are absent underneath. The inflorescence is compact throughout flowering and each plant can have many inflorescences, although flowers open sequentially. Flowers are white, unscented, tube blossom type, with yellow scales. Only the first few flowers on the cyme are bracteate, and style and filament length vary.

This complex has small floral character differences in style length and filament length. Other than that all vegetative characteristics are very similar.

Plants can be distinguished from *M. pygmaea* s.l. in being a larger plant with multiple rosettes. Rosette leaf shape is different with a lamina longer than the winged petiole, whereas in *M. pygmaea* they are more equal in length. Calyx properties differ; in *M. lyallii / elderi*, the calyx is lobed to ½ way, ,with soft, spreading hairs whereas in *M. pygmaea* the calyx is lobed to less than ½ way, and some of the short hairs on the calyx are retrorse. There is a major difference in the flowers which are twice the size in *M. lyallii / elderi*, both in corolla width and length.

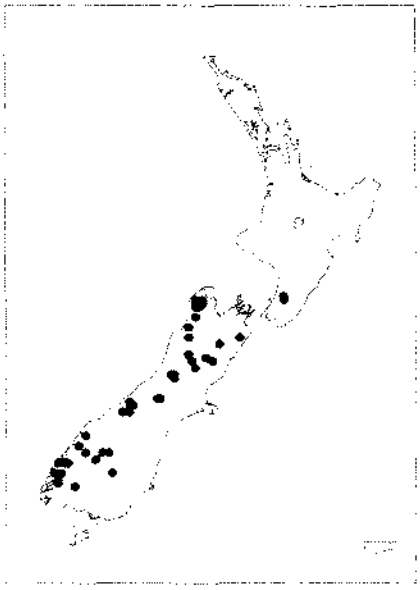
Plants can be distinguished from *M. traversii* by having sequentially opening flowers, larger, flatter corolla lobes and no hairs on the undersides of the rosette leaves. Plants can be distinguished from *M. macrantha,* which is very similar vegetatively, by their smaller, less robust leaves but more reliably by their very different flowers which are only white in *M. lyallii / elderi*.

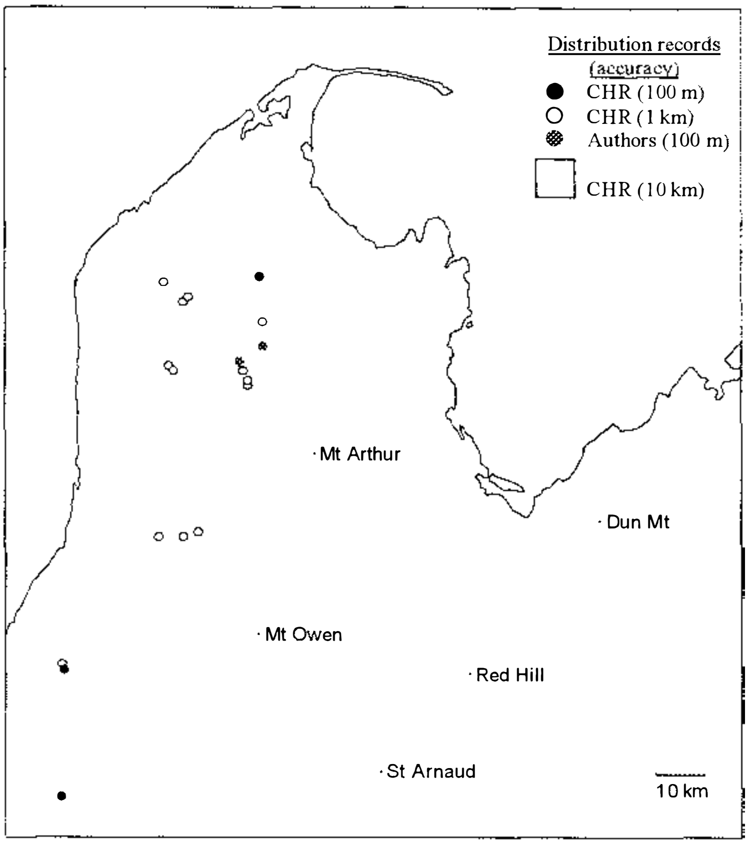
Reproduction and recruitment: Study sites: Kakapo Peak and Mt. Gibbs. Results: herkogamy: 0 – 1.4 mm.

The herkogamy measurements suggest this species has both selfing and non-selfing type flowers on different plants within the populations. This was due to variation in the length of the style, carrying the stigma either above or at the level of the anthers. Variation in filament length and anther exertion was also observed. The herkogamy measured suggests some of the plants in the population will not be affected by density in their reproductive effort.

CHR, 395797, Northwest Nelson, Glasgow Range, 2431-, 5955-, Druce AP, 1986; CHR, 395804, Northwest Nelson, Glasgow Range, 2431-, 5955-, Druce AP, 1986; CHR, 359091, Buller, Glasgow Range, 24319, 59544, Loh G; CHR, 387139, Northwest Nelson, Scarlet Range, Mount Brilliant, 2451-, 5981-, Druce AP, 1981; CHR, 311607, Northwest Nelson, above Lake Aorere, 2453-, 6016-, Druce AP, 1977; CHR, 310505, Northwest Nelson, Mount Centre, 2454-, 6015-, Druce AP, 1977; CHR, 387135, Northwest Nelson, Herbert Range, Mount Herbert, 2456-, 5981-, Druce AP, 1981; CHR, 387136, Northwest Nelson, Herbert Range, Mount Herbert, 2456-, 5981-, Druce AP, 1981; CHR, 387137, Northwest Nelson, Herbert Range, Mount Herbert, 2456-, 5981-, Druce AP, 1981; CHR, 389955, Northwest Nelson, Mount Kendall, 2459-, 5982-, Druce AP, 1983; CHR, 401045, Northwest Nelson, above Lonely Lake, Douglas Range, 2472-, 6025-, Druce AP, 1984; *CHR, 183516, Northwest Nelson, Summit of Lead Hill, Boulder Lake area, 24720, 60348, Ritchie IM, 1968*; CHR, 222760, Northwest Nelson, Mount Goul, 2456-, 6029-, Druce AP, 1973; CHR, 358192, Northwest Nelson, Cobb Valley, Mount Cobb, 2468-, 6015-, Robins I, 1970; CHR, 363561, Northwest Nelson, Kakapo Peak, 2472-, 6020-, Druce AP, 1980; CHR, 365452, Northwest Nelson, South East of Island Lake, 2467-, 6017-, Druce AP, 1980; CHR, 370002, Northwest Nelson, Peel Range, Mount Ranolf, 2469-, 6012-, Druce AP, 1982; CHR, 370034, Northwest Nelson, Peel Range, Mount Prospect, 2469-, 6013-, Druce AP, 1982; CHR, 389957, Northwest Nelson, Mount Kendall, 2459-, 5982-, Druce AP, 1983; **Authors records:** Kakapo Peak, 24726, 60206; Mt Gibbs, 24678, 60174.

Endemicity: Lower N. Is., S. Is.
Reproduction: S & P
Typical population size: S
Threat status: None





All photos: BS

Myosotis traversii Hook. f. var. *traversii*

Ranking: None

Abundance and Distribution: Northern South Island

Habitat: Alpine screes

Threats: None

Distinguishing features: Plants usually consist of several rosettes, the leaves of which have lots of long spreading hairs that are shorter and retrorse underneath. The inflorescence remains compact throughout flowering, which along with synchronous opening of flowers gives it an almost capitate appearance. Each plant can have several inflorescences which along with the capitate cymes produce a large floral display. Flowers are white, unscented, tube blossom type with yellow scales and anthers extending out of the tube on short filaments.

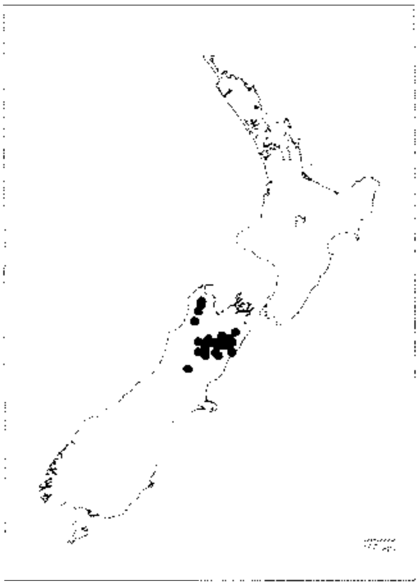
Plants can be distinguished from *M. angustata*, which has similar flowers and inflorescence, by their wider leaves, leaf hairs which are not appressed, are retrorse underneath, and by having hooked hairs on their calyces. Plants can be distinguished from *lyallii / elderi* by the presence of hairs on the undersides of the rosette leaves, smaller (1.5 mm) corolla lobes that are rounded, and more or less capitate inflorescence. Plants can be distinguished from *M. macrantha* by their rosette leaves which have hairs underneath and by their very different flowers which are always white.

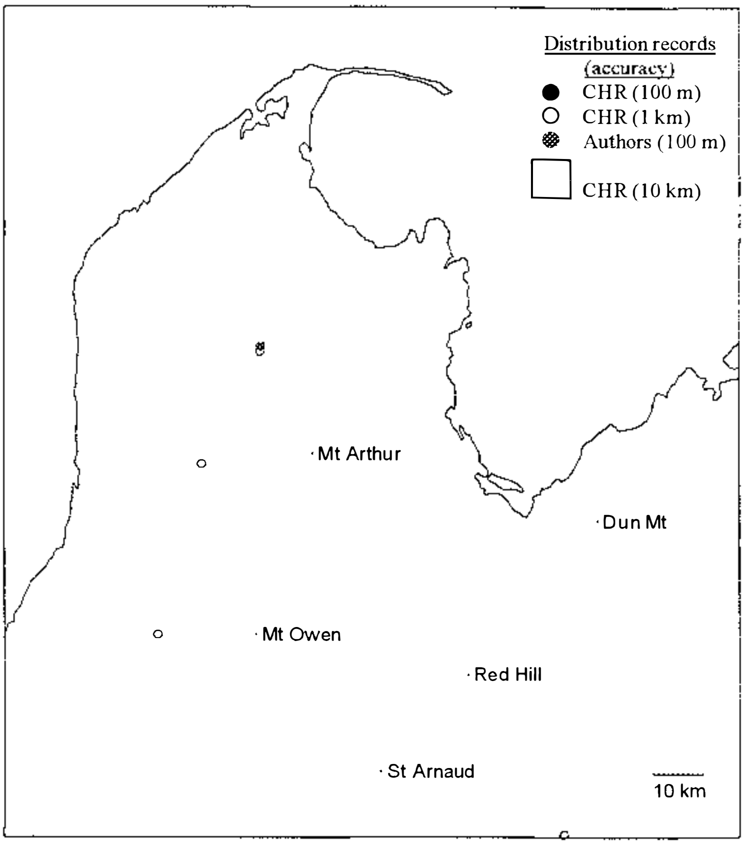
Reproduction and recruitment: Study site: Kakapo Peak. Results: herkogamy: 0 – 1.2 mm.

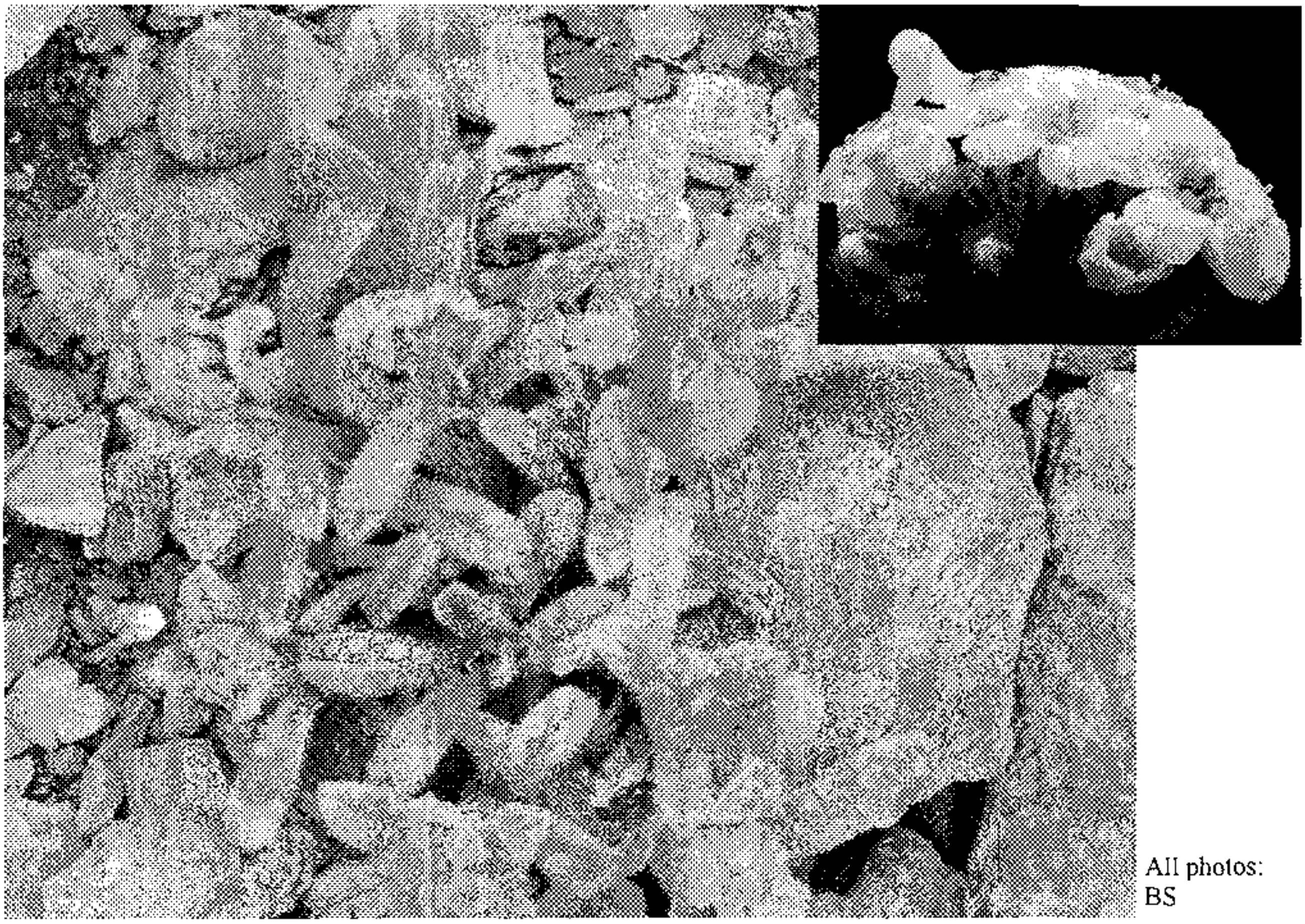
The herkogamy measurements suggest this species has both selfing and non-selfing type flowers on different plants within the population. This was due to variation in the length of the style, carrying the stigma either above or at the level of the anthers. The herkogamy measured suggests some of the plants in the population will not be affected by density in their reproductive effort.

CHR, 252108, Northwest Nelson, Kakapo Peak, 2472-, 6020-, Druce AP, 1970; *CHR, 34872, Kakapo Peak, 2472-, 6020-, Butcher JW, 1946*; CHR, 354916, Northwest Nelson, Matiri Range, The Needle, 2451-, 5961-, Druce AP, 1979; *CHR, 358439, Northwest Nelson, Garibaldi Ridge, 2460-, 5996-, Druce AP, 1980*; CHR, 365454, Northwest Nelson, Kakapo Peak, 2472-, 6020-, Druce AP, 1980; CHR, 387848, Northwest Nelson, Snowden Range, Kakapo Peak, 2472-, 6019-, Druce AP, 1981; CHR, 401807, South-west Nelson, South-south-west of Mount Baldy, 2461-, 5906-, Druce AP, 1984; **Authors records:** Kakapo Peak, 24728, 60206; Kakapo Peak, 24725, 60205; Kakapo Peak, 24726, 60205

Endemicity: Northern S. Is.
Reproduction: S & P
Typical population size: M
Threat status: None







All photos: BS

Myosotis pygmaea Col. var. *pygmaea* and *M. p.* var. *drucei* L.B. Moore

Ranking: None

Abundance and Distribution: Widespread but sparse throughout New Zealand

Habitat: Non-forest, coastal salt turf – alpine

Threats: None

Distinguishing features: Plants in this complex usually consist of single rosettes that are typically small. Inflorescences are prostrate and remain compact throughout flowering. There are not usually more that 3 flowers open at any one time on an inflorescence, and flowers open sequentially. Flowers are small, of the tube blossom type with yellow scales and are bracteate.

Although these two taxa are distinctive in the Nelson region by the traits that distinguish them in Allan (1961), they are not as easily split when encountered outside the Nelson region. For mapping purposes, the distributions are therefore lumped as they can not reliably be separated.

M. p. var. *pygmaea*	*M. p.* var. *drucei*
White corolla, acute lobes, corolla 1.5-3mm wide	Cream –white corolla, obtuse lobes, corolla 3mm wide
Tips of nutlets visible above calyx tips in fruit	Nutlets completely hidden inside calyx
Stiff leaf hairs	Soft leaf hairs

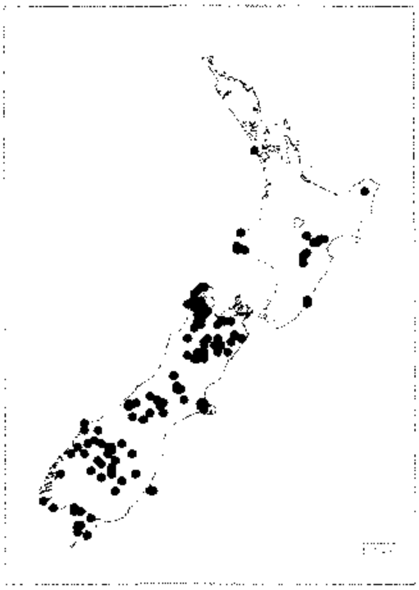
Plants can be distinguished from *M. spathulata / tenericaulis* by their more prostate vegetative habit, and rosette leaves, which do not have a well defined petiole and are usually hairless underneath. Plants can be distinguished from *M. lyallii / elderi* by their more prostrate habit and inflorescence, smaller overall size and single rosette. Their prostrate features distinguish plants from other small white flowered taxa such as *M. a.* 'small white' and *M. forsteri*.

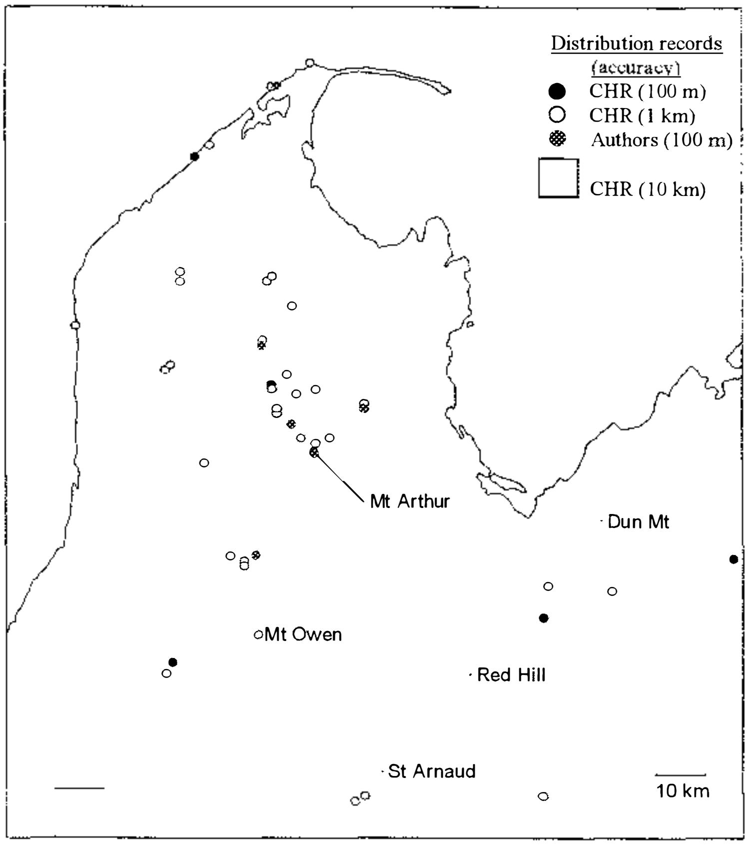
Reproduction and recruitment: Study site: Kakapo Peak and Mt. Arthur. Results: herkogamy: 0 mm.

Herkogamy measurements suggest this species is able to self-pollinate and does not require a vector to achieve pollination. It also suggests open pollinated plants will set unvarying high levels of seed under natural conditions and plants in low density patches will not be pollen limited. This suggests population growth is probably not seed limited.

CHR, 245173, West of Cape Farewell, Wharariki beach, 2482-, 6078-, Druce AP, 1971; CHR, 277811, Northwest Nelson, 2474-, 6073-, Druce AP, 1974; CHR, 285784, Northwest Nelson coast, sandhills creek, 2458-, 6059-, Druce AP, 1975; *CHR, 295291, Isolated Hill, 25905, 59227, Wall A, 1930*; CHR, 313155, Northwest Nelson, north of Heaphy river, 2434-, 6024-, Druce AP, 1977; CHR, 325710, Northwest Nelson, 1 mile south-west of Paturau river mouth, 2461-, 6061-, Druce AP, 1978; CHR, 387724, Northwest Nelson, Mount Hoary Head, 2493-, 6008-, Druce AP, 1980; CHR, 403757, Cobb Reservoir., 2483-, 6011-, Given DR 13218; Given BB, 1983; CHR, 405098, North-west coast, Sandhill Creek., 24586, 60592, Park GN, 1975; CHR, 76086, West Coast south of Farewell spit, Wharariki, 2482-, 6078-, Petterson JA, 1954; *CHR, 87563, Mount Richmond Forest Park, Mount Rintoul, 25296, 59650, Gibbs FG; CHR, 159861, Above Cobb Dam, Lake Sylvester hut, 2479-, 6010-, Druce AP, 1964; CHR, 187736, Northwest Nelson, Top of Mount Arthur, 24833, 59988, Ritchie IM, 1970; CHR, 191796, Mount Arthur, ridge from Flora Hut, 2486-, 6001-, Ritchie IM, 1969*; CHR, 197030, Northwest Nelson, Gouland downs, 2455-, 6033-, Druce AP, 1969; *CHR, 208224, Mount Arthur, 2483-, 5998-, Brereton JA, 1971; CHR, 223074, Richmond Forest Park, Mount Starveall, north of point, 2530-, 5971-, Given DR 71549, 1971; CHR, 227637, Mount Owen, 2471-, 5961-, Simpson MJA 6672, 1972*; CHR, 249602, Mount Owen, 2471-, 5961-, Druce AP, 1972; CHR, 261786, Coast near Big River mouth, 20421, 54259, Johnson PN, 1973; *CHR, 269153, Mount Peel, 2475-, 6007-, Talbot H, 1948*; CHR, 273514, Northwest Nelson, Cobb Valley, 2474-, 6011-, Druce AP, 1974; CHR, 273843, Northwest Nelson, Mount Patriarch, 2468-, 5976-, Druce AP, 1974; CHR, 274161, Northwest Nelson, Cobb Valley, 24744, 60124, Haydock K, 1974; CHR, 277570, Northwest Nelson, Mount Arthur Tableland, 2480-, 6001-, Druce AP, 1975; *CHR, 278288, Gouland Downs, Big River, 245–, 603--, Simpson MJA, 1973*; CHR, 283061, Northwest Nelson, near Boulder Lake, Orator Creek, 2473-, 6033-, Druce AP, 1976; CHR, 283086, Northwest Nelson, Boulder Lake, 2474-, 6034-, Druce AP, 1976; CHR, 283749, Mount Richmond, 2543-, 5970-, Webb CJ 7498; Webb TH, 1974; *CHR, 285634, Northwest Nelson, Luna Lake, 2465-, 5977-, Druce AP, 1974*; CHR, 310484, Northwest Nelson, above Lake Aorere, 2453-, 6016-, Druce AP, 1977; CHR, 310560, Northwest Nelson, Mount Domett, 2452-, 6015-, Druce AP, 1977; CHR, 311791, North West Nelson, Cobb Valley, 2474-, 6012-, Druce AP, 1977; CHR, 355137, Northwest Nelson, Matiri range, head of Bay creek, 2452-, 5953-, Druce AP, 1979; CHR, 355139, Northwest Nelson, Matiri Range, head of Bay Creek, 2452-, 5953-, Druce AP, 1979; CHR, 358525, Northwest Nelson, Garibaldi ridge, 2460-, 5996-, Druce AP, 1980; CHR, 387645, Northwest Nelson, Lockett Range, Mount Lockett, 2477-, 6014-, Druce AP, 1982; CHR, 389081, Northwest Nelson, South Arthur Range, Mount Patriarch, 2468-, 5975-, Druce AP, 1982; CHR, 401041, Northwest Nelson, Douglas range, 2472-, 6021-, Druce AP, 1964; CHR, 401063, Northwest Nelson, Anatoki range, 2478-, 6028-, Druce AP, 1984; CHR, 510682, Matiri Range, Haystack, 24539, 59558, Ford KA M/10, 1994; *CHR, 76093, Gouland Downs, 245--, 603--, Gibbs FG; CHR, 76994, Mount Arthur, near saddle from Gordon Pyramid, 2483-, 6000-, Hay JA, 1952; CHR, 77000, Northwest Nelson, Mount Arthur summit ridge, 2483-, 5998-, Hay JA, 1952; CHR, 87755, Gouland Downs, 245--, 603--, Talbot H, 1956; CHR, 93441, Cobb Valley, Lake Sylvester, 247–, 601--, Simpson MJA, 1957*; **Authors records:** Balloon, 24785, 60044; Kakapo Peak, 24724, 60204; Mt Arthur, 24833, 59987; Balloon, 24784, 60044; Mt. Patriarch, 24710, 59777; Kaihoka Beach, 24755, 60737; Hoary Head, 24935, 60076.

Endemicity: N.Z.
Reproduction: S
Typical population size: S
Threat status: None





Main and lower: BS; Top: AWR

Myosotis australis R. Br.'yellow'

Ranking: None

Abundance and Distribution: Disjunct distribution, central North Island and top ¾ of South Island

Habitat: Alpine, fertile substrates, rock ledges, stable screes, bare rocky pavement, growing in very little soil

Threats: None

Distinguishing features: Plants usually consist of a single rosette, the leaves of which have fine, crowded spreading hairs that are sparser and retrorse underneath. The inflorescence elongates throughout flowering as flowers open sequentially. There are usually not more than three flowers open at any one time on each inflorescence. Flowers are yellow, unscented, tube blossoms with anthers and stigma included within the tube.

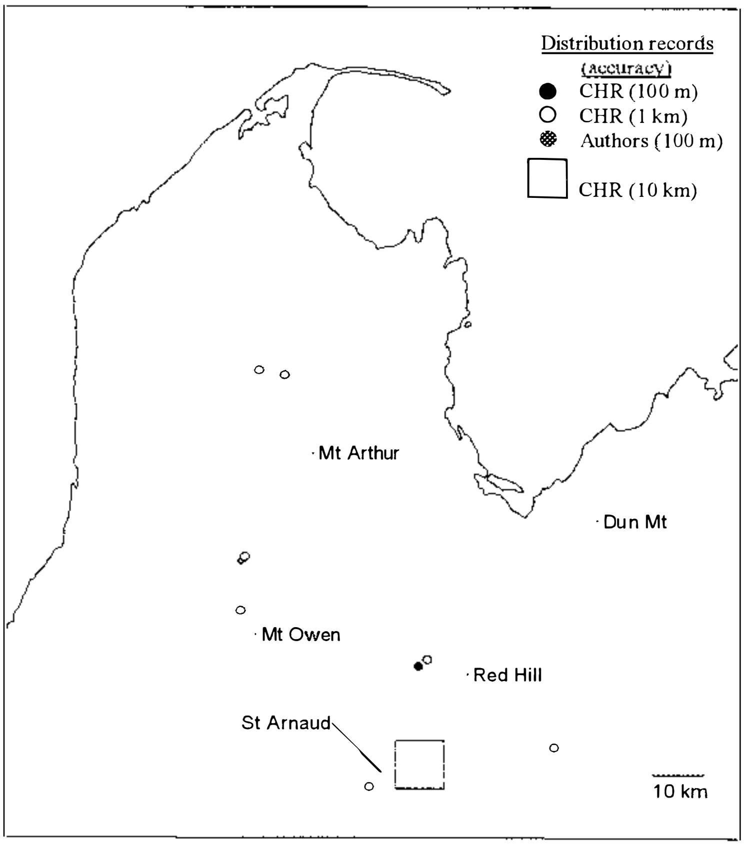
Plants can be distinguished from *M. a.* 'small white' only with flowers. Plants can be distinguished from *M. concinna* by their tube blossom flowers with very short filaments, and rosette leaves which have a good covering of fine hairs that are not appressed.

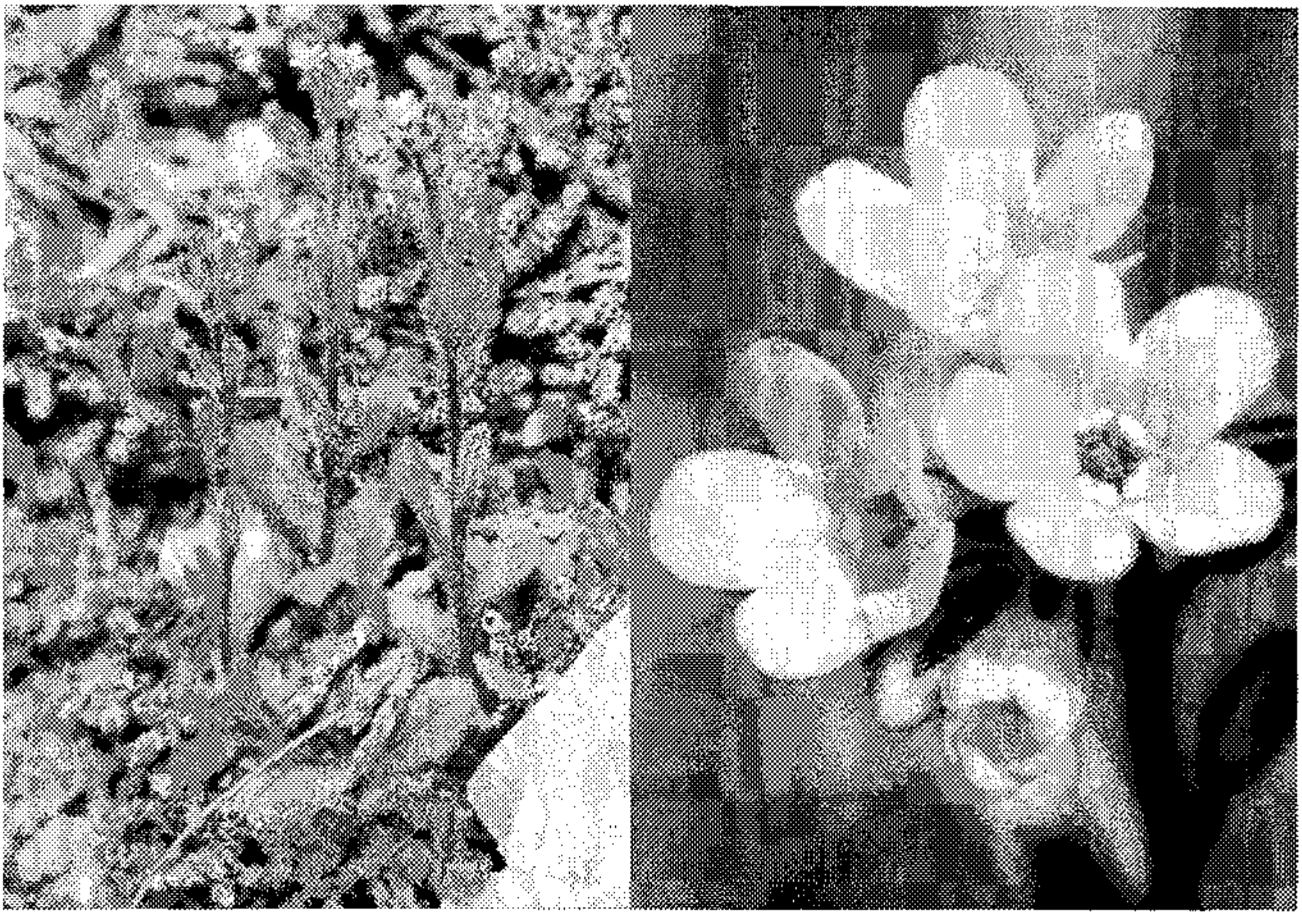
Reproduction and recruitment: Study site: Mt. Patriarch. Results: herkogamy: 0.

Herkogamy measurements suggest this species is able to self-pollinate and does not require a vector to achieve pollination. It also suggests open pollinated plants will set unvarying high levels of seed under natural conditions and plants in low density patches will not be pollen limited. This suggests population growth is probably not seed limited.

CHR, 285305, Mount Patriarch, 2468-, 5976-, Simpson MJA 7391, 1974; CHR, 273792, Mount Patriarch, 2468-, 5976-, Druce AP, 1974; CHR, 273817, Northwest Nelson, North East of Mount Patriarch, 2469-, 5977-, Druce AP, 1974; CHR, 365448, Northwest Nelson, north west of Mount Benson, 2472-, 6015-, Druce AP, 1980; CHR, 387429, Gordon Range, Gordon's Knob, 25048, 59552, Reid JS, 1981; CHR, 387668, Northwest Nelson, Lockett Range, Mount Lockett, 2477-, 6014-, Druce AP, 1982; CHR, 393766, Northwest Nelson, Turks Head Range, 2468-, 5966-, Druce AP, 1983; CHR, 401654, West of Richmond Range, Gordon Range, 2506-, 5956-, Druce AP, 1985; *CHR, 87803, Mount Robert, 2494-, 5930-, Moore LB, 1956*; **Authors records:** Mt. Patriarch, 24689, 59765

Endemicity: Central N. Is & S. Is.
Reproduction: S
Typical population size: S
Threat status: None





Left: AWR; Right: BS

Myosotis australis R. Br. 'small white'

Ranking: None

Abundance and Distribution: This species is found throughout the Nelson region and its distribution extends across to Marlborough. It is known from many sites and is usually sparse.

Habitat: High fertility sites, forest -low-alpine, dry sites, under overhangs, able to tolerate low light levels

Threats: None

Distinguishing features: Plants usually consist of a single rosette, the leaves of which have fine, crowded spreading hairs that are sparser and retrorse underneath. The inflorescence elongates throughout flowering as flowers open sequentially. There are usually not more than three flowers open at any one time on each inflorescence. Flowers are small, white, unscented, tube blossoms with yellow scales. Anthers and stigma included within the tube.

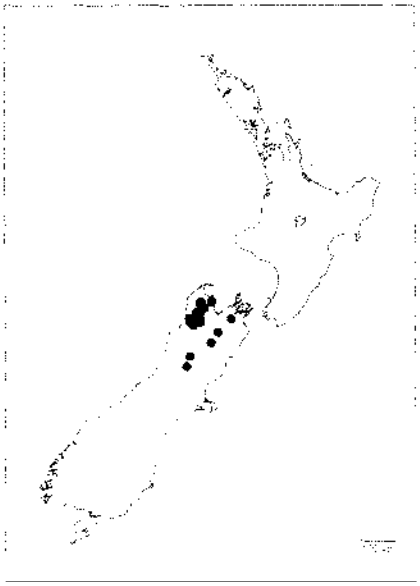
Plants can be distinguished from *M. a.* 'yellow' only with flowers. Plants can be distinguished from other small white flowered species by rosette leaves, inflorescences and habit e.g. Plants can be distinguished from *M. forsteri* by their leaf hairs which are short, stiff and sparse on the upper surface and not retrorse underneath in *M. forsteri*; plants can be distinguished from *M. spathulata / tenericaulis* by their spathulate leaves with well defined petioles and appressed hairs on both sides of the rosette leaves as well as their prostrate, bracteate inflorescence. Plants can be distinguished from *M. pygmaea* by their prostrate habit, prostrate, bracteate inflorescence, broad petioles and absence of hairs on the undersides of their leaves.

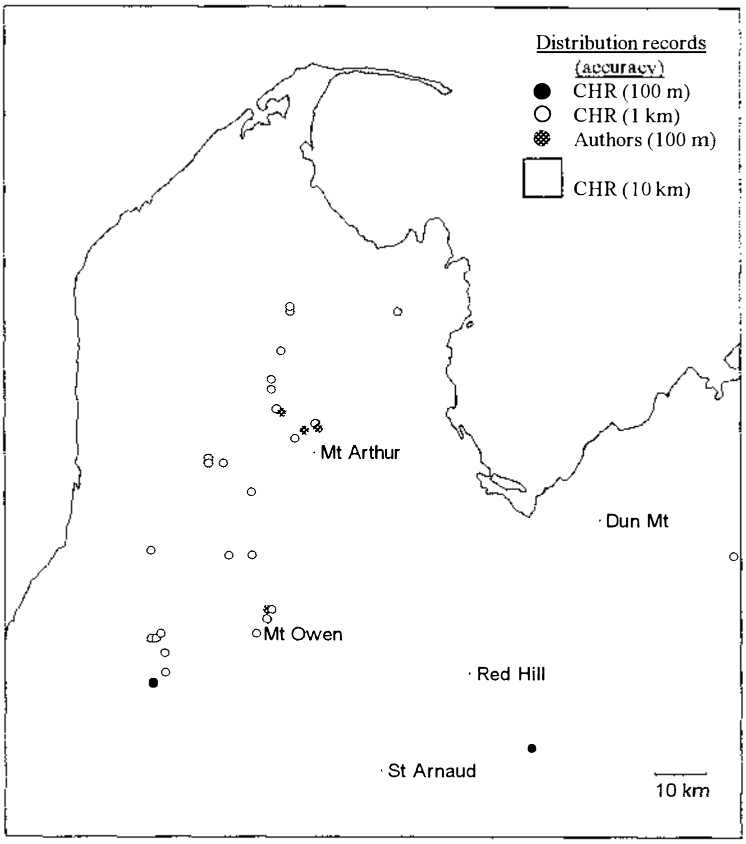
Reproduction and recruitment: Study site: Lake Peel. Results: herkogamy: 0 mm.

Herkogamy measurements suggest this species is able to self-pollinate and does not require a vector to achieve pollination. It also suggests open pollinated plants will set unvarying high levels of seed under natural conditions and plants in low density patches will not be pollen limited. This suggests population growth is probably not seed limited.

CHR, 192301, Northwest Nelson, Pikikuna Range, Canaan Road, 2500-, 6027-, Druce aP, 1969; *CHR, 208221, Mount Arthur district, near turnoff to Salisbury Hut on Flora track, 2483-, 6004-, Brockie WB, 1969; CHR, 227664, Mount Owen, Granity Pass, 2473-, 5964-, Simpson MJA 6699, 1972; CHR, 227666, Mount Owen, Granity Pass, 2473-, 5964-, Simpson MJA 6701, 1972; CHR, 227730, Mount Owen, 2471-, 5961-, Simpson MJA 6765, 1972;* CHR, 249594, Owen, Granity Pass, 2473-, 5964-, Druce AP, 1972; CHR, 249597, Owen Range, foot of Billy Knob, 2474-, 5966-, Druce AP, 1972; CHR, 249692, Owen Range, Blue Creek, 2473-, 5966-, Druce AP, 1972; *CHR, 269137, Mount Peel, 2475-, 6007-, Talbot H, 1948; CHR, 269138, Mount Peel, 2475-, 6007-, Talbot H, 1948; CHR, 269139, Mount Peel, 2475-, 6007-, Talbot H, 1948; CHR, 269140, Mount Peel, 2475-, 6007-, Talbot H, 1948*; CHR, 277552, Northwest Nelson, Mount Arthur Tableland, Rock Shelter, 2481-, 6003-, Druce AP, 1975; CHR, 277571, Northwest Nelson, Mount Arthur Tableland, 2479-, 6001-, Druce AP, 1975; CHR, 277743, Flora Stream, north of Mount Arthur, 2484-, 6003-, Druce AP, 1975; *CHR, 278315, Luna Cirque, 2465-, 5977-, Simpson MJA 7379, 1974*; CHR, 311649, Northwest Nelson, Anatoki Range, 2478-, 6028-, Druce AP, 1977; CHR, 311775, Northwest Nelson, Cobb Valley, 2474-, 6011-, Druce AP, 1977; CHR, 324222, Northwest Nelson, Luna Lake, 2465-, 5977-, Druce AP, 1974; CHR, 324223, Northwest Nelson, Near Mount Patriarch, 2470-, 5977-, Druce AP, 1974; CHR, 355062, Northwest Nelson, Matiri Range, east of Mount Misery, 2451-, 5961-, Druce AP, 1979; CHR, 355063, Northwest Nelson, Matiri Range, east of Mount Misery, 2450-, 5960-, Druce AP, 1979; CHR, 355068, Northwest Nelson, Matiri Range, 2452-, 5957-, Druce AP, 1979; CHR, 355136, Northwest Nelson, Matiri Range, head of Bay Creek, 2452-, 5953-, Druce AP, 1979; CHR, 355238, North West Nelson, Matiri Range, 24500, 59514, Druce AP, 1979; CHR, 358437, Northwest Nelson, Garibaldi Ridge, 2461-, 5996-, Druce AP, 1980; CHR, 358438, Northwest Nelson, , 2461-, 5997-, Druce AP, 1980; CHR, 358479, Northwest Nelson, Garibaldi Ridge, near Sandy Peak, 2464-, 5996-, Druce AP, *1980; CHR*, 365443, Northwest Nelson, South East of Mount Benson, 2474-, 6013-, Druce AP, 1980; CHR, 366178, Northwest Nelson, Matiri Range, Mount Misery, 2449-, 5960-, Druce AP, 1981; CHR, 387140, Northwest Nelson, Allen Range, Mount Zetland, 2449-, 5978-, Druce AP, 1981; CHR, 387828, Northwest Nelson, Snowden Range, 2476-, 6019-, Druce AP, 1981, CHR, 393798, Northwest Nelson, South Arthur Range, Mount Olive, 2470-, 5990-, Druce AP, 1983; CHR, 401004, Northwest Nelson, Anatoki Range, 2478-, 6027-, Druce AP, 1984; CHR, 401367, North Westland, Victoria Forest Park, Blue Grey River, track to Lake Christabel, 2441-, 5865-, Macmillan BH 88/3, Woods EH, 1988; CHR, 401537, Richmond Range, Mount Riley, 2568-, 5977-, Druce AP, 1989; **Authors records:** Gridiron Ck, 24841, 60036; Dry Rock, 24814, 60032; Lake Peel, 24767, 60068; Staircase, 24737, 59665

Endemicity: Northern S. Is.
Reproduction: S
Typical population size: S
Threat status: None





All photos: AWR

Appendix 2

The reproductive biology of Myosotis oreophila: a short report for Alan Mark and Katherine Dickinson

Andrea Brandon[1]

July 2000

Introduction:

Long term monitoring of the rare, high-alpine Dunstan Mountains endemic, *Myosotis oreophila*, has found large fluctuations in population density and size, have occurred during the study period (Stanley *et al.* 1998). Permanent plots were established at the site of the only known population to collect demographic data, which, since 1993, has included such additional information as flower bud initiation, inflorescence number per plant and flowering intensity (Stanley *et al.* 1998). Stanley *et al.* (1998) found that while these flowering characteristics varied over the five year period of the demographic study, they found no obvious relationship to plant recruitment. Population growth does not appear to be affected by any aspect of sexual reproduction. The variation in plant density was thought to be related to environmental factors such as wind exposure and snow lie conditions (Stanley *et al.* 1998).

New Zealand *Myosotis* species studied to date fall into either one of two pollination syndromes based on the degree of herkogamy exhibited by the flowers. Each species either requires a vector to achieve pollination or is able to self-pollinate autonomously (Brandon and Robertson, in prep a). Those species that required vectors to achieve pollination have all been found to suffer Allee effects in that their seed production is disproportionately affected by low local density (Brandon and Robertson, in prep b). In order to determine whether reproductive effort has any effect at all on the demographics of these rare plants, pollination requirements need to be understood. Therefore, to determine pollination requirements and possible density dependence for seed production

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[1] Funding provided by Miss E.L. Hellaby Trust

in *M. oreophila*, the northern Dunstan Mountains population was studied during the last field season.

Methods:

Population: Fieldwork was carried out at the only known locality for this species, in the Northern Dunstan Mountains (G41, 465 871, 1640 m), in February 2000 (Fig. 1).

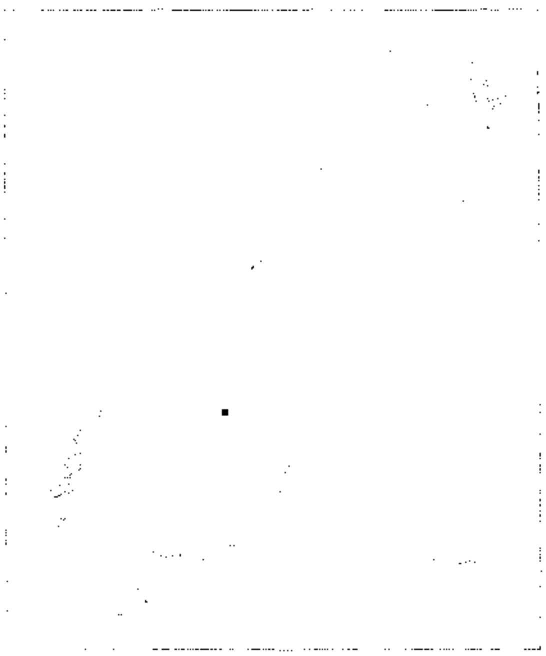

Figure 1: Location of distribution of *Myosotis oreophila*, in the northern Dunstan Mountains, Central Otago, South Island.

Five plants with unopened buds were caged to exclude pollinators. Maturing calyces were collected subsequently and resultant seed set recorded. This was then compared to seed set by unmanipulated, open pollinated plants. Herkogamy was measured using calipers. Seed set counts were made on open pollinated plants for which local flower density was recorded to determine whether local flower density has any influence on seed production.

Data analysis:

The seed set data were analysed using *S-PLUS*. The proportion of seed set was compared between pollinator-excluded flowers and those in open conditions. I examined the effect of local flower density on seed production.

Results and discussion:

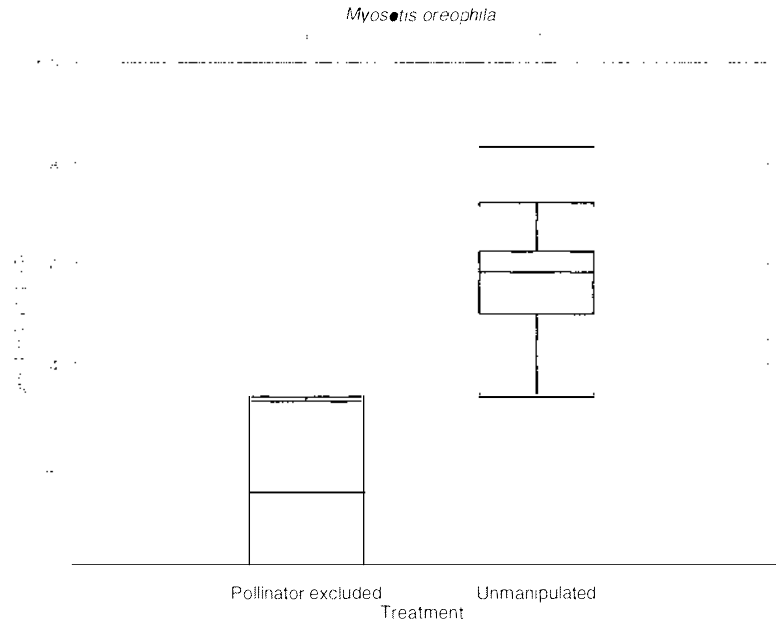

Figure 2: Boxplot of resultant seed from the pollinator excluded plants (left) and that of unmanipulated plants.

Analysis of Variance Table

Response: asprop [asin transformation of the mean proportion of ovules per flower that set seed per plant)]

Terms added sequentially (first to last)

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.8981842	0.8981842	36.43616	4.478796e-006
Residuals	22	0.5423199	0.0246509		

Herkogamy

Table 1: Herkogamy measurements

Plant	Herkogamy (mm)			
1	0.9	1.5	1.4	1.2
2	0.0	0.2	0.0	
3	0.3	0.2	0.0	
4	0.8	1.3	1.2	1.4
5	0.0	0.6	0.8	
6	1.4	1.5	1.2	1.1
7	0.0	0.2	0.8	
8	0.0	0.0	0.3	

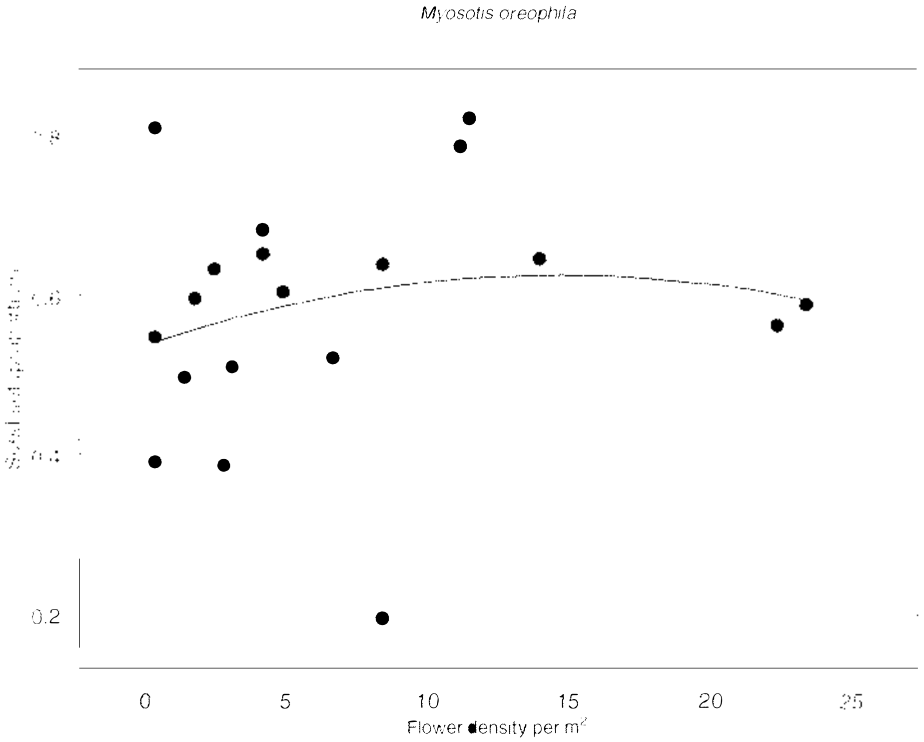

Figure 3: Scatterplot of seed production against local flower density

Analysis of Variance Table on untransformed data

Response: asprop

Terms added sequentially (first to last)

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Flower density	1	0.0105605	0.01056049	0.2836924	0.6011847
Residuals	17	0.6328274	0.03722514		

Analysis of Variance Table on log transformed flower density data

Response: asprop

Terms added sequentially (first to last)

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
log$_{10}$(flower density	1	0.0072393	0.00723925	0.1934568	0.6655956
Residuals	17	0.6361486	0.03742051		

Analysis of Variance Table on polynomial transformed flower density data

Response: asprop

Terms added sequentially (first to last)

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Polynomial (flower density, 2)	2	0.0267349	0.01336746	0.3468391	0.7121042
Residuals	16	0.6166529	0.03854081		

Pollinator excluded plants set significantly less seed than open pollinated plants (P<0.00001). Average seed set for pollinator excluded plants was 0.7 seeds/flower. Average seed set for open pollinated plants was 2.25 seeds/flower. Seed production is significantly affected by the exclusion of pollinator service. Herkogamy was variable within plants sampled, flowers of some plants were always herkogamous, but others were less consistent. Measurements ranged from 0.0 to 1.5 mm. Local density had no effect on seed set by open pollinated plants using untransformed data. Neither log transformation nor a polynomial transformation of flower density made any difference.

Seed set results fall in between the two extremes of the pollinator-requiring type such as *Myosotis monroi* and the selfing type such as *M. forsteri* (see Table 2).

Table 2: Average seed set by other species for comparison (maximum seed set per flower is 4)

Species	Number of seeds set per flower when pollinators are excluded (means)	Number of seeds set per flower by unmanipulated plants (means)
M. oreophila	0.7	2.25
M. monroi	0.16	1.58
M. forsteri	3.8	3.84

The mean number of flowers per inflorescence is 9.5. The mean number inflorescences per flowering plant is 3.2. Therefore, average seed production per flowering plant would be 68.4 (i.e. 2.25*9.5*3.2) for open pollinated plants. In the worst case scenario, if no external pollination occurred, average seed production per flowering plant would be 21.28 (i.e. 0.7*9.5*3.2). Stanley *et al.* (1998) report results from a five year demographic study which estimate the total number of plants in the population have ranged from about 13,000 to 21,800 plants (adult and juvenile) during the study period. Therefore, seed production does not appear to be a limiting factor for population growth of this particularly localized rare plant. In order to be certain, seed augmentation and introduction studies could be carried out to determine whether or not the growth of this population is seed limited.

Herkogamy measurements confirm both vector-requiring and autonomously selfing flower morphologies coexist within the same population and within the same plant. Populations of plants with mixed mating systems can be at an advantage in certain circumstances. In harsh environments, such as at this site in the high alpine zone of the Dunstan Mountains, pollinator availability will vary such that when conditions are severe, selfing individuals may be the only phenotypes reproducing. However, a positive feedback loop can establish, as seed production by self-pollinating individuals is generally high and constant, leading to improved recruitment chances. At times when climatic conditions are favourable, and population demographics are sufficient to attract pollinators, the presence of outcrossing phenotypes in the population can then be beneficial, particularly if inbreeding depression is occurring. In fact the high and consistent seed production of self-fertile plants could allow for considerable selection in the off-spring without showing any detectable effects on demography (Luitjen *et al.* 1998). Wright (1939) considered such a situation to be advantageous to populations in which reproduction is predominantly selfing. He considered occasional crossing could allow for effective selection by genotypes in a "continuously restored field of variability" (Wright, 1939). The mixed mating strategy therefore allows populations to persist in difficult circumstances and then take advantage of more favourable conditions when they occur.

Stanley *et al.*'s (1998) conclusion that environmental factors play an important role in explaining the fluctuations in the demographic parameters that have been measured in this population are therefore supported.

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Appendix 3

Seed set by hand-pollinated flowers of *Myosotis monroi* at the two stages, precocious buds (p) and open flowers (o).

Plant	Stage	Seed set	Ovules	Proportion
1	o	10	16	0.625
	p	8	16	0.5
2	o	21	28	0.75
	p	4	4	1
4	o	7	8	0.875
	p	8	12	0.666667
5	o	4	4	1
	p	9	12	0.75
7	o	11	12	0.916667
	p	8	8	1
9	o	16	20	0.8
	p	4	12	0.333333
12	o	32	36	0.888889
	p	13	20	0.65
13	o	10	12	0.833333
	p	6	8	0.75
14	o	12	12	1
	p	1	4	0.25
16	o	18	32	0.5625
	p	19	40	0.475
20	o	4	4	1
	p	7	8	0.875
21	o	6	8	0.75
	p	9	12	0.75
25	o	10	16	0.625
	p	5	8	0.625
27	o	4	4	1
	p	9	12	0.75
28	o	4	4	1
	p	11	12	0.916667
31	o	16	16	1
	p	27	32	0.84375
32	o	4	4	1
	p	16	16	1
33	o	26	28	0.928571
	p	12	20	0.6
34	o	31	40	0.775
	p	10	16	0.625
35	o	16	16	1
	p	4	8	0.5
36	o	7	8	0.875
	p	6	12	0.5

Means

Stage	Proportion	Seed set/flower
o	0.866903	3.367611
p	0.683829	2.735337

Appendix 4

Pollen load on precocious and fully open flowers of *Myosotis monroi*.

Location	**Pollen load on stigma at precocious phase**	**Pollen load on stigma at end of male phase**
DS	0.00	75.00
DS	0.00	72.00
DS	0.00	103.00
DS	2.00	52.00
DS	0.00	59.00
DS	0.00	64.00
DS	0.00	102.00
DS	0.00	0.00
DS	0.00	8.00
DS	0.00	46.00
DS	6.00	71.00
DS	0.00	23.00
DS	7.00	55.00
DS	0.00	51.00
DS	0.00	55.00
DS	0.00	54.00
DS	0.00	3.00
DS	0.00	0.00
DS	0.00	4.00
DS	0.00	72.00
DS	17.00	104.00
DS	16.00	71.00
DS	0.00	4.00
DS	0.00	0.00
CS	0.00	77.00
CS	0.00	0.00
CS	0.00	10.00
CS	0.00	0.00
CS	0.00	72.00
CS	0.00	42.00
CS	2.00	10.00
CS	0.00	60.00
CS	0.00	39.00
SDM	0.00	38.00
SDM	1.00	15.00
SDM	10.00	87.00
SDM	0.00	6.00
SDM	2.00	42.00
SDM	3.00	41.00
SDM	0.00	5.00
SDM	0.00	3.00
SDM	2.00	3.00

DS = Dun Saddle; CS = Coppermine Saddle; SDM = Southern slopes of Dun Mountain

Appendix 5

Specimens used for morphological measurements in the rarity in New Zealand *Myosotis* study.

M. arnoldii: CHR 258649, CHR 258649, CHR 76183, CHR 269136, CHR 474075
M. angustata: CHR 187735, CHR 387640, CHR 208217, CHR 295297
M. albo-sericea: CHR 416092
M. australis 'yellow': CHR 405242, CHR 405242, CHR 387429, CHR 222987, CHR 491745, CHR 56721, CHR 401654, CHR 69391, CHR 274737
Myosotis australis 'small white': CHR 278315, CHR 269139, CHR 269138, CHR 249692, CHR 311775
M. 'lytteltonensis': CHR 479466, CHR 344951, CHR 344951, CHR 178294, CHR 480453
M. brockiei: CHR 365506, CHR 311722, CHR 335754, CHR 171679, CHR 311717
M. capitata: CHR 49642, CHR 496421, CHR 308227
M. cheesemanii: CHR 475919, CHR 475919
M. concinna: CHR 249718, CHR 335755, CHR 335756, CHR 227661
M. colensoi: CHR 97296, CHR 290735
M. elderi: CHR 56733, CHR 226057, CHR 54371, CHR 222760, CHR 36059
M. eximia: CHR 244028, CHR 63002, CHR 508222
M. explanata: CHR 184602, CHR 328259
M. forsteri: CHR 295263, CHR 334803, CHR 295281, CHR 146371, CHR 355176, CHR 358511, CHR 246338, CHR 323788, CHR 33049, CHR 101430
M. goyenii: CHR 301750, CHR 87810, CHR 439799
M. laeta: CHR 387376, CHR 387462
M. lyallii var. *lyallii*: CHR 67901, CHR 158037, CHR 514986, CHR 223932, CHR 258958
M. lyallii var. *townsonii*: CHR 310505, CHR 387135, CHR 387136
M. macrantha: CHR 314470, CHR 371665, CHR 401060, CHR 76066, CHR 10953, CHR 274159, CHR 295261, CHR 269149, CHR 76067, CHR 283529, CHR 101383.
M. matthewsii: CHR 469640, CHR 469641, CHR 295319, CHR 310255, CHR 311720
M. oreophila: CHR 409172, CHR 409172, CHR 409172
M. monroi: CHR 97282, CHR 60319, CHR 387447, CHR 295249, CHR 295249
M. petiolata var. *petiolata*: CHR 87811, CHR 191795, CHR 208617.
M. petiolata var. *pansa*: CHR 56737, CHR 223568, CHR 367212, CHR 184602, CHR 295315
M. petiolata var. *pottsiana*: CHR 367640, CHR 79073, CHR 508161, CHR 97305
M. pulvinaris: CHR 404251, CHR 404363
M. pygmaea var. *pygmaea*: CHR 208826, CHR 277, CHR 131076, CHR 313155, CHR 325710, CHR 387724, CHR 309606, CHR 245912, CHR 308602, CHR 10961, CHR 56712, CHR 357370, CHR 303844
M. pygmaea var. *drucei*: CHR 131906, CHR 190681, CHR 80165, CHR 311791, CHR 207050, CHR 316348, CHR 364085, CHR 252154., CHR 197031, CHR 249602.
M. pygmaea var. *glauca*: CHR 87808, CHR 189272, CHR 56718.
M. pygmaea var. *minutiflora*: CHR 243997, CHR 473245
M. rakiura: CHR 312741, CHR 177647, CHR 283748, CHR 253085
M. saxosa: CHR 87133, CHR 245866, CHR 210114
M. spathulata: CHR 205235, CHR 131698, CHR 179627, CHR 478284, CHR 288401, CHR 129115, CHR 243992, CHR 131990, CHR 76065B
M. suavis: CHR 233873, CHR 166897, CHR 281096
M. tenericaulis: CHR 354641, CHR 269158, CHR 208219, CHR 221968, CHR 63004
M. traversii var. *cantabrica*: CHR 10955, CHR 328288, CHR 328282, CHR 252799, CHR 252801
M. traversii var. *traversi*: CHR 207133, CHR 365454, CHR 225891, CHR 201499
M. uniflora: CHR 252802, CHR 205291, CHR 499326, CHR 149427
M. venosa: CHR 165798, CHR 131175 a, CHR 97303, CHR 252136, CHR 189160.
M. 'saxatilis': CHR 328265, CHR 84608, CHR 387544, CHR 243814, CHR 249190

Appendix 6

Specimens of *Myosotis forsteri* and *M. venosa* from which measurement data were taken.

Myosotis venosa CHR 245405, CHR 189160, CHR 131175, CHR 132823, CHR 76063, CHR 190975, CHR 252136, CHR 165799, CHR 97303, CHR 165798

Myosotis forsteri CHR 87639, CHR 73753, CHR 87640, CHR 476118, CHR 158977, CHR 295269, CHR 159260, CHR 211330, CHR 244264, CHR 191[illegible], CHR 210188B, CHR 6031[illegible], CHR 259065, CHR [illegible], CHR 252594, CHR 401005, CHR 395141, CHR 210188A, CHR 278246, CHR 245796, CHR 208644, CHR 80168, CHR 110545, CHR 295281, CHR 387045, CHR 511356, CHR 295263, CHR 295224, CHR 94013, CHR 389480, CHR 358511, CHR 468391, CHR 231565, CHR 214852, CHR 513221B, CHR 101430, CHR 86273, CHR 10959, CHR 2357[illegible], CHR 246386, CHR 246338, CHR 355176, CHR 185785, CHR 58011, CHR 113472, CHR 76674, CHR 87558, CHR 110682, CHR 415341, CHR 323568, CHR 370598, CHR 77735, CHR 364290, CHR 334803

Table x Raw data

CHR	Species	Lamina length	Lamina width	Petiole length	Degree of calyx split	Pedicle length	Style length	Filament length	Anther length	Corolla width	Corolla length	Filament attachment	Stigma type
245405	venosa	19	13	26	0.516129	3.125	5.25	2.6875	0.875	6.25	6.71875	at scales	clavate
189160	venosa	16	10	25	0.7	2.8125	6.3125	2.25	1.25	8.28125	7.96875	below scales	clavate
131175	venosa	18	12	23	0.481481	2.03125	6.125	2.1875	1.125	6.09375	6.5625	at scales	clavate
132823	venosa	39	18	34	0.75	4.0625	7.25	2	1.125	7.65625	7.8125	at scales	clavate
76063	venosa	12	9	15	0.571429	2.03125	7.125	1.875	1	7.8125	8.59375	at scales	clavate
190975	venosa	12	11	16	0.75	2.8125	4.0625	1.75	0.9375	6.25	6.09375	below scales	clavate
252136	venosa	16	11	25	0.5	2.65625	4.625	1.625	1	6.09375	6.5625	below scales	capitate
165799	venosa	39	18	34	0.714286	3.125	5.9375	1.5625	1.25	7.8125	8.4375	at scales	capitate
97303	venosa	21	18	25	0.621622	2.03125	6.75	1.5625	1.125	8.125	8.75	at scales	clavate
165798	venosa	41	20	44	0.633333	2.65625	6	1.375	1.125	8.28125	7.96875	at scales	capitate
87639	forsteri	16	10	23	0.608696	2.8125	5.4375	1.25	1.125	5.84375	5.9375	below scales	clavate
73753	forsteri	24	16	25	0.5	1.5625	4.8125	1	1	5.78125	6.09375	at scales	clavate
87640	forsteri	6	3.5	4	0.5625	1.09375	3.4375	0.9375	0.9375	4.84375	5.15625	below scales	clavate
476118	forsteri	25	20	28	0.6	2.8125	3.75	0.9375	0.75	6.25	6.25	below scales	capitate
158977	forsteri	32	17	35	0.6	3	5.125	0.8125	1	9.0625	8.125	below scales	capitate
295269	forsteri	12	9	20	0.545455	2.8125	4	0.8125	0.75	5.625	5	below scales	clavate
159260	forsteri	42	19	30	0.611111	3.125	3.75	0.75	0.9375	5	5	below scales	clavate
211330	forsteri	23	12	55	0.545455	1.5625	3.4375	0.75	1	[illegible]	6.09375	below scales	capitate
244264	forsteri	64	32	50	0.527778	3.4375	4.375	0.6875	0.875	6.25	7.03125	below scales	clavate

CHR	Species	Lamina length	Lamina width	Petiole length	Degree of calyx split	Peduncle length	Style length	Filament length	Anther length	Corolla width	Corolla length	Filament attachment	Stigma type
[illegible]	forsteri	[illegible]	6	8	0.571429	2.34375	3.625	0.625	0.6875	3.375	5	below scales	capitate
210188B	forsteri	55	35	60	0.666667	2.34375	3.6875	0.625	0.8125	7.03125	6.5625	below scales	capitate
60317	forsteri	23	14	38	0.72	2.1875	3.3125	0.625	1	4.6875	5	below scales	capitate
259065	forsteri	14	[illegible]	24	[illegible]	2.34375	[illegible]	0.5625	1	5.46875	5	below scales	capitate
323709	forsteri	24	15	[illegible]	0.48	2.5	5.6875	0.5625	1.25	7.03125	8.59375	below scales	capitate
252594	forsteri	18	17	31	0.5	2.1875	3.875	0.5625	1	6.09375	6.25	below scales	clavate
401005	forsteri	19	13	30	0.555556	2.03125	2.875	0.5625	0.75	3.90625	4.6875	below scales	capitate
395141	forsteri	28	22	31	0.434783	2.96875	3.25	0.5625	0.6875	4.84375	5.3125	below scales	clavate
210188A	forsteri	45	23	45	0.515152	2.1875	4	0.5625	0.75	5	5.78125	below scales	capitate
278246	forsteri	10	8	13	0.6	3.90625	4.125	0.5	0.9375	3.90625	4.375	below scales	clavate
245796	forsteri	19	12	20	0.625	2.65625	3.875	0.5	0.9375	5.625	6.71875	below scales	capitate
208644	forsteri	18	14	28	0.5	2.8125	[illegible]	0.5	1	5.46875	6.40625	below scales	capitate
80168	forsteri	30	23	44	0.6	3.125	4.25	0.5	0.9375	5.9375	6.875	below scales	clavate
110545	forsteri	15	10	20	0.482759	2.34375	3.3125	0.4375	0.9375	3.75	5.46875	below scales	clavate
295281	forsteri	12	9	19	0.5	3.28125	3.4375	0.4375	0.75	5.46875	5.625	below scales	capitate
387045	forsteri	17	12	18	0.636364	1.875	2.375	0.4375	0.5	2.5	3.59375	below scales	clavate
511356	forsteri	26	16	30	0.62963	1.875	3.5	0.4375	0.625	5.3125	5.9375	below scales	capitate
295263	forsteri	16	12	17	0.434783	2.8125	3.25	0.375	0.875	3.90625	5	below scales	clavate
295224	forsteri	14	10	30	0.55	1.875	2.5625	0.375	0.625	2.96875	4.375	below scales	clavate
394013	forsteri	14	11	12	0.5	1.5625	2.4375	0.375	0.75	2.8125	3.4375	below scales	capitate
389480	forsteri	38	22	27	0.424242	2.34375	4.0625	0.375	0.875	6.5625	7.1875	below scales	clavate
358511	forsteri	29	20	30	0.555556	2.65625	2.4375	0.375	0.6875	3.59375	4.6875	below scales	clavate
468391	forsteri	43	23	33	0.652174	3.28125	3.1875	0.375	0.625	2.65625	3.59375	below scales	clavate
231565	forsteri	26	17	62	0.5	2.8125	2.6875	0.375	0.625	3.28125	3.59375	at scales	capitate
214852	forsteri	30	23	25	0.4	2.5	2.6875	0.375	0.75	4.21875	4.375	below scales	clavate
513221B	forsteri	20	11	14	0.4	2.34375	3.125	0.375	0.6875	3.75	4.375	below scales	capitate
101430	forsteri	28	18	27	0.545455	2.34375	2.8125	0.375	0.75	7.03125	7.03125	below scales	clavate
86273	forsteri	9	6	10	0.6	2.5	4.375	0.375	1	4.0625	5.3125	below scales	clavate
10959	forsteri	40	29	48	0.4	1.5625	2.3125	0.3125	0.625	3.75	4.375	below scales	clavate
245774	forsteri	30	19	40	[illegible]	2.65625	3.875	0.3125	0.875	5.46875	6.5625	below scales	capitate
246386	forsteri	30	24	45	0.4	3.59375	3.9375	0.3125	0.9375	6.09375	5.9375	below scales	capitate

CHR	Species	Lamina length	Lamina width	Petiole length	Degree of calyx split	Peduncle length	Style length	Filament length	Anther length	Corolla width	Corolla length	Filament attachment	Stigma type
246338	forsteri	25	15	19	0.428571	2.03125	2.5	0.3125	0.875	3.125	5.46875	below scales	clavate
355176	forsteri	20	21	32	0.521739	3.4375	3.5625	0.3125	0.75	4.21875	4.21875	below scales	clavate
385785	forsteri	22	15	25	0.625	3.59375	2.8125	0.3125	0.5625	3.90625	5.15625	below scales	clavate
358051	forsteri	22	10	17	0.33	2.34375	2.8125	0.3125	0.75	3.75	4.53125	below scales	clavate
113472	forsteri	20	13	18	0.583333	1.875	3.875	0.3125	0.75	3.78125	5.9375	below scales	clavate
76074	forsteri	25	10	32	0.5	1.25	3.125	0.3125	0.5625	1.53125	4.375	below scales	capitate
87558	forsteri	15	10	18	0.612903	2.5	1.875	0.25	0.5625	2.65625	3.125	below scales	capitate
410682	forsteri	28	20	33	0.514241	2.34375	3.125	0.25	0.875	3.59375	4.53125	below scales	clavate
415341	forsteri	36	16	50	0.521739	2.34375	2.8125	0.25	0.6875	2.5	4.84375	below scales	capitate
323568	forsteri	24	13	68	0.642857	2.8125	2.375	0.25	0.4375	4.375	3.90625	below scales	capitate
370598	forsteri	33	16	29	0.761905	1.875	1.5625	0.5	0.4375	2.65625	3.125	below scales	capitate
77745	forsteri	30	19	30	0.657895	2.8125	2.6875	0.4375	0.6875	2.96875	4.375	below scales	capitate
364199	forsteri	42	28	53	0.470588	1.5625	2.4375	0.4375	0.6875	3.90625	4.375	below scales	capitate
334803	forsteri	19	13	28	0.535714	2.8125	3.125	0.125	0.9375	3.28125	5	below scales	clavate

Glossary

Allee effects: Inverse density-dependent effects that occur when a component of individual fitness (e.g. rate of reproduction) decreases disproportionately in response to a decrease in population density or size (Allee, 1951).

Always herkogamous: where the stigma is initially exserted beyond the anthers and remains so.

Autogamous self-fertilization: flowers that can self-pollinate autonomously.

Basicole: Plants that only inhabit base rich sites (e.g. limestone or magnesite).

Brush blossom: These flowers have a narrow corolla tube, with a rim of conspicuous scales and free flat corolla lobes, but differ from the tube blossom type in having anthers and stigma on long exerted filaments and styles.

Calcicole: Plants that only inhabit calcium rich sites (e.g. limestone or marble).

Dichogamy: temporal separation of stigma and anther presentation.

Funnel blossom: These flowers have a wider corolla tube that is more or less funnel shaped. The corolla lobes are spreading rather than flat and often fused above the scales giving the flower a funnel shape.

Geitonogamy: within plant pollinations.

Herkogamy: spatial separation of stigma and anther presentation.

Initially herkogamous: where the stigma initially protrudes but corolla extension lifts the anthers above the stigma during anthesis.

Never herkogamous: where the anthers and stigma are in close contact throughout.

Precocious style: style that protrudes from the closed bud prior to the bud opening.

Protogyny: the form of dichogamy in which stigma presentation precedes anther presentation (opposite of protandry).

Selfing Rate: Rate at which plants are able to produce seeds when pollinator service is excluded.

Tube blossom: These flowers usually have a narrow corolla tube of variable length. The tube is framed by conspicuous corolla scales, with flat, free corolla lobes. Filaments are more or less short carrying anthers near the top of the tube, but the degree of inclusion/exertion varies.

Ultramafic: Rock that has a very high content of iron and magnesium.