

MASTING AND INSECT POLLINATION  
IN THE DIOECIOUS ALPINE HERB *ACIPHYLLA*

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## ABSTRACT

*Aciphylla* species (wild spaniard/speargrass) are an iconic component of the Australasian high country flora, but their reproductive system is enigmatic. They are insect-pollinated dioecious mast seeders (synchronous highly variable seed production), which seems maladaptive. The resource supply to pollinators is highly variable, yet dioecious plants are dependent on pollinators, and dioecious masting requires male and female plants to flower synchronously. Floral display in *Aciphylla* is relatively large, with tall inflorescences bearing thousands of flowers, suggesting that plants would not have the resources to produce such large stalks every year. But why do they have such huge inflorescences in the first place? I tested whether pollinator attraction is providing an economy of scale which favours intermittent production of very large inflorescences, by manipulating floral display size during a high-flowering year and measuring insect visitation rates and seed set (female reproductive success). Using space-for-time substitution and selective removal of male inflorescences, I also tested whether female seed set was affected by distance to flowering male plants (i.e. changes in local pollen availability) to see if flowering asynchrony would reduce pollination success. Bags were used to exclude pollination by insects and test for wind pollination, and hand pollination was done to test for pollen limitation. Insect surveys suggest that *Aciphylla* has a generalist pollination system (to avoid satiating a specialist pollinator during ‘mast’ years’). Male inflorescences received significantly more visits than females, and some seeds were set inside bags (although only 20-30%), suggesting wind pollination may occur at low levels. Seed set rate was higher for taller inflorescences with greater flowering length in *A. aurea* but tall inflorescences with excess flowers led to a decrease in seed set rates in *A. scott-thomsonii*. Hand pollination significantly increased seed set rates although these effects were not as large as expected (e.g. 10% increases from natural to hand-pollinated inflorescences were typical). There was no evidence for resource limitation in any species. Female plants in dense flowering populations had higher seed set rates, and individual floral display size in females was particularly important when females were ‘isolated’ from males. Insect visitation rates were generally higher on inflorescences with a larger floral display, suggesting that display size is important for pollinator attraction. Overall, these results suggest that the pollinator-attraction benefits of such a large floral display (at both the plant and population level) are possibly providing an economy of scale, although the relative effects are small.



*“Few plants are more spectacular than a large  
speargrass in flower.  
A tussocky hillside where hundreds stand like  
great golden candles is a sight not easily  
forgotten”.*

*Moore and Irwin, 1978*

## Chapter 1 - INTRODUCTION

### 1.1 Introduction to the genus *Aciphylla*

*Aciphylla* is a long-lived herbaceous genus in the carrot family Apiaceae, (subfamily Apioideae). There are over 40 species of *Aciphylla* endemic to New Zealand (Allan, 1982) and three endemic to Australia (Pickering & Hill, 2002). Their distribution spans from coastal to mostly montane and sub-alpine. *Aciphylla* is one of the more speciose genera of New Zealand flowering plants (Dawson & Le Comte, 1978). They are an iconic component of the New Zealand high country grassland/shrubland partly because when in flower, the inflorescences stand out like “great golden candles” in the tussocky hillsides (Moore & Irwin, 1978).

#### 1.1.2 *Aciphylla* – a unique genus in the family Apiaceae

Wardle, (2002) describes *Aciphylla* as being “the most bizarre plants of mountain grasslands and herbfields”. Not only is *Aciphylla* a unique component of the New Zealand alpine flora, but they are also unusual among the New Zealand (and global) Apiaceae family. This is because many species have rigid leaf- and bract-segments tipped by needle-sharp spines, and an unusually large number of reduced compound umbels aggregated into narrow elongate inflorescences (Dawson, 1971). Also atypical of the family is that they are dioecious (separate sexes on different plants) (Webb, 1981) and mast-seeding (highly variable and synchronous reproduction) (Mark, 1970), however, resembling most other Apiaceae genera, they are pollinated by insects.

*Aciphylla* has rosettes of rigid spine-tipped leaves and hence are commonly known as ‘wild spaniard’ or ‘speargrass’ (Mitchell *et al.*, 1998), or in maori, ‘taramea’, literally translated to mean “sharp leaves”. Because of this, Leonard Cockayne in 1910, dubbed one of the larger species *Aciphylla colensoi*, “a most formidable plant with bayonet-like leaves a yard long” and quoted “early explorers understandably regarded these as enemies, a solid phalanx of speargrass constituting well-armed opponents, impassable for man or beast” (Moore & Irwin, 1978). All species have at least one rosette of spiny leaves radiating from a crown or rootstock with a deeply descending taproot. Leaves are usually all basal, mostly

compound, and with close-set imbricated sheaths (Allan, 1982), with the lower part of the fleshy sheath often buried beneath the soil surface (possibly a way for this nutrient-rich resource to escape this being eaten by moas, kea, takahe (Atkinson & Greenwood, 1989) and kakapo (Powlesland *et al.*, 2006)). The genus is exceptional because 16 of the species have leaves modified to form groups of rigid needle-sharp spines 0.4-1.5 m in length. The spines are dense and evenly spaced to form a hemispherical tussock-like form (Fig. 1.1). The leaves are tough and fibrous and also have spiny stipules which together form a collar of spines around the base of the leaf rosette (Atkinson & Greenwood, 1989). They have one or more inflorescences of compound, bracted umbels. Bracts have a long sheath with pinnae and are also extremely spiny. Anyone who has ever been tramping or hunting in the high country will know all about the injurious consequences that follow walking into an *Aciphylla* plant (see below).



Fig. 1.1 A rosette of *Aciphylla aurea* (Golden spaniard) showing compound leaves with spiny tips and a young, developing inflorescence with sharp bracts in the centre of the rosette.

## 1.2 Historical background

*Aciphylla* is common in open mountain grasslands, and particularly following fire they can be abundant (Mark, 1970). Pollen and charcoal analysis from south-central South Island Holocene age deposits indicate that *Aciphylla* repeatedly increased its distribution and abundance in southern dryland areas such as the Mackenzie Basin throughout the Holocene. The genus is an important recolonising species in response to drought and local fires which naturally occurred during this time (McGlone & Moar, 1998). Around the period from 800 years BP to 1860 AD, burnt-out *Nothofagus* forests and *Halocarpus* scrub were replaced by *Aciphylla*-rich tussock grassland, however, from 1860 onwards, *Aciphylla* began disappearing, while grasses and *Rumex acetosella* continually increased with European pastoralism (McGlone & Moar, 1998). Additionally, European settlers burnt large areas of *Aciphylla* to travel through some mountain passes (Moore & Irwin, 1978). The current distribution of *Aciphylla* populations in the dryland valleys such as the Mackenzie Basin is likely to be influenced by a combination of fires and droughts of the late Holocene, farming and both pastoral grazing and grazing by introduced mammalian pests.

## 1.3 Taxonomy

There have been a number of attempts at dividing the genus into groups based on various aspects of their morphology, size, and growth habit, amongst other features (e.g. Oliver, 1956; Dawson & Le Comte, 1978). Most recently, D. Glenny (pers. comm. 2005) has been undertaking an extensive, detailed taxonomic revision of the entire genus and has come up with at least ten groups based on various morphological, physiological and evolutionary criteria. Although most authors quote the genus *Aciphylla* as having over 40 species, D. Glenny's revision suggests that there are more likely to be approximately 27 species with a number of varieties. Most groups consist of the smaller and higher-altitude species (previously *Paniculatae* – (Oliver, 1956), referring to inflorescences of “compound umbels on approximate spreading peduncles at the stem apex”). The remaining few groups constitute the larger and more evolutionarily recent varieties (previously *Elongatae* – inflorescences of compound, spiny-bracted umbels arranged along an elongate extension of the central stem (Oliver, 1956)). The research in this thesis focuses on three of the larger

varieties of *Aciphylla* (within Elongatae), with elongated inflorescences. Hereafter, when commenting on features and characteristics generally portrayed by the larger, lower-altitudinal (or montane) species, for simplicity, the term *Aciphylla* will be used (although it is uncertain how specific features mentioned apply to the genus as a whole).

#### **1.4 Why study the reproductive ecology of *Aciphylla*?**

*Aciphylla* has a unique reproductive system in that they are dioecious, insect-pollinated and mast seeding. This is not a set of components that are usually found associated together within one particular breeding system (Proctor *et al.*, 1996). As well as this, they are sexually dimorphic and have been reported to have a male-biased sex ratio (Webb & Lloyd, 1980). Despite having a fascinating ecological background, little is known about *Aciphylla*'s breeding system, hence the need for further investigation into their interesting and bizarre reproductive ecology. The sections below break down each of the aforementioned components of the breeding system and introduce some of the details about their biological significance. The final section will thread these individual components together in relation to *Aciphylla* in order to explain why this reproductive system is interesting and unique.

#### **1.5 Dioecy and sexual dimorphism**

Dioecy, in which separate individuals perform male and female functions, is a relatively rare breeding system among the angiosperms (Vamosi & Vamosi, 2004). Dioecy occurs in about 10% of the world's flora and is especially common in isolated island floras of New Zealand and Hawaii (Webb & Kelly, 1993) and amongst tree species in the tropics (Bawa, 1974). The causes of this high frequency of dioecism in the flora of New Zealand (and islands in general) are not clear. Webb & Kelly, (1993) suggested that the unspecialized pollinating fauna may have encouraged unisexuality as a means of preventing selfing. (See Sakai & Weller, (1999) for an in-depth review on the evolution and maintenance of dioecy). Most dioecious species are insect-pollinated and have on average, smaller flowers than hermaphrodites that are often green or white and inconspicuous with unspecialized pollinators (Proctor *et al.*, 1996). *Aciphylla* fits this description (Pickering, 2000).



Many dioecious species, particularly those in largely hermaphrodite families, have vestigial organs of the other sex present in the flower and the sterile anthers in the females may be part of the attractant in female flowers; some provide sterile pollen, although normally nectar is the attractant as most female flowers do not produce pollen (Proctor *et al.*, 1996). It should be noted that ‘leakage’ in sexuality (“leaky dioecism”) has been reported for some *Aciphylla* species. In these cases, female inflorescences may have a small number of terminal male umbels (D. Glenny, pers. comm. 2005) although it is uncertain as to whether these are functional and whether this can lead to geitonogamous selfing (Newstrom & Robertson, 2005). It must be pointed out that this variation in sexuality was not commonly encountered throughout this research.

Male flowers of dioecious species are often larger and showier than female flowers, produced in greater quantity, and in larger inflorescences (Bawa, 1980), and therefore exhibit sexual dimorphism in sex characters (Webb & Lloyd, 1980; Delph, 1999). This often means that male flowers are more likely to attract the attention of insect pollinators from a distance so that insects may visit them before the females (Proctor *et al.*, 1996) (essential for successful pollination). Some *Aciphylla* species have also been shown to exhibit these traits (e.g. in Australia: *Aciphylla glacialis* (Pickering, 2001), and in New Zealand: *A. aurea*, *A. monroi*, *A. poppelwellii*, and *A. scott-thomsonii* (Webb & Lloyd, 1980)). While dioecy almost completely eliminates selfing in plants, it also increases the plant’s reliance on animal (or wind) movements of pollen between individuals of both sexes for successful reproduction (Bawa, 1980).

The New Zealand flora generally possesses a high number of sexually dimorphic taxa compared with global levels (Webb & Kelly, 1993). There have also been numerous reports of dimorphism in terms of male-biased sex ratios in New Zealand alpine plant species, particularly for *Aciphylla* (e.g. Lloyd & Webb, 1977; Webb & Lloyd, 1980). This departure from equality may arise from gender differences in life histories such as costs of reproduction, and male versus female function (i.e. pollen and ovule production respectively) (Delph, 1999).

## **1.6 Insect pollination**

Pollination is the transfer of pollen from an anther to a stigma. The movement of pollen between plants by insects (entomophily) is by far the most common pollination system in the world (Proctor *et al.*, 1996). In many hermaphrodite plants (which make up 80% of all flowering plant species) this can happen within the same flower or on the same plant, but in dioecious species, it is fundamental for pollen to be moved between plants, from male to female. The plant-insect pollination system is a mutualism (DeAngelis & Holland, 2006), where the insects rely on the flowers for food (pollen and/or nectar), while the plant relies on the insect to move its pollen between plants in order for successful outcrossing to occur (Bronstein, 2004). (Note: an insect may be in a mutualistic relationship with a plant in some ecological circumstances, while it could be parasitic in others, (see Thomson, 2003 and Holland *et al.*, 2004). So, plants provide floral rewards that entice insects to visit their flowers in order for them to carry pollen away or inwards for successful fertilisation. If the plant has a specialist pollinator, it needs to provide a reliable food resource through time in order for the plant-pollinator mutualism to be maintained, and the plant can be susceptible to pollen limitation if the pollinator becomes rare in the environment (Ladley *et al.*, 1997). A generalist pollinator system (see Newstrom & Robertson, 2005), however, may allow the plant to get away with providing more variable rewards in space and time, as the insect pollinators are not necessarily dependent on it as a food source, although there is the additional problem of plants competing with each other for generalist pollinators, which can lead to pollen limitation in some cases (Robertson, 1895; Waser, 1978; Campbell, 1985; Feinsinger & Tiebout (III), 1991).

### ***1.6.1 Insect pollination and the importance of floral display***

Visual and olfactory cues are used by plants to signal and attract insect pollinators (Proctor *et al.*, 1996). For plants with individual flowers which are grouped together in inflorescences such as *Aciphylla*, it is the inflorescence rather than the individual flower that is the effective functional unit for pollinators, enhancing the floral display to attract pollinators (Proctor *et al.*, 1996). A large floral display attracts insects from a distance, particularly unspecialised insects which are characteristic as pollinators of dioecious

species. The importance of the plant's floral display for pollinator attraction has been reported numerous times in the literature (e.g. Willson & Price, 1977; Robertson, 1992; Johnson *et al.*, 1995; Andersson, 1996; Armstrong, 1997; Kawarasaki & Hori, 1999; Lortie & Aarsen, 1999; Pickering, 2000; Irwin *et al.*, 2004; Mitchell *et al.*, 2004; Kudo & Harder, 2005; Orellana *et al.*, 2005; Brunet & Sweet, 2006; Buide, 2006). Floral display not only attracts pollinators at the plant level, but has also been shown to attract pollinators at the population-level (e.g. Augspurger, 1980; Kato & Hiura, 1990; Jennerston, 1993; Bosch & Waser, 1999; Lofgren, 2002; Forsyth, 2003; Kirchner *et al.*, 2005). This is commonly achieved through reproductive synchrony (Crone & Lesica, 2004), and the resulting economies of scale (Norton & Kelly, 1988; Crone & Lesica, 2004).

### **1.7 Mast seeding**

An important aspect of the reproductive ecology of *Aciphylla* is that they are mast-seeding, (also referred to as mast flowering, supra-annual flowering, mass-fruiting or masting (Kelly, 1994)). Masting has been generally defined as the synchronous highly variable seed/flower production among years by a population of plants (Kelly, 1994). Masting is a population phenomenon that results when individual plants within a population synchronise their reproductive activity (Haase, 1986) by concentrating reproductive effort into a large floral display during “mast” years at the expense of little or no flowering during “non-mast” years (Crone *et al.*, 2005).

This masting phenomenon has been witnessed for centuries because of phenomena such as the mass over-production of acorns some years - a massive food crop affecting mice, birds and other wildlife, and the virtual absence of these crops in other years, effectively starving these animals (Koenig & Knops, 2005). These events were not hard for people to miss and were especially important for farmers who fed acorns to their livestock. Records for this phenomenon go as far back as the mid-17<sup>th</sup> century (Koenig & Knops, 2005). The term “mast” comes from the German word, *mást*, for nuts/acorns of forest trees that have accumulated on the ground, especially for those used as food for fattening swine (Janzen, 1971; Kelly & Sork, 2002). This German word for “fat” was then incorporated into the Old English language (Bodsworth, 2003) and took on a similar meaning. It was Janzen, (1971, 1976) however, who was among the first to draw detailed scientific attention to mast

seeding (but see also Salisbury, 1942 and Silvertown, 1980), and defined it as the “synchronous production of seed at long intervals by a population of plants”. He suggested masting served to reduce levels of loss to seed predators (see section 1.8.3.1). His definition suggests that there are just two types of years: seeding (mast) years and non-seeding years - a concept referred to as ‘strict masting’ (Kelly, 1994). However, most polycarpic plants do not display this bimodal pattern of strict masting, instead falling along a continuum of variability levels (Herrera, 1998a; Kerkoff & Ballantyne (IV), 2003) from ‘strict’ to ‘normal’ masting (see Kelly, 1994). Hence, in this study, mast-seeding will be considered in the light of Kelly’s (1994) more general definition: “the synchronous highly variable seed production among years by a population of plants” because technically most plant species are not known to be strictly masting (Herrera, 1998a).

Masting occurs most frequently in long-lived plants which are less affected by the costs of not reproducing in some years (Kelly, 1994). It is a worldwide phenomenon and is found in plants from many different taxonomic groups and from most parts of the world (Isagi *et al.*, 1997). Mast-seeding seems to be common in temperate forest trees (Silvertown, 1980) and herbs (Mark, 1970; Campbell, 1981; Brockie, 1986; Webb & Kelly, 1993; Kelly *et al.*, 2001) and present in some tropical forest trees (Janzen, 1971). It also appears to be especially prevalent in the New Zealand flora (Webb & Kelly, 1993; Kelly, 1994). The ultimate reasons for the high masting frequency in New Zealand are mysterious, but Schauber *et al.* (2002) note that “New Zealand’s long history before humans and mammalian herbivores arrived may have enabled even herbs and grasses to evolve long life spans, which are necessary for masting to be a viable strategy”. Masting is a marked feature in several New Zealand alpine plant genera (e.g. *Aciphylla*, *Celmisia*, and *Chionochloa*) (Mark, 1970; Mark & Adams, 1973; Campbell, 1981) and is more pronounced at higher altitudes (Webb & Kelly, 1993). The snow tussock *Chionochloa* (a co-inhabitant with *Aciphylla* in New Zealand alpine grasslands) is one of the best-studied examples of a mast-seeding species, gaining large selective benefits from masting through predator satiation (see Kelly & Sullivan, 1997; Kelly *et al.*, 2000; Rees *et al.*, 2002).

Masting in itself is paradoxical phenomenon (Kelly & Sullivan, 1997; Koenig *et al.*, 2003) and the evolution of masting is puzzling because it imposes a number of clear selective disadvantages (Kelly *et al.*, 2000) including lost opportunities for reproduction (Waller, 1979) and more severe density-dependent mortality among seedlings produced in mast

years (Hett, 1971; Augspurger, 1981; Kelly *et al.*, 2001). Masting affects the timing of flowering and fruiting as well as critical aspects of the plant's life cycle such as pollination and dispersal (Tisch, 1996). Therefore, masting has important consequences for the community and ecosystem, because animals that rely on pulsed resources (such that masting plants provide) for a food source such as pollen, nectar or seeds, will face an unreliable food supply (Curran & Leighton, 2000; Ostfeld & Keesing, 2000; Schaubert *et al.*, 2002). Thus it is important to understand plant reproductive patterns in order to understand the dynamics of a whole system.

## **1.8 Why do some plant species mast when such high costs are involved?**

### **1.8.1 Proximate versus ultimate reasons for masting**

It is important to differentiate between proximate and ultimate causes of masting. Proximate causes refer to those factors governing the synchrony in flowering/fruiting between individual plants, and the magnitude and timing of masting events (Sork & Bramble, 1993; Isagi *et al.*, 1997). It essentially considers the question: how do plants mast? Factors such as weather and resources for example, are likely to be proximate causes for masting (Kelly & Sork, 2002). Proximate causes therefore do not drive the selection for masting, but may provide the initial variation on which selection can act (Waller, 1993). Proximate reasons for masting, such as spatial synchrony and cues for flowering are not the focus of this study, but should be an important consideration for future research in this area because of the likely effects of global warming on flowering variability and its inter-trophic consequences (see McKone *et al.*, 1998). Ultimate explanations for masting deal with the question: why mast? There are two general categories that attempt to answer this question. The first involves a non-evolved response, while the second involves evolutionary hypotheses to explain this phenomenon.

### **1.8.2 Non-evolved hypothesis to explain mast-seeding**

One of the earliest explanations for mast-seeding was that the plants had no choice i.e. that masting was a non-evolved response to a naturally variable environment (Busgen &

Munch, 1929). This is now known as the resource matching hypothesis of mast seeding (Kelly, 1994; Kelly & Sork, 2002), which implies that mast years are simply good years for all aspects of the plant, and hence growth and reproduction are expected to be positively correlated with each other (Kelly & Sork, 2002). This hypothesis has largely been ruled out by the fact that variability in reproductive output is considerably greater than the variability in environmental factors in many mast-seeding systems (Koenig & Knops, 2000) and by the switching of resources from growth to reproduction during mast years, (Norton & Kelly, 1988; Kelly & Sork, 2002; Monks & Kelly, 2006). Also at many sites, masting species grow side-by-side with species which have relatively constant seed output from year to year (Campbell, 1981; Webb & Kelly, 1993), indicating that the environment alone is not responsible for masting behaviour. Therefore, because masting could not merely be a side effect of resources that vary from year to year, mast seeding appears to be predominantly an evolved strategy in which there are some overall lifetime reproductive benefits to the plant of focusing reproduction in some years at the expense of not reproducing in others (Norton & Kelly, 1988; Koenig & Knops, 2000; Kelly & Sork, 2002), but see Kerkoff (2004).

### ***1.8.3 Evolutionary hypotheses to explain mast-seeding***

The alternative ultimate explanations for masting consider the evolutionary selective pressures that may have led a particular plant species to exhibit masting behaviour. There are a number of hypotheses that have been put forward as selective forces for plants producing variable, synchronous seed crops. For all the hypotheses, the common element is that the selective advantage occurs through an economy of scale (Janzen, 1971; Norton & Kelly, 1988) whereby large reproductive efforts are more efficient than smaller ones, so plants reproducing in step with mast years will have higher fitness (Kelly & Sork, 2002). The benefits of these large synchronous reproductive events accrue by overcoming some environmental constraint such as pollen limitation or seed predation (Kerkoff, 2004). Among the many hypotheses for masting, there are two which have by far received the most support: the predator satiation and the wind-pollination hypotheses. Below I briefly review six of the most common evolutionary-based hypotheses which have been suggested as favouring mast seeding, but it is the final two that are of most significance to this study.

### 1.8.3.1 *Predator satiation*

The predator satiation hypothesis states that large, intermittent seed crops reduce losses to seed predators (Janzen, 1971, 1978; Silvertown, 1980). Seed consumers are effectively starved during intermast intervals and swamped during mast events, driving large fluctuations in consumer abundance and increasing the potential fitness benefit of masting (Curran & Leighton, 2000; Schauber *et al.*, 2002). Studies show that predator satiation has been found to occur in a range of plants such as *Chionochloa* (snow tussocks), (Kelly & Sullivan, 1997; McKone *et al.*, 1998; Kelly *et al.*, 2000; Rees *et al.*, 2002), oaks, and beeches (Janzen, 1971) by a variety of animals from insects to mammals (Kelly & Sork, 2002). These, among many other examples, show evidence for a lower seed predation rate in high-seed years.

### 1.8.3.2 *Wind-pollination*

The wind-pollination hypothesis states that masting should be strongly selected in species that can achieve greater pollination efficiency through synchronised above-average flowering effort (Nilsson & Wastljung, 1987; Smith *et al.*, 1990; Kelly & Sork, 2002). Plants that synchronously produce masses of flowers and pollen once every few years, are likely to experience higher pollination rates and fruit set than plants that produce average amounts of flowers and pollen each year (Koenig & Knops, 2005). Masting plants showing improved reproductive success from wind-pollination are many, and include Lodgepole pine (*Pinus contorta*) (Smith *et al.*, 1990), rimu (*Dacrydium cupressinum*) (Norton & Kelly, 1988), and the beeches (*Fagus* (Nilsson & Wastljung, 1987) and *Nothofagus* (Kelly *et al.*, 2001)). Theoretical models by Satake & Iwasa (2002) suggest that pollen limitation could be a crucial driving force behind highly variable and synchronous seed production that is so characteristic of wind-pollinated masting species (but this remains to be empirically supported (Sork *et al.*, 2002; Koenig & Ashley, 2003)).

#### 1.8.3.3 *Animal fruit dispersal and large seed size*

The following two hypotheses involve selection at the fruit level, where firstly, large fruit *crops* could result in wider dispersal by generalist animal dispersers or scatterhoarders (Christensen & Whitam, 1991) and secondly, larger fruit *size* could increase variability in seed number between years (although this does not require synchrony among individuals) (Kelly, 1994). So far there exists little or no empirical or theoretical support for these hypotheses and they will not be examined in this study.

#### 1.8.3.4 *Accessory costs of reproduction*

This hypothesis proposes that small reproductive efforts are energetically inefficient because of high accessory costs (Kelly, 1994). It is based upon the idea that relatively indirect costs associated with reproduction decrease (per seed produced) with an increasing size of the reproductive effort, thus it is energetically more efficient to reproduce less often but at a higher level (at the individual plant level). High accessory costs could lead to individual plants producing occasional large reproductive efforts being favoured. This hypothesis does not require synchrony at the population level, thus accessory costs may help select for masting in association with more direct selection forces, but cannot explain masting at the population level (Kelly, 1994).

#### 1.8.3.5 *The animal pollination hypothesis*

The animal pollination hypothesis suggests that when the majority of individual plants flower synchronously, they are likely to attract more pollinators than they could individually and in this way encourage pollination. For wind-pollinated species, it has been frequently shown that percent fruit set is higher when flowering density is higher (see Kelly & Sork, 2002). Researchers thus suggest that mast flowering is more likely to evolve in wind-pollinated species because increased pollen availability enhances pollination success, while in animal-pollinated species, increasing flowering may saturate the pollinators (Norton & Kelly, 1988; Smith *et al.*, 1990). This was supported by Herrera (1998) and Kelly & Sork (2002) who surveyed hundreds of masting datasets and showed that wind-



pollinated species were significantly more variable in seed output (higher mean CV's (coefficient of variation)) than animal-pollinated species. Therefore, to date there is almost no convincing evidence of pollination economies of scale for masting, animal-pollinated species because the general school of thought is that animals are more likely to be satiated by large crops (Koenig & Knops, 2005) providing diseconomies of scales (Herrera *et al.*, 1998b; Kelly & Sork, 2002).

Despite this, if animals are attracted by a large floral display, masting could improve pollination (Norton & Kelly, 1988; Kelly, 1994). However, if the system involved a specialised plant-insect pollination mutualism, how would the sole pollinator species overcome the variability in flowering between years? One would expect that a specialised plant-pollinator system would be highly co-evolved, as the pollinator would require timing its reproductive cycle with that of the plant (like specialised seed/flower predators of *Chionochloa* spp. which undergo prolonged diapause, see McKone (2001)). A specialist pollinator is likely to be more affected by variable reproduction of its food source in terms of satiation during high flowering years and starvation during others. Another possibility however, is if the plant has a specialist pollinator with a very short generation time that is able to breed up during a mast event, as seems to happen with thrips-pollinated Dipterocarps in Southeast Asia (Ashton *et al.*, 1988). On the other hand, if the plant is pollinated by a suite of generalist animals (common for insect-pollinated plants (Proctor *et al.*, 1996)), masting and animal pollination would be more likely to work, particularly if pollinators are attracted to large floral displays. For insect pollination economies of scale to occur would require a generalist fauna that favour large floral displays and would not become satiated.

### **1.9 The bizarre reproductive ecology of *Aciphylla***

Little is known about the reproductive ecology of *Aciphylla*, although they are a ubiquitous and well-known component of the high country (high alpine to lower montane) grassland flora. What is known, is that they exhibit mast seeding; are insect-pollinated and dioecious and together this presents a paradoxical situation. Firstly, insect-pollinated plants should not provide an inconstant resource supply as this could lead to either a break-down of the mutualism between the pollinator and the plant, caused by pollinator satiation during

“mast” events and subsequent starvation during years of little or no flowering. Dioecy requires synchrony because male and female plants to flower at the same time in order to achieve successful pollination. This synchrony could either be achieved through constant average reproduction each year, (which is typical of most insect-pollinated dioecious systems), *or* by occasional synchronous large floral displays (i.e. masting) if there exist other selective forces encouraging the plant to have significantly large floral display size, such that it can’t be produced every year. The latter is seen in *Aciphylla*, and it is hypothesised that in order to avoid pollinator satiation, *Aciphylla* must overcome this paradox by having a generalist pollinator fauna, and that pollinator economies of scale could be one of the important selective forces behind the large floral display size in the first place.

### **1.10 Research Aims**

This study aims to test the hypothesis that pollinator economies of scale are an important selective force for the large population-level flowering effort in *Aciphylla* and that the high costs of reproduction that come with floral investment explain the reasons for masting in this genus. This can be tested at two scales: at the individual plant level it is hypothesised that a larger floral display size will have increased reproductive success due to enhanced attractiveness to insect pollinators; while at the population level, masting synchrony leads to more inflorescences (increased population-level reproductive effort), and improved pollination success due to pollinator economies of scale. Specifically, the outline of this thesis is as follows:

- 1) Chapter 2 aims to test the hypothesis that pollinator economies of scale are an important selective force for the large floral display size in *Aciphylla*. This chapter will be separated into two sections: Part (A) tests whether variation in plant-level floral display size affects seed set rate in female *Aciphylla* inflorescences using both natural variation in floral display size and manipulative space-for-time substitution experiments, and simultaneously tests for pollen and resource limitation. Part (B) investigates whether variation in population-level flowering effort affects female seed set, again employing both natural and manipulative experimental approaches.

- 2) Chapter 3 applies the hypotheses outlined above to test whether variation in (A) plant-level floral display size and (B) population-level flowering effort affect pollinator visitation rates to female *Aciphylla* inflorescences, and whether this relates to seed set rates (Chapter 2). Furthermore, it explores whether pollinators are more attracted to inflorescences of male or females. Additionally, I provide the first comprehensive description of the insects associated with flowers of *Aciphylla* species.
- 3) Chapter 4 investigates aspects of sexual dimorphism in the dioecious *Aciphylla*. I test whether there is evidence for sexual dimorphism in terms of a male-biased sex ratio, among other morphological and phenological characteristics in flowering *Aciphylla* to determine whether these differences relate to sex-specific pollination success (Chapter 3).
- 4) Chapter 5 discusses the implications associated with each of the above chapters and how they relate, in the context of *Aciphylla* and in the wider ecological framework.

## **Chapter 2- Does floral display size affect reproductive success in *Aciphylla*?**

### **2.1 INTRODUCTION**

As outlined in Chapter 1, large-leaved, montane *Aciphylla* species including *A. aurea* and *A. scott-thomsonii*, among others, are long-lived, dioecious (male and females are individual plants), mast-seeding (Mark, 1970; Campbell, 1981) herbaceous plants, that have tall showy inflorescences with thousands of flowers. *Aciphylla* are thought to be primarily pollinated by insects and wind (Dawson, 1971; Webb, 1986) although the relative importance of these agents as effective pollen vectors has rarely been tested experimentally in *Aciphylla* (but see Brookes & Jesson, 2006). In many genera worldwide, numerous studies link pollination success to floral display size at both the individual plant- (e.g. Vaughton & Ramsey, 1998; Orellana *et al.*, 2005; Buide, 2006) and population- (Kato & Hiura, 1990; Kirchner *et al.*, 2005; Kindlmann & Jersakova, 2006) levels, in both wind- (e.g. Smith *et al.*, 1990; Kelly & Sullivan, 1997; Kelly *et al.*, 2001) and insect- (e.g. Davila & Wardle, 2002; Grindeland *et al.*, 2005) pollinated systems. In many flowering plants, reproductive success depends on the ability to attract animal pollinators (Vaughton & Ramsey, 1998) and this is frequently achieved through having a large floral display (Kudo & Harder, 2005). In *Aciphylla*, biological and/or environmental pressures over time appear to have selected for large floral display size and synchronous variable reproduction i.e. masting, (Kelly, 1994). This suggests that the lifetime benefits gained by having a large floral display and reproducing only some years must outweigh the costs of forfeiting reproduction during others (Janzen, 1971; Silvertown, 1980) and also must be more effective than having a constant, but reduced, floral display size each year (Norton & Kelly, 1988; Koenig *et al.*, 2003).

#### **2.1.1 The importance of floral display size at multiple levels in *Aciphylla***

Floral display in large-leaved, montane *Aciphylla* species is interesting at multiple levels, including the inflorescence, plant and population scales. Firstly, inflorescences are particularly tall with thousands of flowers (e.g. up to 15,300 flowers for a female inflorescence, and averaging 9,746 flowers for an average size female inflorescence of 1.9 metres for *A. scott-thomsonii*, see Appendix 1). Within an inflorescence, there is natural

variation in the proportion of the inflorescence with flowers present relative to the entire height of the stalk (Fig. 2.1). There is also variation in inflorescence height with a continuum of short to tall inflorescences present within a population. On the individual inflorescence, there are also other structures that may be important for floral display such as the spiky floral bracts. The role that bracts play during flowering is not certain, but there is some possibility that they could be influential in pollinator attraction and/or seed production in some way. Secondly, at the plant level, inflorescence number varies between plants. Plants have one to many rosettes and each rosette can make a single inflorescence. One to three inflorescences is common for a female plant during a flowering year, and 1-4 for males, but in some cases there can be up to 10 inflorescences per plant. Thus variation in plant-level floral display also exists within a population.



Figure 2.1 *Aciphylla glaucescens* (Cow Creek, Waihopai Valley, Marlborough) provides a clear example of the variation in floral display size within an inflorescence during a high-flowering year. All inflorescences here are relatively tall, but on some stalks, flowers are not presented until quite a distance up the rachis (e.g. front-right), while on others, flowers begin just above the leaves (e.g. centre-left). Photo courtesy of Barry Hope.

Finally, floral display varies prominently from year-to-year in large montane *Aciphylla* species. In plants generally, not all species are highly variable in seed production (i.e. ‘mast’ years with most individuals flowering, interspersed with ‘non-mast’ years with little or no flowering), but instead plant species tend to fall along a continuum of variability levels (Webb & Kelly, 1993; Kelly, 1994; Herrera, 1998a) from ‘strict masting’ to ‘normal masting’, i.e. highly variable to nearly constant (Kelly, 1994). This variability in the timing and effort of flowering differs between populations of *Aciphylla* depending on species, altitude, latitude and resource availability (Pickering & Hill, 2002). Therefore, floral display in terms of population effort also varies between years and different populations at different sites. Floral display can be examined at these multiple levels simultaneously to assess the relative importance that the variation in floral display at each level has on reproductive success in *Aciphylla*. First however, we must consider the evolutionary and ecological significance of floral display size and its importance in reproductive success in plants, particularly in mast-seeding, dioecious, entomophilous breeding systems.

### **2.1.2 Measuring floral display at the inflorescence and plant level**

Animal-pollinated plants are expected to evolve strategies that maximise the efficiency of pollen transfer to and from the plant. Plants have evolved a number of mechanisms that increase the probability that visitors will move pollen among plants to ensure outcrossing (Marr *et al.*, 2000). The evolution of dioecy has probably been the most successful of these (Lloyd, 1975; Charlesworth & Charlesworth, 1978; Sakai & Weller, 1999), however, this still requires the plant to employ mechanisms for successful pollinator attraction. One such strategy may be to develop large showy floral displays that attract more pollinators (Proctor *et al.*, 1996; Grindeland *et al.*, 2005). There is a wealth of literature providing evidence that floral display size plays an important role in the reproductive success of many plant species worldwide, (e.g. Andersson, 1996; Le Corff *et al.*, 1998; Kawarasaki & Hori, 1999). In particular, many studies have reported a positive relationship between visitation rates of pollinators and floral display size at the level of the individual plant (e.g. Kato & Hiura, 1990; Grindeland *et al.*, 2005; Kudo & Harder, 2005; Brunet & Sweet, 2006). There is also evidence for this among species in the Apiaceae family (e.g. Pickering, 2001; Davila & Wardle, 2002).

The timing of pollen availability (Harder & Wilson, 1994) and nectar production (Marden, 1984) as well as the amount of reward offered by a plant can determine both the rate of visitation and the subsequent behaviour of flower visitors and therefore have consequences for pollen transfer (Robertson *et al.*, 1999).

Floral display can be measured in a number of ways, including flower number (Robertson *et al.*, 1999), flower or inflorescence display size (Abe, 2000), or showiness (Ashman *et al.*, 2004). The notion that large floral displays exist to attract pollinators is referred to as the pollinator attraction hypothesis (Sutherland, 1987). This is analogous to the animal-pollinator hypothesis of masting (Kelly, 1994) (see Chapter 1). Pollinator attraction is critical to the reproductive success of the majority of flowering plants (Kearns *et al.*, 1998) however, the payment for attracting pollinators can be costly, both in the amount of resource invested (Pyke, 1991) and by attracting nectar robbers (Irwin *et al.*, 2004).

If a larger floral display can attract more visitors, does this actually lead to an increase in seed set? Some researchers hypothesise that seed-set on female plants is a direct indicator of insect attraction (Ortiz-Perez *et al.*, 2004). In reality however, factors such as pollinator efficiency, pollen carryover and resource availability must also be taken into account. While larger floral displays may attract more insect pollinators, in self-compatible hermaphrodite or monoecious plants, a large proportion of visits made by pollinators is likely to occur between flowers on the same plant, resulting in geitonogamous self-pollinations rather than cross-pollinations (Robertson, 1992; Mitchell *et al.*, 2004). Therefore, in bisexual plants, the effect of increased geitonogamy with increased floral display may be considered an unavoidable detrimental effect of large displays (Hessing, 1988). Given that *Aciphylla* is completely dioecious (Webb, 1979) the detrimental effects of geitonogamy from a larger floral display will not be seen. Nevertheless, dioecious species with showy floral displays do not necessarily escape from pressures that may be detrimental to their overall reproductive success. Dioecious species often have sexually dimorphic floral displays, with a common pattern of males being larger and/or showier than females (Delph, 1996; Eckhart, 1999) (see Chapter 4). If insects are more attracted to showier floral displays and better floral rewards, and visitation rates are disproportionately higher on males than females as many studies have shown (e.g. Le Corff *et al.*, 1998; Vaughton & Ramsey, 1998; Pickering, 2000; Voight *et al.*, 2005), this reduces the chance of females being pollinated (Bawa, 1980). This could be detrimental for female seed set

particularly when pollinator abundance is low (Vamosi & Otto, 2002) due to increased competition for pollinators. In *Aciphylla*, male inflorescences are generally showier than females (Pickering, 2000), therefore this species is an ideal candidate to additionally test whether inter-sexual competition for pollinators exists and whether this has consequences for female reproductive success (tested in Chapter 3). Furthermore, in dioecious plants generally, investment in reproduction is usually considerably higher for females than males (Webb & Lloyd, 1980; Pickering, 2000; Pickering & Arthur, 2003; Wheelwright & Logan, 2004). This has also been shown for *Aciphylla* species (Hogan *et al.*, 1998) and is therefore likely to play an important role in the floral display size of female plants (see Chapter 4).

#### ***2.1.2.1 The importance of resource and pollen limitation in Aciphylla – does female success depend on individual reproductive effort?***

In this study, it is hypothesised that larger reproductive effort (increased floral display) increases reproductive success (i.e. seed set) due to increased pollinator attraction. However, it is anticipated that these effects may not be so straightforward due to the opposing effects of resource limitation that are also likely to be acting on display size. Therefore, these effects must be considered in order to make assumptions about the relative importance of floral display size, pollinator attraction and resource limitation.

Bateman's Principle states that males are limited by the ability to find mates due to competition for mating opportunities while females are limited by resources required for offspring provisioning (Bateman, 1948). "However, in flowering plants this principle appears too simplistic because if male searching for mates (including pollen dissemination via external agents) is not sufficiently successful, then the reproductive success of both sexes will be limited by the number of matings rather than resources" (Burd, 1994 pp. 83).

Limitations of female success due to inadequate pollen receipt appear to be a common phenomenon in plants (Vaughton & Carthew, 1993; Burd, 1994; Ashman *et al.*, 2004; Newstrom & Robertson, 2005). Across species, supplemental pollen often leads to increased fruit set, especially in plants that are highly self-incompatible such as dioecious species, suggesting that inadequate pollen receipt is a primary cause of low fecundity rates in many perennial plants (Hirayama *et al.*, 2005; Knight *et al.*, 2006; Pias & Guitian, 2006).



It was mentioned above that, as well as pollen, resources are likely to be playing an important role in the reproductive success of female plants (Horvitz & Schemske, 1988). In animal-pollinated plants, resources must be allocated for both pollinator attraction and provisioned for successful offspring (seed or fruit) production (Ashman *et al.*, 2004). Plant populations are expected to evolve traits where taller inflorescences with more flowers may promote increasing rates of pollinator visits and act to optimise fecundity, while resource limitation may offset the ability for taller inflorescences to set more seeds (Haig & Westoby, 1988). Therefore floral display size can be thought of as an optimal investment between pollinator attraction and fecundity.

Thus there exists a dynamic interplay between the two opposing forces, requiring tradeoffs between large reproductive effort and successful offspring provisioning, i.e. while taller inflorescences attract more pollinators and may achieve higher rates of seed set, eventually resources may become limited no longer enabling all ovules that have been fertilised to set seed (Haig & Westoby, 1988). Because of the conflicting dynamics between the two, how can we separate them to test for the relative importance of pollinator attraction and resource limitation?

Resource limitation has been inferred when supplemental pollination does not increase seed production (Brookes & Jesson, 2006). To simultaneously test for pollen and resource limitation in this study, flowers are removed (0, 40 and 80% removal) to create three levels of floral display size within populations of *Aciphylla*, experimentally imitating natural variation in floral display size within a population. As previously mentioned, *Aciphylla* has a large floral display consisting of inflorescences capable of reaching 3 metres tall with thousands of flowers. In an important paper by Haig and Westoby (1988), they presented a graphical model in which ovule fertilisations rise with increased allocation to pollinator attraction (increased flowering length) while at the same time ovule maturation ability (seed set rate) declined because enhanced attraction diverts resources from seed and fruit production. Figure 2.2 depicts the hypothesised effects of pollen and resource limitation scenarios on seed set rate.

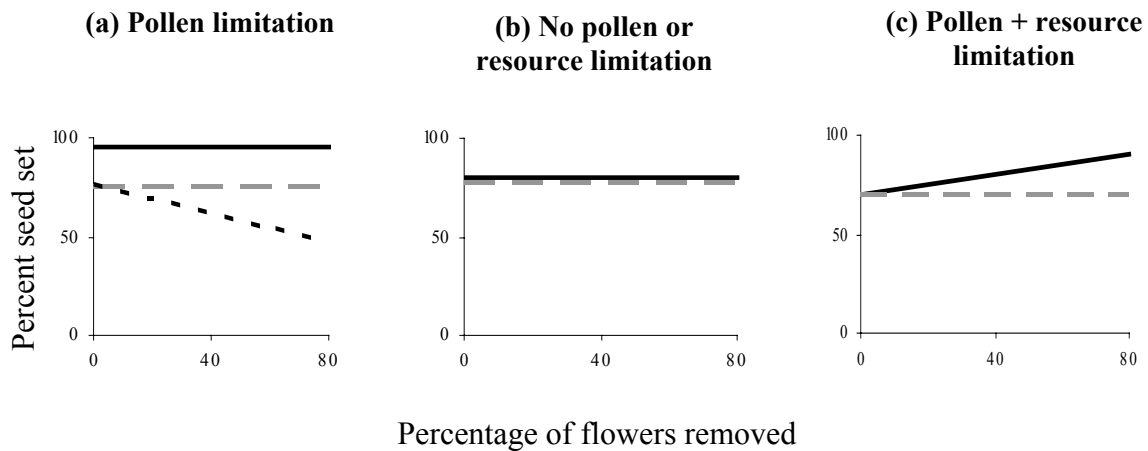


Fig. 2.2 Expected effects of pollen and resource limitation when supplemental pollen is added. Supplemental pollination (—), natural pollination (---) and induced pollen limitation by removing flowers (- - -). Under scenario (a) supplemental pollination resulting in an increase in seed set indicates pollen limitation. Also shown is the effect of inducing pollen limitation by removing flowers. As flower number decreases, floral attraction is reduced and thus lower seed set is expected. Under scenario (b) there is no increase in seed set when pollen is added. Under scenario (c) when the plant is simultaneously pollen and resource limited, an increase of resources to the remaining flowers and supplemental pollination would induce greater increases in seed set than only being limited by one factor. (Adapted from Brookes & Jesson, 2006).

While this study does not attempt to test for the effects of resource limitation *per se* by supplementing resources, by removing flowers it increases the amount of resources available per seed on inflorescences with fewer flowers. Therefore, this study employs both supplementary pollination and flower removal experiments to test for both pollen and resource limitation simultaneously in *Aciphylla* by testing the above model explicitly. As well as this, factorially designed manipulative experiments which provide more flexibility and power to test proposed hypotheses, were used to test for the effects of floral display size on pollinator attraction (Chapter 3) and reproductive success (this Chapter) at multiple levels.

### 2.1.3 Measuring population-level flowering effort – pollinator efficiency

The benefits of having a large floral display at the plant level may scale up to the level of the population, meaning that population flowering effort (i.e. pollinator economies of scale)

could also be important for reproductive success. Because *Aciphylla* show masting behaviour, there is a larger number of plants flowering overall in the population during high-flowering years. Does masting benefit reproductive success of individual females in *Aciphylla* by increasing population-level pollinator attraction through economies of scale?

Masting plant species are known to show clear benefits from masting when they are wind pollinated (Norton & Kelly, 1988; Kelly *et al.*, 2001). Concentration of pollen production in mast years increases the probability of pollination for wind-pollinated species (Nilsson & Wastljung, 1987). On the other hand, relatively few species that display masting behaviour are insect-pollinated (Kelly & Sork, 2002; Crone *et al.*, 2005). The negative effect of extreme fluctuation in plant reproduction on the stability of populations of pollinators might help to explain why most plant taxa that mast are wind-pollinated rather than animal-pollinated (Smith *et al.*, 1990). Many other authors regard animal pollination to be disadvantageous in a mast-seeding system due to pollinator satiation (see Chapter 1 and review in Kelly, (1994)). Masting has been assumed to swamp specific pollinators by producing such large temporally-concentrated flowering crops. However, if animals are more attracted by large floral displays, masting could improve pollination (Kelly, 1994) and overall lifetime reproductive fitness. This could be achieved by having a diverse and generalised pollinator fauna that does not rely on the masting species as a sole food resource, and are not satiated then starved, but is highly attracted to that species in the years that it does flower.

If flowering effort is so great during mast years that the masting species can ‘out-compete’ other generalist-pollinated plant species in the community for pollinators, then masting may be a successful method for ensuring overall lifetime reproductive fitness, because the costs of forfeiting reproduction some years must be outweighed by the benefits of concentrating flowering effort into certain years.

Because *Aciphylla* is dioecious, pollination requires male and female plants to flower synchronously. Individual reproductive success in obligate outcrossers is profoundly influenced by the presence of conspecifics in space and time, and individual plants may succeed best when they synchronise with others (Howe & Westley, 1986). Plant population size and density can influence the interactions between plants and pollinators and affect reproductive success (Schemske, 1980; Kirchner *et al.*, 2005) by affecting the quantity and

quality of pollination services received (Kunin, 1993). Dense stands of a flowering plant may collectively attract more pollinators as opposed to small patches or low population size, because small or sparse stands may be less apparent and/or offer low pollen and nectar rewards (Rathcke & Lacey, 1985; Bosch & Waser, 1999).

The effects of population-level flowering (pollinator attraction effort) on an individual plant can be observed in rare plant species, isolated plants or populations, naturally sparse populations or in a naturally low flowering year of masting species. For instance, in low flowering years, when only a small proportion of individuals flower, and are out of synchrony with the majority of the population (Smith *et al.*, 1990), this could have negative consequences for the individual in terms of pollinator attraction and reproductive success if the flowering population is the “attraction unit” (Groom, 1998; Forsyth, 2003). The effects of this can be stronger when a masting species has highly self-incompatible individuals (Forsyth, 2003) such as dioecious *Aciphylla*. For individual female plants flowering out of synchrony, these ‘isolation’ effects could be particularly detrimental to reproductive success when they are effectively isolated from the pollen supply (i.e. male plants) (Groom, 1998). In this scenario, pollen and/or pollinator limitation are expected to be important, particularly if population flowering effort is important for pollinator attraction (Kato & Hiura, 1990; Bosch & Waser, 1999; Kirchner *et al.*, 2005).

The effects described above can be studied using long-term datasets over time to measure changes in flower production between years. However, because of the time frames under which many studies are conducted, long-term experiments may not be practical. Therefore, by manipulating the natural flowering effort, flowering density and sex-ratio within a mast year using space-for-time substitution methods (e.g. Nilsson & Wastljung, 1987) as an alternative to long-term studies, it is possible to imitate a low-flowering year to test the effects of variation in floral display on reproductive success.

Many studies have investigated the effects of floral display on reproductive success but few have tested for the relative importance of floral display size for pollinator attraction and seed set at multiple levels (but see Kato & Hiura, 1990; Bosch & Waser, 1999; Grindeland *et al.*, 2005).

## 2.1.4 Objectives

### 2.1.4.1 Part (A): Floral display at the level of the individual plant

The first part of this chapter attempts to test for the importance of female floral display size on reproductive success through increased pollinator attraction at the plant-level, while simultaneously testing for the importance of pollen and resource limitation. It is hypothesised that an increased floral display will lead to increased female reproductive success (seed set) through pollinator attraction but only if resources and/or pollen are not limiting factors. Experiments were conducted during a high-flowering year using both experimental manipulations and natural measures of floral display size within a population. More specifically the questions were:

- How is seed set rate affected when bags are applied to inflorescences to exclude insect pollinators?
- How does variation in floral display size affect rates of seed set in female *Aciphylla* inflorescences? How does this vary when display size is measured by each of the following?
  - flowering length
  - inflorescence height
  - percent flower removal
  - number of inflorescences
- Is pollen limitation occurring at the level of the individual plant?
- Are floral bracts playing an important role in reproductive success and is seed set rate affected when bracts are removed?

#### **2.1.4.2 Part (B): Population level flowering effort - male to female flowering densities**

The second aim was to investigate the effects of male floral densities (i.e. local pollen availability) and asynchronous flowering on female reproductive success. ‘Space-for-time’ substitution was used to simulate the reduction of floral densities that would occur during a low-flowering year and test what would happen to female reproductive success if male to female flowering synchrony was reduced. It is hypothesised that by removing showy attractive male inflorescences from the population, and decreasing population level floral display in *Aciphylla*, this will lead to a decrease in reproductive success. More specifically:

- How is reproductive success in female inflorescences affected with increasing distance to the nearest flowering male plants (i.e. distance from pollen source)?
- Is pollen limitation occurring in female inflorescences that are more isolated from flowering male plants?

Table 2.1 outlines each predictive term used in this study and the hypothesised directions of the relationships between the each floral display term and seed set rate, and provides an explanation for each of these predictions based on the hypotheses discussed in the section above (i.e. pollen and/or pollinators are limited and larger floral displays are more attractive to pollinators).

Table 2.1 Expected relationships between each predictive term and the response variable, seed set rate. The first six terms represent floral display at the level of the individual plant, followed by an interaction between percent flower removal and pollination treatment, (as performed in statistical models in this chapter testing how each predictor affects seed set rate in female *Aciphylla* inflorescences). Explanations for expected direction of relationships are based on the assumption that a bigger display size increases the number of insect pollinators and subsequently will experience higher seed set. <sup>1</sup> The double signs next to flower removal indicate where there were 3 levels of removal. The first sign represents the direction of the relationship between the control (0%) and 40% removal and the second between 0% and 80% removal. <sup>2</sup> The interaction between percent flower removal and pollination treatment is complex and cannot be simply represented by a single symbol (see Fig. 2.2).

	<b>Predictive terms</b>	<b>Expected relationship</b>	<b>Explanation</b>
Floral display at the individual-plant level	Hand pollination	+	Hand pollination = higher seed set, natural pollination = lower seed set if pollen limited
	Flower removal (0,40, 80%)	- / - -	Smaller floral display, less attractive to insects = lower seed set <sup>1</sup>
	Flowering length (cm)	0	Should be cancelled out by the effects that percent flower removal has already explained in the model
	Inflorescence height (cm)	+	Taller inflorescences attract more insects, therefore more pollen received will give higher seed set
	Number of inflorescences	+	More inflorescences on a plant attracts more insects = higher seed set
	Floral bract removal	-	Bract removal may deter pollinators, and/or remove resources required to set seed = lower seed set
	Hand pollination x flower-removal (%) interaction	NA <sup>2</sup>	Refer to Fig. 2.2 and text in section 2.1.2.1
Floral display at the population level	Local pollen index	+	Females closer to pollen (males), insect visit males more often, females visited more by insects closer to males if pollen is the main attraction = increase seed set
	Increasing distance to males	-	Females further from males, less local pollen, less insects, fewer visits = lower seed set
Other experiments	Pollinator-exclusion bags	-	Bags prevent insect pollinators from delivering pollen = low or zero seed set

## 2.2 Site and species selection

The number of flowering *Aciphylla* populations during the summer field season of 2004/2005 limited the number of sites available for study. An initial survey to find potential flowering populations around the middle of the South Island was carried out in late October 2004. Sites selected were Lewis Pass (Lat/Long: 42° 22.713 S, 172° 24.021 E), Burkes Pass (Lat/Long: 44° 5.565 S, 170° 34.792 E) and Hakataramea Pass (Lat/Long: 44° 18.667 S, 170° 34.292 E). The three site locations were at the extreme northern (Lewis Pass) and southern (Burkes and Hakataramea Passes) limits of the Canterbury region of the South Island of New Zealand (Fig. 2.3). The sites were all at low alpine passes with easy access and within a 200 metre altitudinal range of each other. See Table 2.2 for information on study sites.

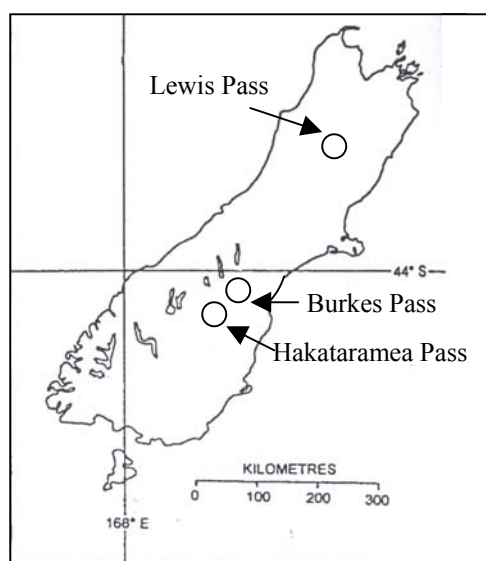


Fig. 2.3 Map of the South Island of New Zealand showing the three study site locations. All sites are located within the wider Canterbury region, with Lewis Pass at the northern boundary and Burkes and Hakataramea Pass at the southern boundary.



Table 2.2 Summary of site information for the three study areas used in this research including site location and description, elevation in metres above sea level (m.a.s.l.), and a brief description of climate, geology, soils, vegetation and background/modifications of the general area. Data sourced from Land Environments of New Zealand (LENZ), (Leathwick *et al.* 2003) and surveys from Ecological Regions and Districts of New Zealand (McEwen 1987).

Site	Location description	Elevation (m.a.s.l)	Climate	Geology and soils	Vegetation	Historical background
Lewis Pass	Boggy grassland area either side of road at the Pass (near tarn at start of St James Walkway). Conservation status: Forest Park.	860 m	Cool, moist, warm summers, rainfall 2800-4800mm p.a. (higher at the Pass)	Parent material: mostly Triassic Torlesse greywacke & argillite mountains; gravelly glacial outwash alluvial soils on river flats, fertility: moderate; drainage: good	Stable streams in valley floors with open grasslands of fescue tussocks, and bogs on terraces have <i>Dracophyllum</i> , <i>Carex</i> , <i>Phormium</i> , <i>Sphagnum</i> , etc	Change from continuous beech forest to patchy forest, some burning from sheep graziers
Burkes Pass	Burkes Pass Scenic Reserve, near roadside Tekapo Highway (SH1)	700 m	Subhumid hill climate with cool to cold winters and mild dry summers; rainfall 800-1200 mm p.a. ; NW winds prevail	Mostly Mesozoic-Paleozoic Torlesse greywacke and argillite, some schists, tertiary gravels & marine deposits, Pleistocene loess-covered gravels; subsoils pale-coloured, compact and droughty in summer	Tussocklands with silver/fescue tussock, scrub - matagauri, <i>Coprosma</i> , introduced grasses	Mostly modified by repeated burning beginning in Polynesian times & grazed by both domestic and wild animals
Hakataramea Pass	Alongside Hakataramea River, between Grampian and Dalgety Ranges, McKenzie district, South Canterbury. Farmed (merino sheep).	900 m	Semi-arid to sub-humid inland climate with cold winters and mild to hot summers; rainfall less than 600-1200 m p.a.	Landform: rolling to steep mountains either side of Pass, parent material: mainly Paleozoic Torlesse greywacke and schist - some older basalts, argillite conglomerates, alluvium and loess, basic volcanic and tertiary rocks, including limestone; subsoils at Pass pale, compact & droughty; fertility: moderate, drainage: near river - wet and boggy	Modified and depleted tussocklands with heavy weed infestation - hawkweeds & broom. Scrub in gullies: matagauri, fescue/silver tussock, <i>Gaultheria</i> , <i>Bulbinella</i> , range of introduced grasses	Mostly modified by repeated burning beginning in Polynesian times & grazed by both domestic and wild animals

### 2.2.1 Study species

At these sites, three species were studied: *A. aurea* at Burkes and Hakataramea Passes, *A. scott-thomsonii* at Hakataramea Pass, and *A. 'lewis'* at Lewis Pass. These taxa are described below.

#### 2.2.1.1 *Aciphylla aurea* W.R.B. Oliver

*Aciphylla aurea* (also known as golden spaniard) is abundant mainly east of the main divide of the South Island (Wardle, 2002) from Marlborough through to Southland. Habitat ranges from montane to sub-alpine tussocklands and shrublands, usually on well-drained hill slopes, ridges, outwash surfaces and around rock outcrops (D. Glenny, pers. comm. 2005). Plants form 1-10 rosettes of golden yellow-green tussocks up to 1.3 m in diameter and up to 70 cm tall.

Inflorescences consist of a tapering rachis (stalk) up to 1.8 m tall. There can be up to 10 inflorescences on a plant, but 1-4 is more common. The rachis supports compound lateral umbels, each with multiple umbels and umbellules branching from the peduncle. These lateral branches start from above the leaves, continuing to the apex, subtended by yellow linear bracts which are long (18-37 cm) and consist of a sheath (29-37 mm length) and long spiky stipules and leaflets. The rachis is hollow and the lower 1-20 bracts are usually sterile. Bract and umbel length decreases towards the apex. Bracts at the upper half of the inflorescence are reflexed at the top of the sheath to point downwards, while the stipules point upwards to create a “cage” of spines (D. Glenny, pers. comm. 2005). See Fig. 2.4 for a diagram of an *Aciphylla* inflorescence.

The winged fruits consist of two mericarps, forming a laterally compressed schizocarp (Webb & Simpson, 2001). Fruits in *A. aurea* are dark brown. While the entire female inflorescence is presented at the same time, flower maturation is basipetal with the top of the inflorescence maturing first (Brookes & Jesson, 2006). Flowering begins from early to mid December at montane altitudes.

Although formidable, this plant is susceptible to browsing by animals such as hares, rabbits and livestock (Allan, 1982), along with a suite of invertebrates such as weevils and various Lepidopteran larvae, many of which are *Aciphylla*-specific herbivores.

#### **2.2.1.2 *Aciphylla scott-thomsonii* Cockayne & Allan**

Common and widespread in Otago and South Canterbury, the natural habitat of *A. scott-thomsonii* is sub-alpine scrub, extending slightly into alpine tussocklands, shrublands and scrub on south-facing colluvial hill slopes. It is most common beside streams or in flushes at valley heads (D. Glenny, pers. comm. 2005). *A. scott-thomsonii* is often found in association with *A. aurea* (at damper, more fertile and scrubby habitat) and like numerous members of the New Zealand Apiaceae, these two species can readily hybridise (Dawson & Le Comte, 1978). Care was taken to avoid using hybrid specimens for purposes of this research.

*A. scott-thomsonii* are typically much larger than *A. aurea* and form huge tussocks with long, glaucous, extremely sharp pinnate leaves up to 1.5 m long (Allan, 1982). Inflorescences can reach an impressive 4 metres tall with yellow-green fruits. Reproductive arrangement is very similar to *A. aurea* (see above). Flowering in *A. scott-thomsonii* begins early to late December and fruit ripens by mid-February (D. Glenny, pers. comm. 2005).

#### **2.2.1.3 *Aciphylla* ‘lewis’**

The northern limit of *A. scott-thomsonii* is recorded as Arthur’s Pass in mid-Canterbury (Lat/Long: 42° 56.633 S, 171° 33.942 E), (Mark & Adams, 1973). However, D. Glenny (2005), currently revising the taxonomy of this group, has collected plants as far north as Kowhai Saddle near Kaikoura that are more similar to *A. scott-thomsonii* than any other *Aciphylla* species. The form at Deer Valley near Lewis Pass represents a viable, reproducing population very similar to *A. scott-thomsonii*, but with aspects of the next most similar species, *A. colensoi*. D. Glenny (2005) suggests that it is difficult to draw a boundary between *A. scott-thomsonii* and *A. colensoi* at Lewis Pass. Differences in leaf and inflorescence morphology have been noted by D. Glenny (2005). This population will be

referred to as *A. 'lewis'* from this point on. Leaf voucher specimens (of all study species) were collected, pressed and mounted, then deposited in the University of Canterbury Biological Sciences herbarium. Refer to specimen numbers #38835 to #38838 LMY.

Like *A. scott-thomsonii*, *A. 'lewis'* grows alongside streams and rivers in extremely wet conditions amongst other bog-dwelling plants such as flax (*Phormium tenax*), fescue tussocks and *Sphagnum*. The extent of its distribution is not yet confirmed, although is probably restricted to wet riversides east of the Main Divide around the greater Lewis Pass area. Plant size and colour is similar to that of *A. scott-thomsonii* but inflorescences and bracts are a bright yellow and fruits are yellow-green. Flowering in *A. 'lewis'* typically begins around mid to late November.

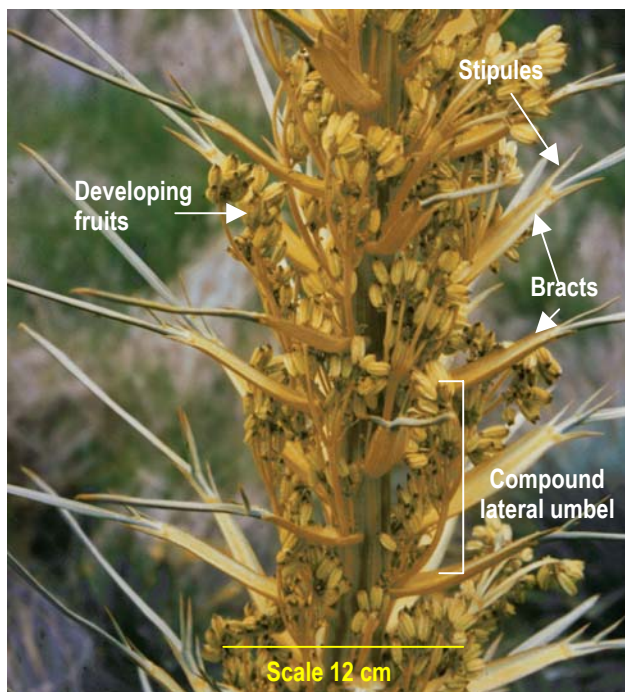


Fig. 2.4 Mid-section of an inflorescence stalk of *Aciphylla scott-thomsonii* showing detail of spiky bracts with stipules, subtending compound lateral umbels made up of clusters of flowers (umbellules) which are beginning to develop into fruits in the above example.

## **2.2.2 Field work**

The experiments were carried out at all three field locations over the summer of 2004/2005, beginning late November when flowering commenced, through until early March when fruit and seed set. Burkes and Hakataramea Passes were experiencing a heavy flowering year therefore those populations were sufficiently large to perform manipulative experiments in the field. At Lewis Pass, flowering *Aciphylla* plants were sparser and there were relatively fewer in the population over a larger area, representing a relatively low flowering year.

## **2.3 METHODS**

### **2.3.1 Part (A): Floral display at the level of the individual plant**

An experimental area (plot) was roughly measured out at each site. These plots varied in size between sites because a large enough area to encompass sufficient numbers of flowering plants for experimentation was needed, as well as leaving enough non-experimental (control) plants. *Aciphylla scott-thomsonii* habitat is usually limited to wet sites near streams and rivers therefore Hakataramea and Lewis Pass sites with *A. scott-thomsonii* and *A. "lewis"* respectively were very long and relatively thin compared to *A. aurea* populations (refer to Tables 2.3 & 2.4 for quantitative site information). The following floral display experiments and the effect that they had on seed set rates all involved female inflorescences only.

#### ***1) How do pollinator-exclusion bags affect seed set?***

To see whether seed set was affected when insect pollinators were excluded, fine mesh curtain bags (approximately 0.5 mm mesh diameter) were used to cover inflorescences. These were put on at the beginning of the season when inflorescences were small and in an early developmental stage, with bracts covering unopened flowers. Bags were tied at the base of the inflorescence as close as possible to the point of attachment to the rosette. As

inflorescences grew taller, bags were extended so as to prevent stalks from bending over or becoming damaged during growth and elongation. Bags were sufficient to exclude all insects however mesh size was not small enough to exclude pollination by wind. Control inflorescences were also tagged nearby or on the same plant for comparison with bagged plants. Thus this method was used to test for seed set by mechanisms such as wind pollination, selfing or apomixis (although selfing is unlikely given that plants in this study were always strictly dioecious). At Hakataramea Pass however, one plant consisting of mostly female flowers but with some male flowers present, was discovered. This plant was noted as hermaphroditic and bagging was used to test for possible self-fertilisation.

## ***2) Does floral display size affect seed set rate?***

This question was approached in four ways. The first employed manipulative field experimentation where 0, 40 and 80% of flowers were removed from inflorescences to test whether seed set rate was affected by the varying proportion of flowers along the stem. Percent flower removal was hence treated as a factor. The second two approaches measure continuous variation in flowering length i.e. the length of inflorescence with flowers present, and inflorescence height in relation to seed set rate, i.e. total length from ground to apex. The fourth measures display size on the plant level as a whole, by using the number of inflorescences on the plant as an indicator of display size.

### *a) Flower removal experiment*

Three levels of flower removal were chosen to represent variation in floral display size in each population. These were 0, 40 and 80% removal with flowers being removed from the lower part of each inflorescence, leaving the flowering portion remaining at the top. To test for pollen limitation, supplemental pollen was added to some inflorescences in each level of flower removal treatment. Flower removal and pollen supplementation experiments were set up factorially creating more flexibility and power to test the proposed questions.

Treatments were assigned randomly to female plants that had at least 2 inflorescences to enable paired design sampling. Within the same plant, one level of flower removal treatment was performed on both inflorescences (e.g. 40% removal) with one inflorescence receiving supplemental pollen and the other remaining naturally pollinated. This design was useful for controlling between-plant effects.

The flowering length of the inflorescence was measured from the lowest bract to the apex and the appropriate length of flowers to be removed was calculated as 0, 40 or 80% off the total flowering portion. Flowers were snipped off using fine scissors and care was taken to avoid damaging the stem. Because inflorescences had not usually grown to full size at the time of first flower removal with late expansion concentrated at the tip, flowering length was re-measured between 2 and 3 weeks later and any more flowers that needed removing, were removed.

#### *b) Flowering length*

Flowering length, i.e. the flowering portion of the inflorescence (cm), was measured after flower removal treatments were performed. This was done in order to run statistical analyses to test whether the effects of the absolute value of flowering length (rather than the percentage removed – as above) was a better expression of floral display size and if there was any effect on seed set rate. Flowering length was recorded at the end of the season immediately prior to harvest.

#### *c) Inflorescence height*

The entire length of the inflorescences from the base to the tip of the apex was referred to as inflorescence height (cm). Measurements of inflorescence height were recorded at the end of the field season just prior to harvest.

d) *Number of inflorescences*

The number of inflorescences on each treatment plant was recorded and used in statistical analyses to test whether this accounted for any variation in seed set rate.

3) *How does the removal of floral bracts affect seed set?*

To determine whether the spiny, sterile floral bracts were having any effect on seed set rates, bracts were removed on some inflorescences. Again, plants with at least 2 inflorescences were randomly selected from the population in order for paired design sampling to be used. On one inflorescence, all bracts were removed, and the other was left as a control for comparison. Bracts were snipped off from the entire length of the inflorescence, leaving about 2 cm beneath the base of each peduncle to avoid damage to the stem or floral parts. (See Table 2.3 for all manipulative treatments, plot size, number of replicates, etc).

Table 2.3 Quantitative information for floral display experimental manipulations performed in the field. The six manipulated flower/pollination treatments include 0, 40 and 80% flower removal each with and without hand pollination. Bract removal and bag treatments also included controls for each. (\* = no hand-pollination performed at Lewis site due to weather, hence only 3 treatments – 0, 40, and 80% flower removal). In total 252 female inflorescences with various treatments were harvested from the sites below.

Site	Species	Site dimensions (m)	Type of treatment	No. of treatments	No. of Replicates	Total no. of inflorescences
Burkes Pass	<i>A. aurea</i>	127 x 236	Flower/pollination	6	8	48
			Bract removal	2	9	18
			Bag	2	6	12
Hakataramea Pass	<i>A. aurea</i>	500 x 220	Flower/pollination	6	8	48
			Bract removal	2	8	16
			Bag	2	7	14
Hakataramea Pass	<i>A. scott-thomsonii</i>	1200 x 50	Flower/pollination	6	6	36
			Bract removal	2	6	12
			Bag	2	5	10
Lewis Pass	<i>A. 'lewis'</i>	800 x 230	Flower/pollination	3 *	8	24
			Bract removal	2	3	6
			Bag	2	4	8
<b>TOTALS</b>						<b>252</b>



#### ***4) Does local pollen availability affect seed set rates?***

To determine whether local pollen densities were affecting female reproductive success, the distance between each female plant used in the above experiments and the closest four flowering male plants was measured. The number of inflorescences on each of the four nearest male plants was recorded. This information was used to construct a weighted local pollen index, indicating local densities of pollen from nearby male inflorescences. The following formula was devised:

$$\text{Distance-weighted local pollen index (DWPI)} = \frac{s_1}{d_1^2} + \frac{s_2}{d_2^2} + \frac{s_3}{d_3^2} + \frac{s_4}{d_4^2}$$

where  $s$  is the number of male inflorescences on the four nearest male plants and  $d$  is the distance in metres to the plant (indicated by the subscripts given). The DWPI decreases with the square of the distance and increases linearly with the number of inflorescences on each male. A larger value results when females are close to males with many inflorescences.

### **2.3.2 Part (B): Population level flowering effort - male to female flowering densities**

#### ***Isolated-female experiment***

The aim of this study was to reduce the local density of flowering male plants to imitate a low mast year when female and male plants might flower out of synchrony, or are separated by increasing distance. To investigate the effects of male floral densities (i.e. pollen availability) on female reproductive success (seed set), floral densities were manipulated at separate plots at Burkes and Hakataramea Passes by removing male inflorescences. At Lewis Pass, the population structure was such that there were a number of flowering female plants that were naturally isolated (i.e. far away from flowering male plants) therefore no experimental removal of flowering males was carried out.

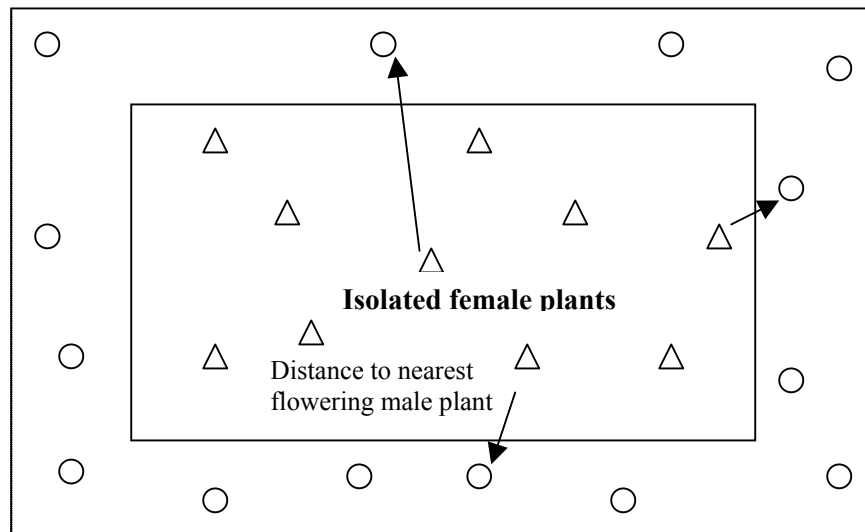


Fig 2.5 Diagrammatic representation of isolated female experimental design to investigate the effects of male floral densities on female reproductive success. All flowering male inflorescences removed from plot (inner rectangle), leaving only female plants at varying distances to male plants. Female plants (Δ) in the centre of the plot are more isolated from male plants (○) than females nearer the edge of the plot. Arrows represent distance between female and nearest flowering male plant outside plot. This distance was measured for each female.

One plot was marked out at each site, where all developing male inflorescences inside the plot were removed. Only female inflorescences remained inside the plot and males were left randomly distributed around the edge of the plot. Female plants were thus at varying distances to the nearest flowering male (see Fig. 2.5). Within the plot, female plants with 2 or more inflorescences were randomly selected and tagged to enable paired design sampling (see Table 2.4 for plot information). The position of each female plant was mapped, as well as the position of each flowering male plant around the outskirts of the plot. The distance between each experimental female and the nearest flowering male plant outside the plot was measured. On each experimental plant, one inflorescence was given supplemental pollen treatment and the other was left naturally pollinated. The hypothesis was that pollen limitation (hand-pollinated seed set – control seed set) would be stronger in females further from males. Hand pollination was performed 3 separate times over approximately 3 weeks (see below).

Table 2.4 Plot information for isolated-female experiment. Pollen limitation was tested for by pollen supplementation i.e. hand pollinated. (\* = no hand-pollination performed at Lewis Pass site due to adverse weather).

Site	Species	Plot size (m <sup>2</sup> )	Number hand pollinated	Number naturally pollinated	Total number of inflorescences	Dist. range to nearest male (m)
Burkes Pass	<i>A. aurea</i>	7,650	20	21	41	1-60
Hakataramea Pass	<i>A. aurea</i>	42,780	18	18	36	1-200
Lewis Pass	<i>A. 'lewis'</i>	184,000	0*	38	38	1-500

### 2.3.3 Pollen limitation

To determine whether seed set of female plants in all of the experiments outlined above at Burkes and Hakataramea Passes was limited by the availability of pollen, supplemental pollen was applied by hand to one inflorescence from each pair of treatments. Hand (supplemental) pollination was done by whacking a section of male inflorescence with a substantial pollen load over the female inflorescence while covering the non-hand-pollinated stalk with a paper bag to avoid accidental pollination.

Hand pollination was repeated on each inflorescence three separate times over approximately three weeks during December 2004, to encompass variation in flower longevity and stigma receptivity over the course of the flowering season. Hand pollination was performed only on non-rainy days to avoid pollen loss and wastage. Because there were no days without rain during field work at Lewis Pass, hand pollination was not performed there.

Male inflorescences used for hand pollination were taken from outside the experimental plots, 50 to 300 metres away from the boundary to avoid altering the natural structure of the population inside the plot. Naturally pollinated inflorescences were left untouched. After male inflorescences were used for hand pollinating females, they were discarded over 300 metres away from the plot.

### **2.3.4 Other field measurements**

For each of the 377 experimental inflorescences, a number of measurements were made at the: 1) inflorescence, and 2) plant level. These were used to compile detailed data matrices to gain a more in-depth understanding of how both experimentally manipulated and natural variables affect reproductive success in *Aciphylla*.

1) All inflorescences were harvested between late February and early March when fruit and seeds were filled but not ripe enough to be dispersed. The height of each inflorescence was re-measured and a categorical estimate of aphid densities on inflorescences was recorded (low, medium or high). Each inflorescence was then carefully cut near the base of the rachis and placed into separate paper for storage until seed sorting and counting began. Some were stored in a freezer, but due to limited space, others were dry-stored.

2) Plant-level measurements recorded were plant area, plant leaf height, rosette number and number of inflorescences. Diameter was measured as the greatest diameter across the clump formed by all rosettes belonging to a single plant (length, L), and the diameter perpendicular to that (width, W). To calculate the area of the plant, the formula for measuring an ellipse was used  $(L + W / 2)^2 \pi$ . Plant leaf height was measured using the distance from the leaf base at ground level to the tip of the tallest leaf. These measurements were used to test the effect of plant size on reproductive success.

Other plant species flowering at each site were also noted along with the weather each day field work was done. Insect visitation rates to every inflorescence in all of the experiments were also recorded with multiple observations (see Chapter 3).

### **2.3.5 Lab work**

#### **2.3.5.1 Measuring reproductive success – counting seeds**

Flowers that had been pollinated had developed into fruits by the time plants were harvested. Reproductive success in females was measured by counting the proportion of

fruits that made seeds ('filled') vs. the proportion that did not make seeds ('unfilled'). Each 'fruit' is technically described as a laterally compressed schizocarp consisting of 2 mericarps, each containing one seed (see Fig. 2.6). For this study, seed set not fruit set was measured by counting each mericarp individually, therefore each mericarp had either zero or one seed (i.e. 'filled' or 'unfilled' respectively). For every inflorescence a sub-sample of seeds were sorted and counted by hand (see below for sub-sampling methods). A total of 122,811 seeds were counted. Aside from the 2 categories of whether a fruit was 'filled' or 'unfilled' with a seed, the proportion of predated seeds (both fully and partially) was also recorded. Predated seeds were assumed to have once been 'filled' before they were eaten by an invertebrate seed predator, and hence were also counted as 'filled'.

This measure of reproductive success in female plants slightly differed to methodology used previously by Brookes & Jesson (2006). Rather than testing each seed for viability to identify germinability, the simpler categorisation of seed 'filled' or 'unfilled' was employed. Proportion of filled seeds will from now on be referred to as 'percent seed set'. This method is frequently used for quantifying pollination efficiency and although it is not a direct measure of pollination success, it does set a lower limit (Tisch, 1996). This method was used due to large sample sizes and time constraints. Slightly different methods of seed counting were employed for *A. aurea* and *A. scott-thomsonii* as explained below.

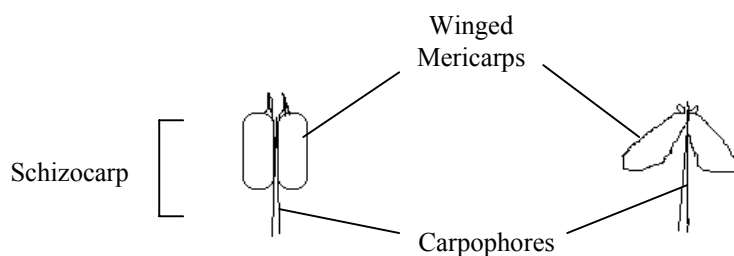


Fig. 2.6 Intact developing fruit, or schizocarp, (left) consisting of 2 winged mericarps, each containing one seed. The carpophore is the centre stalk that attaches to the mericarps. The picture on the right shows the fruit at maturity with mericarps still attached prior to dispersal.

### *Aciphylla aurea* and *A.* ‘lewis’

For each inflorescence, all fruits were removed from the entire length of the inflorescence by shaking them off the stalk into a paper bag. The contents of the bag were shaken well to mix them, then a random sub-sample of 250-350 developed and undeveloped fruits were selected out for counting. The number of seeds that fell into each category (filled, unfilled and eaten) was recorded. I counted only 250-350 seeds per inflorescence, as trials showed that the ratio of filled: unfilled did not change with increasing number counted beyond that.

### *Aciphylla scott-thomsonii*

For *A. scott-thomsonii*, all seeds were counted from every fifth lateral umbel along the axis of the inflorescence. This method was used to obtain an idea of whether seed set rate varied along the inflorescence. Only the grand mean seed set rate data for each inflorescence however, will be presented in this section. See Appendix 1 for more detail of seed set along the inflorescence and how flower number per umbel can be accurately predicted from its particular location along the rachis.

#### **2.3.5.2 Germination experiments**

Sub-samples of seeds from all species and sites were randomly selected from the experimental inflorescences in an attempt to germinate some of the seeds. This experiment began in late August 2005. The purpose of this was to test the assumption that ‘filled’ fruits were viable and ‘unfilled’ fruits were not. Because the seeds had been dry-stored since harvest in late-February to early-March, soaking them before sowing was recommended (Metcalf, 1995). One hundred filled and 100 unfilled fruits were separated out for each species from each site and soaked in warm water overnight. These were then surface-sterilised in 50% bleach solution for 10 minutes and a soak/rinse cycle in distilled water was performed 3 times to clean off the bleach. Seeds were placed into sterilised Petri dishes lined with damp germination paper, sealed with plastic wrap and refrigerated for 4 weeks to imitate cold winter field conditions. In mid-October, seeds were removed from cool storage and Petri dishes were then left at room temperature and kept moistened for ten months.

### 2.3.6 Statistical Analyses

Statistical analyses were performed in the statistical package R version 1.5.1 and in Microsoft Excel 2003. Generalised Linear Models (GLM's) were used to examine the influence of the various treatments on rates of seed set. For all GLM's with the response variable 'seed set', a binomial error distribution with a logit link function was used because there were two possible outcomes for the fate of a fruit: 'filled' or 'unfilled'. Because the assumption of independence necessary for use of tests based on the chi-squared distribution appeared to be breached, the fit of all models was tested using F-ratios.

For each experiment performed at each site, a large multi-variate model was created to test for the effects of each predictor on seed set rate. Model selection was attempted in multiple ways, with the aim of fitting a model that could best explain variation in seed set rate and that would allow for comparison between sites. Model construction was performed by initial inclusion of all factors and variables of interest. The order of predictors in the model was varied for each site until a generic order that could be used for all sites was selected. Predictors lacking significant explanatory power were then removed from the model. For both the floral display size (A) and isolated-female (B) experiments, five predictors were used in the final model in the following order: *pollination treatment*, *local pollen availability* (using DWPI or distance to nearest male inflorescences), *percent flower removal*, *flowering length*, and *inflorescence height*. See Table 2.1 for predicted relationships of all explanatory predictors and seed set rate (the order of variables are shown in the GLM tables in section 2.4).

When testing various models to select the most appropriate one to explain variation in seed set rate, *percent flower removal* (as a factor) explained little or no significant variation in the data on its own at most sites. When the variable *flowering length* was added to the model after *percent flower removal*, it usually explained a greater proportion of the variance while *percent flower removal* explained little or none. Of the few sites where *percent flower removal* explained the most variation, *flowering length* explained almost none. Therefore, these two predictors were highly correlated, as expected. *Flowering length* was essentially the same predictor as *percent flower removal* (because most of the variation in final flowering length was due to experimental removal, rather than variation among

unmanipulated inflorescences), but it expresses it as a continuous variable rather than a factor with three levels of flower removal. Different sites responded to either one or the other of these two predictors measuring “variation in the amount of flowers on an inflorescence”, but were both retained in the final model to maintain consistency between sites.

Apart from analysing the effects of floral display on seed set rate by site and species (for floral display at multiple levels) using multi-variate models, some analyses were also done by treatment and presented graphically to allow for more convenient comparison between sites and species. The effect of the interaction between pollen supplementation and flower removal (testing for simultaneous pollen versus resource limitation), was presented using two separate graphs. The first compared seed set rate against *percent flower removal* as a factor for supplemental and natural pollination, while the second compared seed set rate and *flowering length* (cm) as a continuous variable for supplemental and natural pollination.

## 2.4 RESULTS

### 2.4 Part (A): Effects of insect-exclusion bags, plant-level floral display, hand-pollination, and local pollen on seed set

#### 2.4.1 Mean seed set rates of control inflorescences at all sites

Seed set rates for control inflorescences varied between sites (Fig 2.7). Overall, *Aciphylla scott-thomsonii* at Hakataramea Pass (HP) had the highest mean seed set rate of 87%, while also at Hakataramea, seed set in *A. aurea* was relatively high (75% at HP1 and 72% at HP2). Seed set at Lewis Pass (LP) was the next highest (66%). *Aciphylla aurea* at both Burkes Pass (BP) sites experienced the lowest levels of seed set (57% BP1 and 53% at BP2).



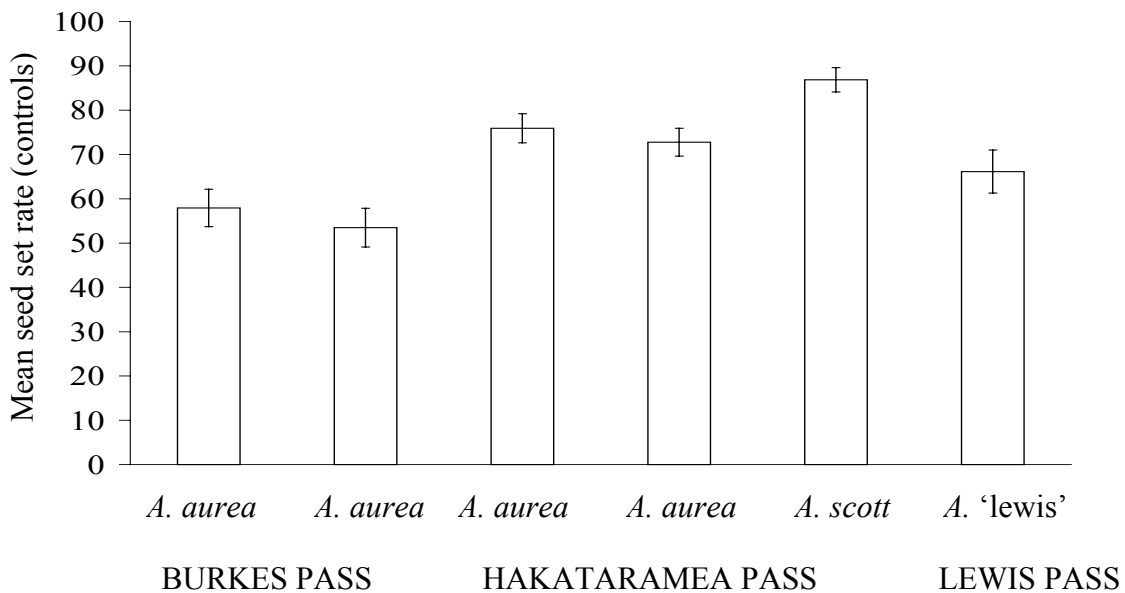


Fig 2.7 The natural variation in seed set rates amongst sites using control inflorescences, including all experiments ( $\pm 1$  SE). The two bars for *A. aurea* at both Burkes Pass and Hakataramea Pass indicate mean seed set rates for both experiments: floral display size (left) and isolated female (right).

#### 2.4.2 Effects of bagging on seed set rate

Bagged inflorescences excluding insect pollinators experienced a highly significant decrease in seed set rate, compared with controls, at all sites (Table 2.5; see also Fig. 2.8). Seed set inside bags was expected to be zero or nearly so, yet in three cases mean seed set rates inside bags were over 20%, which is surprisingly high, but still significantly less than non-bagged plants suggesting insects played a vital role in pollen movement.

Table 2.5 Mean seed set rates of bagged versus control inflorescences. Statistical values given are for the effects of bagging on seed set rate from binomial GLM's. (Abbreviations: BP = Burkes Pass, HP = Hakataramea Pass and LP = Lewis Pass).

Species and sites	Mean seed set rate (%)		F (d.f)	% Deviance	
	Control	Bag		explained	P-value
<i>A. aurea</i> BP	52.0	23.8	354.24 (1,11)	50.33	< 0.0001
<i>A. aurea</i> HP	76.8	37.5	430.31 (1,9)	44.96	< 0.0001
<i>A. scott-thomsonii</i> HP	91.4	23.7	3067.4 (1,5)	92.68	< 0.0001
<i>A. 'lewis'</i> LP	79.1	9.2	1320.9 (1,8)	91.99	< 0.0001

### 2.4.3 Effects of floral display on seed set

#### 2.4.3.1 Analyses by site

##### Burkes Pass – *Aciphylla aurea*

At Burkes Pass, all predictive terms had a significant effect on seed set rate. Inflorescence height was the most significant predictor and accounted for over 13% of the variation in seed set rate (Table 2.6). As expected, taller inflorescences had a higher rate of seed set. Local pollen abundance (DWPI) also explained significant variation in seed set rates, and as expected, an increase in local pollen abundance contributed to a higher seed set rate. Hand pollinated inflorescences also set significantly more seed than those left openly pollinated, although this explained only 1.7% of the variation in the model. Flower-removal treatment significantly decreased seed set rate, suggesting that with an increase in the number of flowers along the inflorescence stem, comes an increase in seed set rate. Therefore, results demonstrate that taller inflorescences with more flowers have a higher rate of seed set than shorter ones at Burkes Pass, and seed set rate is further increased when there is more local pollen available. The model accounted for 25.7% of the variation in seed set rates at Burkes Pass (ranging from 33-92%, see Fig. 2.8). There was also a significant interaction between pollination and percent flower removal (see Fig. 2.9).

Table 2.6 Analysis of Deviance Table for *A. aurea* at **Burkes Pass** showing variation in seed set rate as a response to five predictive terms. The terms DWPI (Distance-Weighted local Pollen Index), flowering length and inflorescence height are non-manipulated variables while the terms pollination (hand vs. natural) and percent flower removal (0, 40 & 80%) are manipulated factors. Finally shown is the interaction between the two manipulated factors – pollination and flower removal. The double signs next to flower removal indicate where there were 3 levels of removal. The first sign represents the direction of the relationship between the control (0%) and 40% removal and the second between 0% and 80% removal. † = refer to Fig. 2.8 for pollination x flower removal effects.

Terms added	Df	F	Resid. Df	Resid.dev	% Dev. expl.	P-value	Slope
NULL			45	1426.73			
Hand pollination	1	23.62	44	1403.11	1.66	<0.001	+
DWPI	1	98.91	43	1304.20	6.93	<0.001	+
Flower removal (%)	2	16.22	41	1271.75	2.27	<0.001	- / - -
Flowering length (cm)	1	10.37	40	1261.38	0.73	0.0013	-
<b>Inflorescence height (cm)</b>	1	191.95	39	1069.43	13.45	<0.001	+
Pollination x Flower removal	2	4.68	37	1060.08	0.66	0.0093	†

#### Hakataramea Pass – *Aciphylla aurea*

The most significant variable affecting seed set in *A. aurea* at Hakataramea Pass was inflorescence height, although the direction of this relationship was not as expected. As inflorescence height increased, seed set rate significantly decreased (Table 2.7). Percent flower removal also significantly decreased seed set rate as expected. Hand pollination significantly increased seed set along with greater local pollen supply (DWPI). A pollination x flower removal interaction also produced significant effects on seed set rates (see Fig. 2.9 for detail). Overall, this model (Table 2.7) accounted for 26.3% of the variation in *A. aurea* seed set at Hakataramea Pass (which ranged from 42-95%, see Fig. 2.8).

Table 2.7 Analysis of Deviance Table for *A. aurea* at Hakataramea Pass showing variation in seed set rate as a response to five predictive terms. Terms and symbols are as for Table 2.6.

Terms added	Df	F	Resid. Df	Resid.dev	% Dev. expl.	P-value	Slope
NULL			39	811.9			
Hand pollination	1	13.83	38	798.07	1.70	<0.001	+
DWPI	1	20.94	37	777.13	2.58	<0.001	+
Flower removal (%)	2	35.04	35	707.05	8.63	<0.001	- / - -
Flowering length (cm)	1	0.05	34	707	0.01	0.82	-
<b>Inflorescence height (cm)</b>	1	92.32	33	614.67	11.37	<0.001	-
Pollination x Flower removal	2	8.10	31	598.47	2.00	<0.001	†

#### Hakataramea Pass – *Aciphylla scott-thomsonii*

In *A. scott-thomsonii* at Hakataramea Pass, the most significant outcome was that with an increase in flowering length there was significant **decrease** in seed set rate (Table 2.8). The variable flowering length explained more than 40% of the variation in seed set, accounting for over four fifths of the variation that the entire model explained (which was 49.4%). The range in seed set rate was 63-97% in *A. scott-thomsonii*. Taller inflorescences had a higher seed set rate, although this explained only 4.7% of the deviance (Table 2.8). Hand pollination only slightly increased seed set rate (see Fig. 2.8), although this was highly statistically significant. Local pollen abundance was relatively unimportant at this site, as was percent flower removal, although results significantly showed that percent seed set increases with more flowers, as expected. Thus there is strong evidence for inflorescences that are tall but with fewer flowers having higher seed set in *A. scott-thomsonii*.

Table 2.8 Analysis of Deviance Table for *A. scott-thomsonii* at **Hakataramea Pass** showing variation in seed set rate as a response to five predictive terms. Terms and symbols are as for Table 2.6.

Terms added	Df	F	Resid. Df	Resid.dev	% Dev. expl.	P-value	Slope
NULL			30	1965.61			
Hand pollination	1	13.65	29	1951.96	0.69	<0.001	+
DWPI	1	5.19	28	1946.77	0.26	0.023	-
Flower removal (%)	2	21.94	26	1902.89	2.23	<0.001	- / - -
<b>Flowering length (cm)</b>	1	801.09	25	1101.81	40.76	<0.001	-
Inflorescence height (cm)	1	93.24	24	1008.57	4.74	<0.001	+
Pollination x Flower removal	2	6.88	22	994.8	0.70	0.001	†

#### Lewis Pass – *Aciphylla* ‘lewis’

The total variation in seed set rate explained by the Lewis Pass model was just 13.7%, with seed set values ranging from 2-96%. The most significant explanatory term was flowering length which accounted for around 8.5% of the variation in seed set in *A. ‘lewis’* (Table 2.9). Seed set was greater with an increase in flowering length. Distance to the nearest flowering male plant (pollen availability) was also highly significant, with isolated female inflorescences experiencing lower rates of seed set. Also statistically significant was an increase in seed set rate with increasing inflorescence height. Pollen limitation was not tested for at Lewis Pass although with mean seed set rates of around 65% (see Figs 2.7 to 2.9) it would have been interesting to test whether seed set rates would increase if supplementary pollen was applied.

Table 2.9 Analysis of Deviance Table for *A. 'lewis'* at Lewis Pass showing variation in seed set rate as a response to four predictive terms. Terms and symbols are as for Table 2.6. Note: no pollination treatment was carried out at Lewis Pass.

Terms added	Df	F	Resid. Df	Resid.dev	% Dev. expl.	P-value	Slope
NULL			20	1611.49			
Nearest male	1	58.05	19	1553.44	3.60	<0.001	–
Flower removal (%)	2	14.65	17	1538.79	0.91	<0.001	– / +
<b>Flowering length (cm)</b>	1	136.53	16	1402.26	8.47	<0.001	+
Inflorescence height (cm)	1	10.9	15	1391.36	0.68	<0.001	+

*The effect of bract removal on seed set*

Inflorescences with bracts removed had a significantly higher seed set on *A. aurea* at Burkes Pass, *A. scott-thomsonii* at Hakataramea Pass and on *A. 'lewis'* at Lewis Pass. Bract removal on *A. aurea* inflorescences at Hakataramea Pass however, had a weak but significant negative effect on seed set (Table 2.10).

Table 2.10 The effects of removing inflorescence floral bracts on seed set rate. Statistical values given are for the effects of bract removal (as a factor) on seed set rate, using binomial GLM's. (Abbreviations: BP = Burkes Pass, HP = Hakataramea Pass and LP = Lewis Pass).

Species and sites	Mean seed set rate (%)		% Deviance explained	F (d.f)	P-value
	Control	Bract removal			
<i>A. aurea</i> BP	51.96	58.79	1.7	14.7 (1,15)	<0.001
<i>A. aurea</i> HP	75.43	71.1	2.1	4.2 (1,11)	0.04
<i>A. scott-thomsonii</i> HP	86.87	89.17	1.3	5.7 (1,10)	0.02
<i>A. 'lewis'</i> LP	66.14	83.99	12.3	64.7 (1,13)	<0.0001

### **2.4.3.2 Analyses by treatment and graphical representation to compare sites**

The following figures depict graphically, the effect of each predictive term on its own, by site. The graphs are based on the same datasets from which the above generalised linear models were created, but linear models were fitted to show the trend from each main effect. The graphs do not take into account the variation explained by the other variables in the multivariate models, but they are useful for showing overall trends and making site comparisons. The above GLM's should be referred to for statistical values.

#### *Flower-removal, pollination, and bagging effects on seed set*

Overall, seed set rate was relatively high across all sites with the exception being for bag treatments where seed set rates were consistently lower than all other treatments (Fig. 2.8). Seed production in open-pollinated inflorescences was, in most cases, significantly less than in hand-pollinated inflorescences (Tables 2.6 – 2.9), although the magnitude of this effect was small. Across sites, this pattern was evident at the 0% and 40% flower removal levels, but inflorescences with 80% flower removal were not affected (Figs. 2.8 and 2.9).

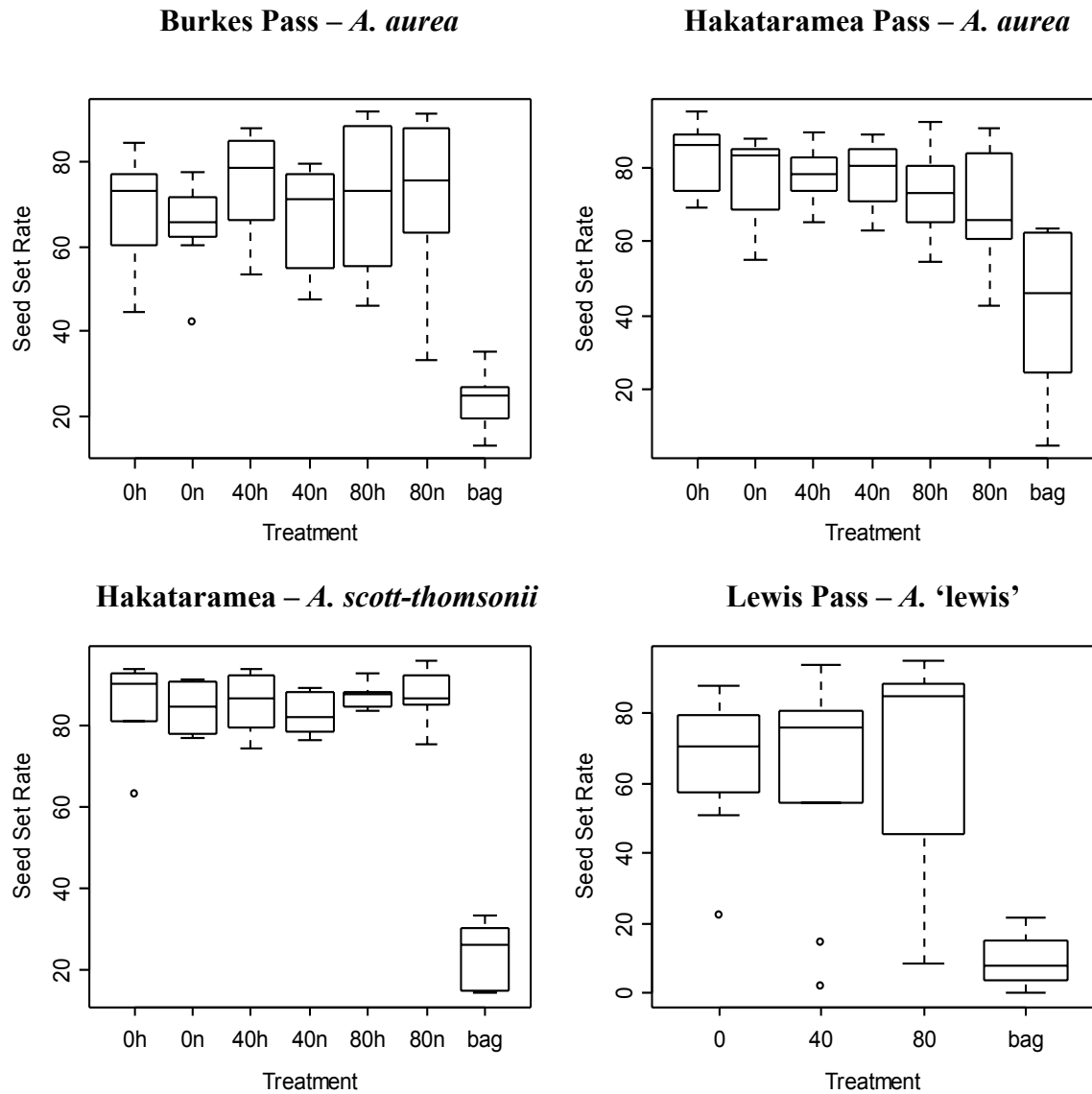


Fig. 2.8 Box and whisker plots for all sites showing the variation in seed set rates for each level of flower removal, pollination and bag treatment. The middle horizontal line within the box shows the median seed set rate. Also shown are the upper and lower quartiles (lines either side of the median), and whiskers encompassing 95% range of the data (designated by dashed lines), with outliers that fall beyond the 95% range/whiskers (dots). See Tables 2.6 to 2.9 for statistical tests.

*Simultaneously testing for the effects of resource and pollen limitation on seed set*

According to the multi-variate site GLM's, for *A. aurea* at Burkes and Hakataramea Passes, the factor *percent flower removal* was a stronger predictor of seed set rate than the continuous variable *flowering length*, while *flowering length* was a significantly stronger predictor for *A. scott-thomsonii* than *percent flower removal* (Tables 2.6, 2.7 and 2.8



respectively). Both terms fundamentally represent the same variable but each was measured in a different way (see Methods section). This suggests that Fig 2.9 is more appropriate for interpreting the relative importance of simultaneous pollen and resource limitation for *A. aurea* while Fig. 2.10 is more appropriate for *A. scott-thomsonii*.

#### *Percent flower removal and hand pollination*

Figure 2.9 depicts the effects of the interactions between hand pollination and flower removal. If both pollen and resources simultaneously limited seed set, hand pollination of plants with 80% flower removal treatment would result in an increase in seed set compared to the control (see Fig. 2.2). Overall, there was no evidence of resource limitation in any of the *Aciphylla* species in this experiment because there was no significant increase in seed set rates for hand pollinated inflorescences with flowers removed (i.e. inflorescences with more resources per flower). This suggests that the biological importance of resource limitation was not particularly relevant within one flowering year when measured using percentage of flowers removed.

To some degree, results for *A. aurea* at Hakataramea Pass conformed to the expected effects that the pollinator attraction hypothesis would have on seed set rates, i.e. inflorescences with more flowers had higher seed set rates (Fig. 2.9). This outcome did not agree with the predictions made by the pollen and resource limitation model (see Fig. 2.2c) as there was no evidence for resource limitation, because inflorescences with 40 or 80% of the flowers removed did not have higher seed set rates relative to the controls. This suggests that other factors, not tested for here, were possibly affecting the ability of inflorescences with flowers removed to set more seed despite theoretically having more resources allocated per flower to develop seeds.

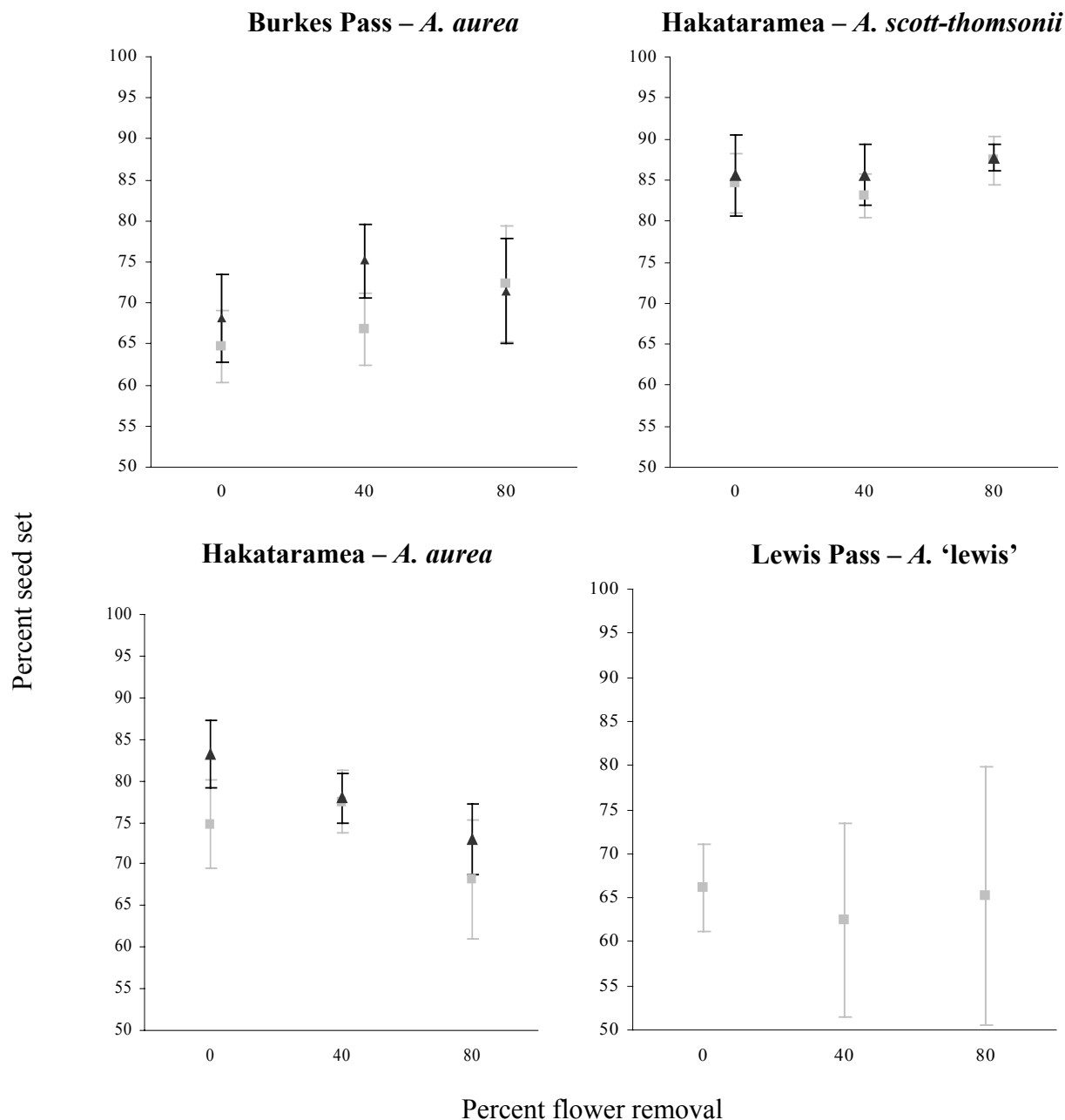


Fig. 2.9 Interaction plots showing the effects that simultaneous pollen and resource limitation following flower removal and hand pollination ( $\pm 1$  SE) had on seed set rates. Dark triangles ( $\blacktriangle$ ) represent hand pollinated inflorescences and light squares ( $\blacksquare$ ) represent naturally pollinated inflorescences. Note: no hand pollination performed at Lewis Pass due to weather. See Tables 2.1 to 2.4 for statistical significance values.

Consistent among most sites however, was evidence for pollen limitation, although in biological terms, this effect was weak. Natural pollen limitation can be inferred when inflorescences with zero percent of flowers removed have higher rates of seed set when supplementary pollinated. This was evident among all three sites where inflorescences

were hand pollinated (Fig. 2.9) but the effects were very small, particularly in *A. scott-thomsonii* (see Table 2.8).

### *Flowering length and hand pollination*

Flowering length (after manipulations) varied between sites and species: *Aciphylla aurea* BP ranged from 5 cm to 90 cm ( $\bar{x} = 43 \text{ cm} \pm 2.9 \text{ SE}$ ,  $n = 62$ ), *A. aurea* HP ranged from 6 cm to 110 cm ( $\bar{x} = 50 \text{ cm} \pm 3.3 \text{ SE}$ ,  $n = 55$ ), *A. scott-thomsonii* ranged from 21 cm to 152 cm ( $\bar{x} = 50 \text{ cm} \pm 5.1 \text{ SE}$ ,  $n = 50$ ) and *A. 'lewis'* LP ranged from 13 cm to 128 cm ( $\bar{x} = 60.6 \text{ cm} \pm 5.6 \text{ SE}$ ,  $n = 31$ ). Overall, the effects of increasing flowering length on seed set rates in *A. aurea* and *A. 'lewis'* were very small with the direction of the relationship differing between sites.

The effects of hand pollination were also small, although as expected, adding pollen did increase seed set compared with open-pollinated inflorescences (Fig. 2.10). For *A. aurea* at Burkes Pass there was a slight negative effect of flowering length on seed set, while in *A. 'lewis'*, an increase in flowering length was important in contributing to higher seed set rates. However, the direction this relationship at Lewis Pass appears to be strengthened by the outliers at the bottom-left of the graph. These were inflorescences that had stunted growth and relatively low seed set compared with the rest of the Lewis Pass population. There was considerable scatter in the *A. aurea* data at Hakataramea with no significant effect of flowering length on seed set rates (Table 2.7). In the multivariate GLM (Table 2.8), flowering length was important in explaining a significant amount of the variation in seed set rate in *A. scott-thomsonii*, however, Fig. 2.10 shows the relationship between flowering length and seed set to be quite weak in contrast. In the multivariate GLM, flowering length is probably having the main effect on seed set, as the table allows for explanation by other variables. In *A. scott-thomsonii* the effects of hand pollination slightly decreased with increasing flowering length, again suggesting alternative factors may have been influencing the ability for inflorescences with flowers removed to set more seed as would be expected (Fig. 2.10).

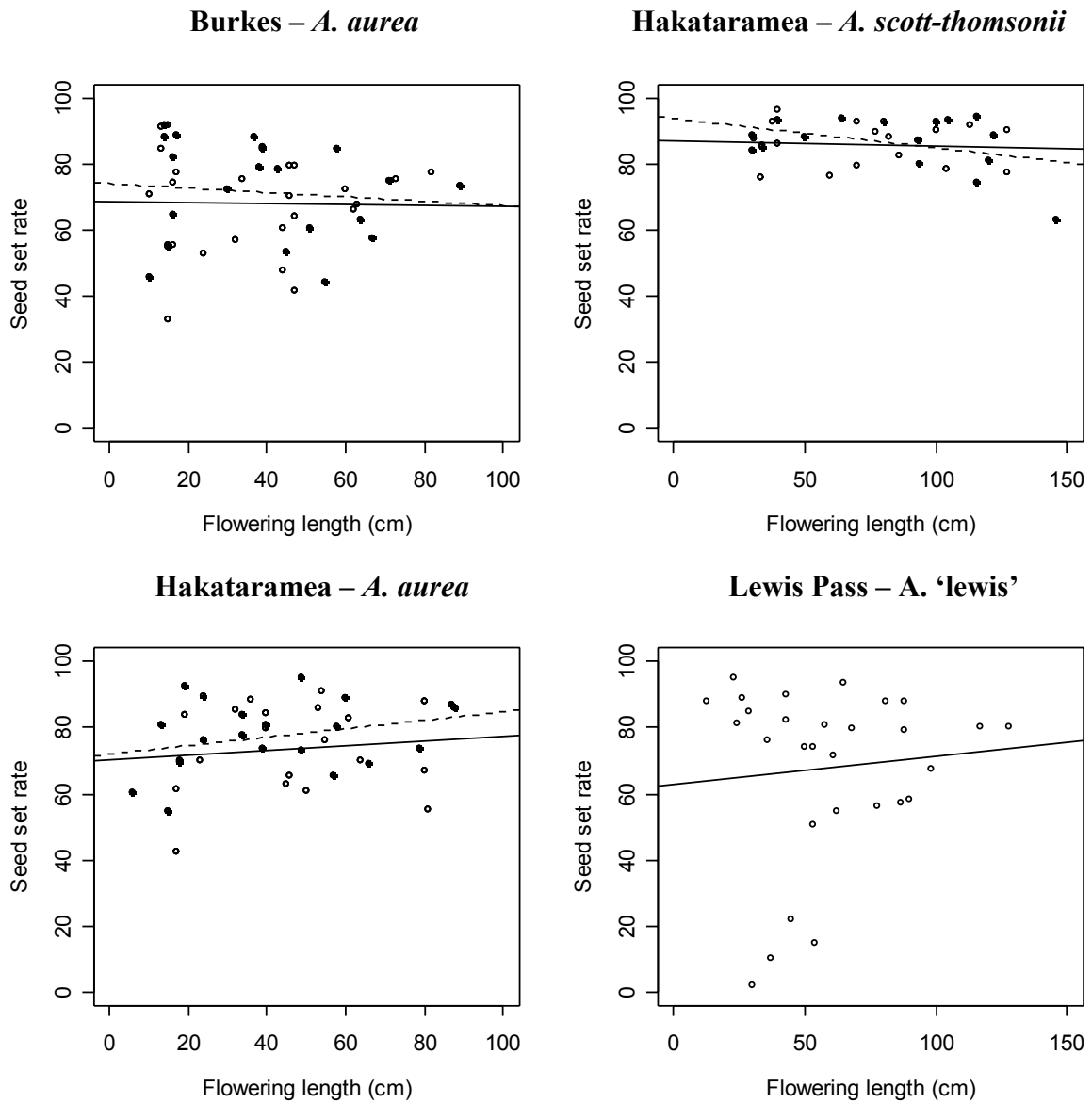


Fig. 2.10 The effect of inflorescence flowering length on seed set rate for hand vs. natural pollination treatment at all sites. Dashed lines (----) and solid symbols represent hand-pollinated inflorescences and unbroken lines (—) lines and hollow symbols represent control inflorescences. Note: x-axis ranges are standardised between the two *A. aurea* sites, and between *A. scott-thomsonii* and *A. 'lewis'* sites. No hand pollination was performed at Lewis Pass due to weather. See Tables 2.6 to 2.9 for statistical significance values.

### *The effect of inflorescence height on seed set*

Mean inflorescence height varied between sites and species: *A. aurea* BP ranged from 80 cm to 155 cm ( $\bar{x} = 122.2 \text{ cm} \pm 2.3 \text{ SE}$ ,  $n = 62$ ), *A. aurea* HP ranged from 62 cm to 190 cm ( $\bar{x} = 141.5 \text{ cm} \pm 3.4 \text{ SE}$ ,  $n = 55$ ), *A. scott-thomsonii* HP ranged from 90 cm to 280 cm ( $\bar{x} = 212.4 \text{ cm} \pm 5.5 \text{ SE}$ ,  $n = 50$ ) and *A. 'lewis'* LP ranged from 74 cm to 164 cm ( $\bar{x} = 128 \text{ cm} \pm 4.7 \text{ SE}$ ,  $n = 31$ ). The relationship between inflorescence height and seed set rate is shown in Figure 2.11. Seed set rate increased with inflorescence height in all sites except *A. aurea* at Hakataramea. This relationship was strongest for *A. aurea* at Burkes Pass and positive, though weakly significant in *A. scott-thomsonii* and *A. 'lewis'* (see Tables 2.6 to 2.9). A small number of outliers are evident in the bottom two graphs of Figure 2.11. There are occasional inflorescences with very low seed set, for unknown reasons. The outlier present at the *A. aurea* site at Hakataramea Pass was an inflorescence subjected to intense caterpillar damage.

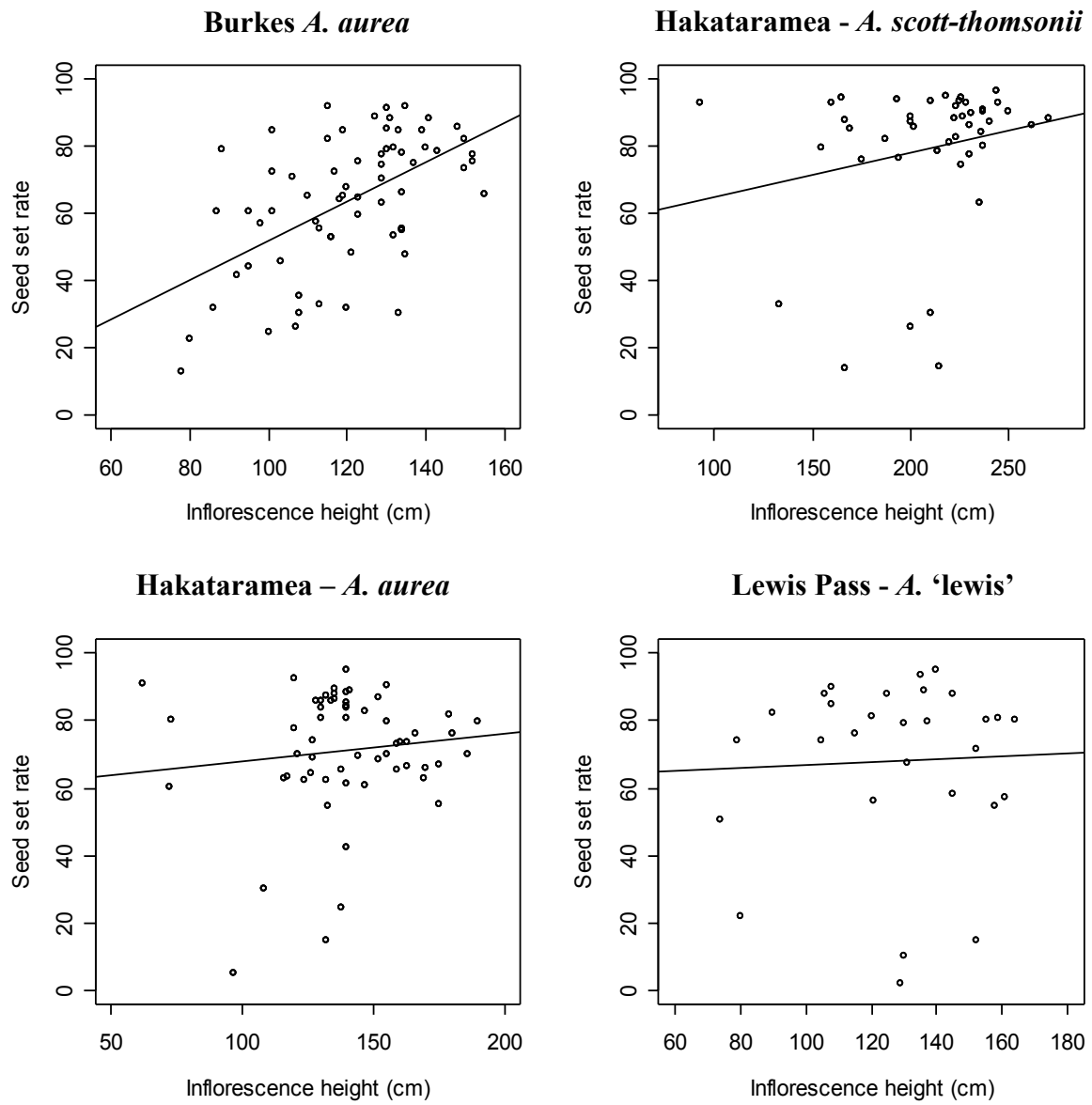


Fig. 2.11 The effect of inflorescence height on seed set rate at all sites. See Tables 2.1 to 2.4 for statistical significance values. Axes are standardised to the same intervals to allow for comparison between sites.

## 2.4 Part (B): Effects of female isolation, hand-pollination and pollen on seed set

### 2.4.4 Analyses by site

#### Burkes Pass site 2 – *Aciphylla aurea*

For the isolated female experiment at Burkes Pass, where local pollen supply was experimentally manipulated by removing male inflorescences, close to 40.5% of the total variation in seed set rate of *A. aurea* could be accounted for by the model (Table 2.11). There was no experimental removal of flowers within an inflorescence at this site or at Hakataramea site 2 (also *A. aurea*). Seed set rates ranged between 18-81% at Burkes Pass site 2. Natural (un-manipulated) flowering length was the most significant variable, explaining almost 35% of the variation in seed set. Also significant was distance to male inflorescences (an indicator of the local pollen abundance). As expected, the further from flowering males, the lower the seed set rate. Hand pollination only slightly increased seed set rates and inflorescence number had a very weak negative effect on seed set.

Table 2.11 Analysis of Deviance Table for isolated female experiment in *A. aurea* at Burkes Pass (site 2). All terms added in to the GLM are non-manipulated variables apart from hand pollination which was a manipulated treatment.

Terms added	Df	F	Resid. Df	Resid.dev	% Dev. expl.	P-value	Slope
NULL			37	1622.18			
Hand pollination	1	19.23	36	1602.95	1.19	<0.001	+
Nearest male	1	75.33	35	1527.62	4.64	<0.001	-
No. inflorescences	1	6.5	34	1521.12	0.40	0.011	-
<b>Flowering length (cm)</b>	1	554.89	33	966.24	34.21	<0.001	+
Inflorescence height (cm)	1	0.43	32	965.81	0.03	0.51	+

## Hakataramea Pass site 2 – *Aciphylla aurea*

The most significant variable affecting seed set rate in *A. aurea* at Hakataramea Pass site 2 was inflorescence number. Plants with more inflorescences achieved higher rates of seed set (Table 2.12) as expected. Inflorescence height significantly decreased seed set rate, while flowering length significantly increased seed set, but only weakly. Hand pollination did not further improve seed set, and distance to nearest flowering male plant had a very weak but significant negative effect. Seed set rates ranged between 49-92% and the model accounted for only 16.4% of the variation observed in seed set rates in *A. aurea* at Hakataramea Pass.

Table 2.12 Analysis of Deviance Table for isolated female experiment in *A. aurea* at Hakataramea Pass (site 2). All terms added in to the GLM are non-manipulated variables apart from hand pollination which was a manipulated treatment.

Terms added	Df	F	Resid. Df	Resid.dev	% Dev. expl.	P-value	Slope
NULL			32	709.6			
Hand pollination	1	0.35	31	709.25	0.05	0.55	+
Nearest male	1	5.7	30	703.55	0.80	0.02	–
<b>No. inflorescences</b>	1	64.01	29	639.55	9.02	<0.001	+
Flowering length (cm)	1	5.38	28	634.17	0.76	0.02	+
Inflorescence height (cm)	1	40.6	27	593.57	5.72	<0.001	–

Distance to males was also highly significant for *A. 'lewis'* at Lewis Pass, with isolated females having lower seed set rates. This result was presented in section 2.4.3.1 (see Table 2.9)

### *Are isolated females pollen limited? The effects of local pollen abundance on seed set*

The Distance-Weighted local Pollen Index (DWPI) value gets bigger with increasing proximity to males with more inflorescences, therefore this represents a more abundant local pollen supply. In *A. aurea* at both Burkes and Hakataramea Passes, DWPI had a



significant positive effect on seed set rates at sites of un-manipulated male and female densities, indicating that proximity to males (and hence synchronous flowering) is important in *A. aurea*, with females not being too isolated from a pollen source to ensure adequate seed set (Fig. 2.12).

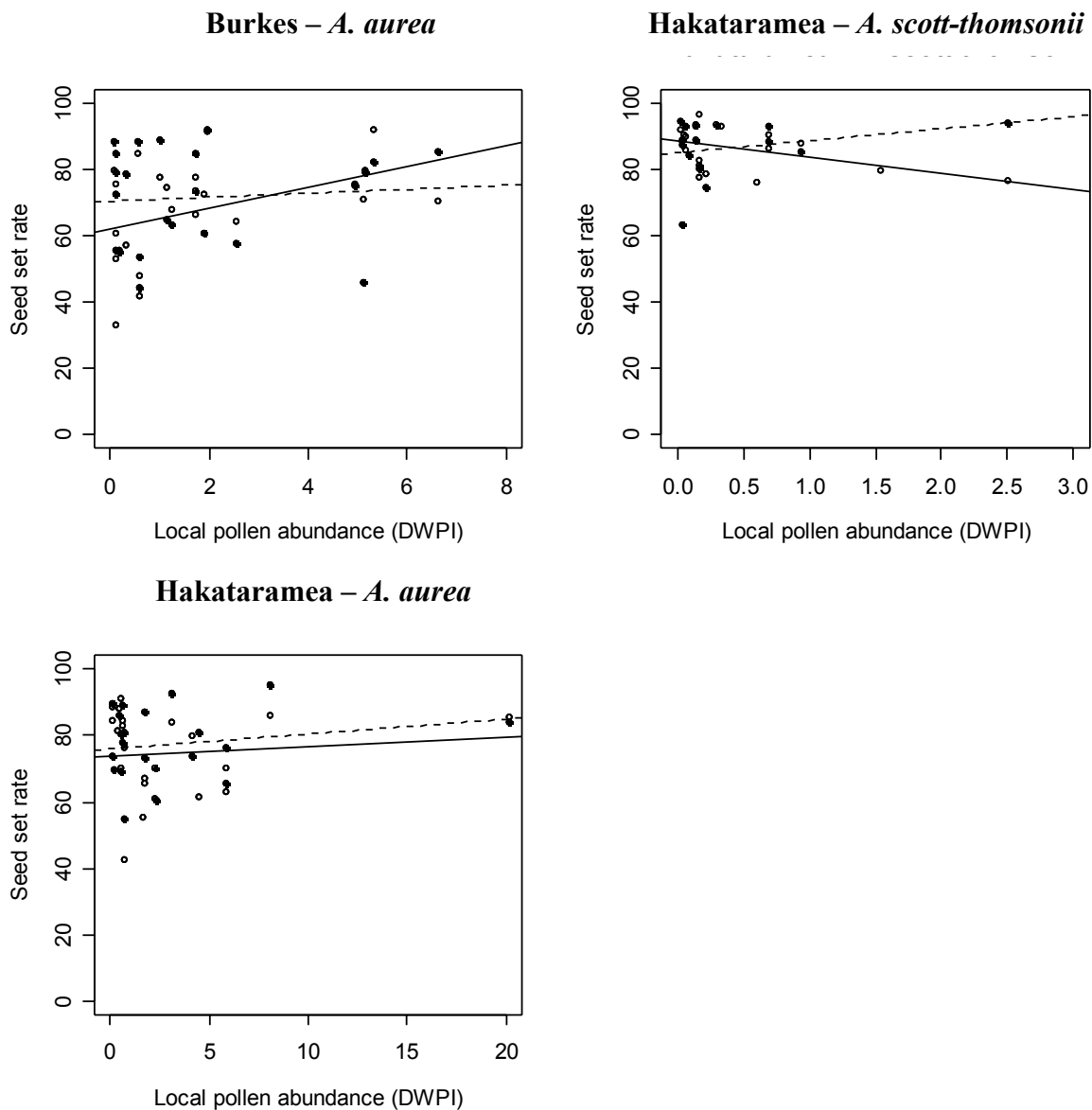


Fig. 2.12 The effect of local pollen abundance (measured with the Distance-Weighted local Pollen Index, or DWPI) on seed set rate at Burkes and Hakataramea Passes. Larger DWPI values indicate higher local pollen availability. Dashed lines (----) and solid symbols represent hand-pollinated inflorescences and unbroken lines (—) lines and hollow symbols represent control inflorescences. See Tables 2.6 to 2.8 for statistical significance values.

This is supported by the manipulated isolated female experiment results for *A. aurea* where seed set rate decreased with increasing distance from the nearest flowering male plants at a larger scale (Fig. 2.13). The same trend was also present for *A. 'lewis'*, with isolated females experiencing lower seed set rates. Local pollen abundance did not improve rates of seed set in *A. scott-thomsonii*.

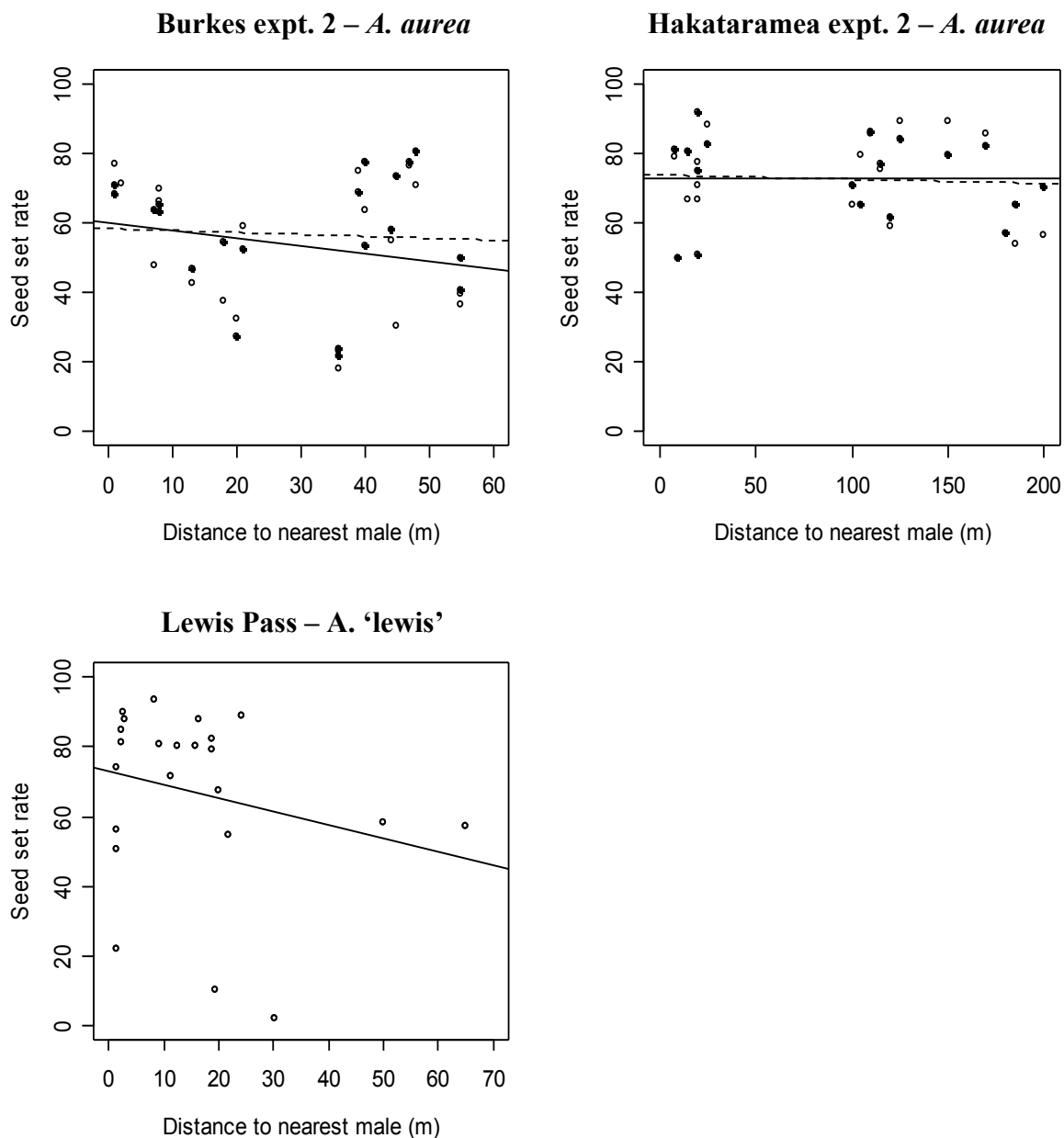


Fig. 2.13 The effects of increasing distance to the nearest male inflorescence on seed set rate at isolated female sites, where male to female flowering ratios were experimentally manipulated. Dashed lines (----) and solid symbols represent hand-pollinated inflorescences and unbroken lines (—) lines and hollow symbols

represent control inflorescences. Note the difference in degree of isolation between the same species at different sites. No hand pollination was performed at Lewis Pass due to weather.

#### **2.4.5 Seed germination**

Although seed germination tests were conducted to determine the germination rates of ‘filled’ seeds and to see whether any of the seeds classified as ‘un-filled’ germinated, there was insufficient time to yield such results. Only two *A. aurea* seeds from Hakataramea and one *A. aurea* seed from Burkes Pass germinated during the 8 month period, all of ‘filled’ seeds. There was no germination of ‘unfilled’ seeds. *Aciphylla* seeds are usually known to remain dormant for a substantial period of time and often germinate over a 3-4 year period (Metcalf, 1995).

#### **2.4.6 Summary**

Overall, there were many statistically significant effects of floral display, hand pollination and local pollen abundance on seed set rates. Many of these effects were however, biologically weak. The directions of the effects were mostly as expected (see Table 2.2), but there were notable exceptions.

Table 2.13 presents the overall direction of the significant relationships between seed set rates and predictive variables compared to the expected direction of the relationships. Most consistently, hand pollination increased seed set rate at all sites. Also as expected, seed set rate nearly always increased with either more local pollen (DWPI) or decreased with increasing distance to male inflorescences, i.e. female plants closer to males improved seed set rate. This suggests that pollinator attraction is important in *A. aurea* and *A. ‘lewis’*, as an increase in local pollen supply lead to higher reproductive success. The only exception to this pattern was *A. scott-thomsonii* at Hakataramea Pass where seed set was not significantly affected by local pollen supply in biological terms.

In most cases, removing flowers negatively affected seed set rates, meaning that inflorescences with more flowers remaining had higher seed set rates with the exception again being *A. scott-thomsonii* (Table 2.13). In sites where flower removal was not performed or where percent flower removal did not explain any variation in seed set rate,

an increase in natural flowering length was significantly associated with an increase in seed set rate, reiterating that floral display size is important for pollinator attraction. Figures 2.9 and 2.10 provided no convincing evidence for resource limitation at any site. Also relatively consistent among sites and in accordance with the expected direction, increasing seed set rates were significantly associated with taller inflorescences. Both *A. aurea* sites at Hakataramea Pass were the exception to this trend however.

Table 2.13 Hypothesised directions of relationships between seed set rate and predictors, and the actual outcomes from the GLM summary tables. 0 represents a non-significant result for a particular term and blank spaces indicate terms that do not apply at certain sites. Lewis Pass was used for both the flower removal and isolated female experiment. This was a non-manipulated isolated female experiment site (i.e. females were naturally far from males). The double signs next to the term ‘percent flower removal’ indicate where there were 3 levels of removal. The first sign represents the direction of the relationship between the control (0%) and 40% removal and the second between 0% and 80% removal. Since percent flower removal and flowering length are representing essentially the same variable (length of inflorescence with flowers on), and only one usually explained significantly more variation than the other in each site model, the stronger effect is highlighted. Site and species abbreviations: BP = Burkes Pass, HP = Hakataramea Pass, LP = Lewis Pass, *A. scott-th* = *A. scott-thomsonii*, and 1 and 2 refer to experiments 1 and 2 (floral display size and isolated female experiments respectively).

Predictive terms	Expected direction of relationship	Flower removal experiment					
					Isolated female experiment		
		<i>A. aurea</i> BP	<i>A. aurea</i> HP	<i>A. scott-th</i> HP	<i>A. lewis</i> LP	<i>A. aurea</i> BP 2	<i>A. aurea</i> HP 2
Hand pollination	+	+	+	+		+	+
DWPI	+	+	+	-			
Nearest male (m)	-				-	-	-
No. of inflorescences	+					-	+
Flower removal (%)	- / - -	- / - -	- / - -	- / - -	- / +		
Flowering length (cm)	0 (or +)	-	0	■	■	■	■
Inflor. height (cm)	+	+	-	+	+	+	-

In summary, pollen provisioning and close proximity to natural pollen sources increased seed set across almost all species and sites. Moreover, seed set rates increased with taller

inflorescences, and overall seed set also increased with greater flowering length in *A. aurea* and *A. 'lewis'* but not in *A. scott-thomsonii*.

## **2.5 DISCUSSION**

In terms of the reproductive strategy employed by *Aciphylla* i.e. irregular mass flowering, overall high seed set rates seem to suggest that this reproductive strategy works well for female plants at least in this high-flowering year. Bagged inflorescences had low seed set rates, confirming that insects are important pollen vectors in *Aciphylla*. In most cases, inflorescences with a larger floral display (either taller in height and/or with more flowers along the rachis) had higher rates of seed set (although the intricacies of these results are discussed below). Hand pollinated inflorescences with fewer flowers did not have a significantly higher seed set rate relative to hand pollinated inflorescences with no flowers removed, therefore the hypothesis that flower removal would increase resources available per flower for seed set was not supported. Hence there was no evidence for resource limitation when defined in this sense. Pollen limitation was evident across all sites because seed set improved with hand pollination, although the increase was generally small.

### **2.5.1 Part (A): Floral display at the plant level**

#### **2.5.1.1 *The importance of insect versus wind as pollen vectors in Aciphylla***

Results provide strong evidence that *Aciphylla* species are pollinator-dependant as the addition of insect-excluding mesh bags significantly reduced seed set rates to an average of 23%. This is an important finding, as it was unclear in the literature what the relative importance of wind versus insects as pollinator was (Dawson, 1971; Webb, 1986; Mitchell *et al.*, 1999). In *A. aurea* at Hakataramea Pass however, mean seed set inside bags was over 37%. This was unexpectedly high but a possible explanation for high bag seed set rates at this site is that because this was the last site to be set up, some ovules may have already been fertilised (due to the more developed stage of some of the flowers) before the bags were put on. On the other hand, Lewis Pass was the first site where bags were set up, and this is reflected by the very low levels of seed set that occurred (ca. 9%). The reasons for the 23% of seeds that were made inside bags at the other sites are unclear but there are a number of likely explanations. Plants could potentially make seeds inside bags due to

either: wind pollination (because mesh bags almost certainly excluded insects but possibly not wind-blown pollen); apomixis (reproduction by seeds formed without sexual fusion); or by putting bags on after some flowers were receptive and ovules had already been fertilised. Note that I checked that leaky dioecy (some male flowers inside the female bags) was not occurring. These possibilities should be explored in more detail to determine whether any wind pollination or apomixis is occurring, as this could have important consequences for reproductive assurance of *Aciphylla* species in pollinator-limited environments.

#### ***2.5.1.2 The importance of plant-level floral display (and how it is measured) on reproductive success***

According to the pollinator attraction hypothesis, increased floral display size could enhance female fitness as more offspring could be produced (Davila & Wardle, 2002; Grindeland *et al.*, 2005). If resources are not limiting, female reproductive success can be increased through greater allocation to floral display (Ehlers *et al.*, 2002) although there may be a threshold size for females over which increases in size do not increase seed set due to the costs of offspring provisioning (Vaughton & Ramsey, 1998). This study showed that overall, decreasing the size of the floral display in terms of reducing flowering length, decreased seed set. This was true for all species and sites apart from *A. scott-thomsonii* at Hakataramea Pass, where seed set was significantly increased with a decrease in flowering length. Before discussing the importance of floral display for reproductive success in *Aciphylla*, we must first consider the ways in which we define and measure floral display.

Results varied slightly when floral display was measured using different methods. For *A. aurea*, the factor *percent flower removal* was a better measure of variation in the amount of flowers along the stem than the continuous variable *flowering length*, as it explained significantly more variation in seed set. On the other hand, variation in seed set for *A. scott-thomsonii* and *A. 'lewis'* was explained significantly by *flowering length* and not *percent flower removal*. In some cases, *inflorescence height* was the best predictor of seed set, while unexpectedly, *number of inflorescences* on a plant was rarely a significant predictor of seed set, hence its exclusion from the site models for experiment 1 (flower removal). This study therefore emphasises the importance of the methodology and statistical analyses

employed in this type of research, where results and outcomes may vary depending on how the variable of interest is defined and approached.

With the exception of *A. scott-thomsonii*, these results suggest that decreasing floral display (shorter inflorescences and smaller flowering length) reduces attractiveness to insect pollinators (tested in Chapter 3). A greater floral display has been associated with increasing pollinator visits in both hermaphroditic and dioecious plants (Andersson, 1996; Donnelly *et al.*, 1998) but for self-incompatible and dioecious plants, this is particularly important given their obligate requirement for outcross pollen. *Aciphylla* being dioecious requires out-crossing and this appears to be achieved effectively by having a large floral display. In *A. aurea* and *A. 'lewis'*, taller inflorescences with more flowers experienced greater seed set during this high-flowering year. The effects of this were further amplified when the local natural pollen abundance was greater.

*Aciphylla scott-thomsonii* on the other hand was more successful when inflorescences were taller but with fewer flowers. This provides an ideal explanation for the common occurrence of inflorescences within populations that have extraordinarily tall inflorescences while often only having a low proportion of the inflorescence bearing flowers (see Fig. 2.1). This appears to be a clever strategy on the plant's behalf because while being taller was almost always associated with having higher seed set rates, being taller *and* having more flowers was not. Therefore the may plant effectively get away with advertising for pollinators by simply being taller, avoiding the costs of having to put un-necessary (expensive) effort in to ovule production. The problem here however, is that accessory costs per flower (or seed) could potentially become very high for stalks with few flowers, and it may rapidly become uneconomic if flowering length is reduced and the stalk is large. This is probably overcome by the fact that the stalk is largely made of carbon, while the more expensive reproductive structures (i.e. seeds) require more nitrogen (Hogan *et al.*, 1998). Furthermore, inflorescence structures on *Aciphylla*, including carpels (in females), spiny bracts, and stalks (males and females) can contribute to their own carbon and energy costs by photosynthesising (Hogan *et al.*, 1998). Therefore for species with extremely large inflorescences such as *A. scott-thomsonii* and *A. glaucescens*, because seed set is typically very high (see Hogan *et al.*, 1998)), this natural tendency for plants to produce tall stalks with fewer flowers is probably an ideal adaptation for pollinator attraction and is an optimal trade-off between display size and fecundity.

These results additionally suggest that there are negative effects on seed set rates in *A. scott-thomsonii* on some inflorescences with excess flowers (i.e. there was no further increase in seed set beyond a certain flowering length, even when hand pollinated). Alternative reasons for having a high flower-to-fruiting ratio exist and will be discussed in Chapter 5.

Resource availability, pollen availability, predation, adverse climatic conditions and genetic conditions may all act to constrain plant fertility (Charlesworth, 1989). Of these constraints, resource availability and pollen quantity and quality are generally considered the principal determinants of among-plant variation in female reproductive success (Haig & Westoby, 1988; Pias & Guitian, 2006). Some authors contend that pollen-limitation is common (Bierzychudek, 1981; Ackerman & Montalvo, 1990) while others contend that reproduction in most species is resource limited (Howe & Westley, 1986). Haig and Westoby (1988) argued that if floral display size is an optimal investment between pollinator attraction and fecundity, then a plant should be simultaneously limited by both resources and pollen. Because seed set rates in treatments with flowers removed (i.e. increased resources) was not greater than seed set in controls when supplemental pollen was added, there was no support found for simultaneous resource and pollen limitation in *Aciphylla aurea*, *A. 'lewis'* or *A. scott-thomsonii*. However, seed production in open-pollinated inflorescences was in most cases, significantly less than in hand-pollinated inflorescences, suggesting that pollinators are failing to supply maximal pollination services. By providing supplementary pollen, mean seed set rate was (statistically) significantly higher, although in most cases, this was probably not biologically as significant, (i.e. mean seed set rate for hand-pollinated inflorescences was never more than 10% greater than the natural rate in open-pollinated inflorescences). This will be discussed further in Chapter 5.

### **2.5.2 Part (B): Population level flowering effort**

At the population level, males were removed from a large area, leaving female inflorescences at varying distances from the nearest pollen source. This tested whether female plants were sufficiently attractive to encourage visitors from further distances and



effectively aimed to understand the effects on reproductive success of individuals flowering out of synchrony during a low-flowering ('non-mast') year.

Isolation, measured by the distance to the nearest flowering conspecific male, was a good predictor of pollen limitation at isolated female experiment sites, because seed set of individual female plants decreased with isolation. In the site GLM for the isolated female experiment in *A. aurea* at Burkes Pass, *nearest male* was the second most significant predictor of seed set rate after while natural *flowering length*. Hand pollination also significantly increased the seed set rate of isolated females at this site.

At Hakataramea Pass, inflorescence number per plant was a significant predictor of seed set rate for *A. aurea*. Female isolation was also important here but much less so than at Burkes Pass and surprisingly, hand pollination did not significantly increase seed set. It is likely that the effects of inflorescence number were significant at this site because of the relatively low flowering densities in that particular area, suggesting that floral display at the plant-level may play a more important role in pollinator attraction when population densities are lower. Also at Hakataramea Pass, overall seed set rates for *A. aurea* were higher than at Burkes Pass. Because seed set rates were extremely high in *A. scott-thomsonii* at Hakataramea, this suggests they were highly attractive to insects. Perhaps by flowering in synchrony with a highly successful con-generic, *A. aurea* is reaping the benefits from being close-by. Alternatively, these differences in fecundity between the two nearby sites could be attributed to variation in site productivity (but this was not tested here).

At Lewis Pass, where there was natural female isolation due to the sparse nature of the population, seed set also significantly decreased with isolation. This was the second-most important variable after flowering length, in predicting seed set rate. Overall, taller inflorescences with more flowers were more successful, and there were increased benefits to this when females were isolated from males.

These results show that the negative effects of isolation and asynchronous flowering are evident, but remain small in this study. The scale of the experimental design was most likely too modest to reflect the magnitude of the outcomes likely to occur during natural low-flowering years. High-flowering densities of *Aciphylla* remaining outside the

experimental area possibly also influenced the overall outcome of these experiments. Natural low-flowering years of course vary in their degree of flowering density, female isolation and sex-ratio, but the overall scale would generally be larger. The outcomes of this study however, are indicative of what would occur during a natural low-flowering year with isolated individuals occurring naturally at much greater distances from conspecifics, and provide a helpful insight into the negative effects on individuals if they flowered during a 'non-mast' year. The overall conclusion is that the insect pollinators of *Aciphylla* appear to move readily over the scales used in this experiment (up to several hundred metres), albeit with some decrease in presumed pollen delivery rate (equivalent to rates of seed set), tested in Chapter 3.

Monitoring the effects of pollen limitation on naturally low-flowering years remains to be tested but would provide a better insight into whether this is an important selection pressure for masting in *Aciphylla* species. Ashman *et al.* (2004) suggest that a larger response to experimental pollen supplementation should occur in populations or species that experience greater variance in pollen acquisition or have smaller costs, therefore in masting species like *Aciphylla*, this should be expected between years.

### **2.5.3 Conclusions**

#### ***The overall importance of floral display and densities for female reproductive success***

There is much evidence for floral display size playing an important role in the reproductive success of many plant species worldwide, (e.g. Andersson, 1996; Kawarasaki & Hori, 1999). Most studies have considered the importance of display size at the plant level, but there are only a small number that test for the relative importance of floral display at both the plant level and on a larger scale at the population and community levels (e.g. Kato & Hiura, 1990; Bosch & Waser, 1999; Grindeland *et al.*, 2005). To date, there have been almost no studies showing that reproductive success can be achieved through economies of scale in masting, insect pollinated, dioecious species (but see Forsyth, 2003). Therefore this research provides one of the first insights that this paradoxical breeding system works effectively by testing whether both plant and population level factors influence reproductive success in *Aciphylla*.

Haig and Westoby's (1988) argument that plants should evolve to optimally allocate resources towards pollinator attraction and seed maturation seems to hold true to a certain degree for the *Aciphylla* populations studied here. The way they appear to have achieved this is through masting. A number of insect-pollinated plant species with large floral displays suffer pollen limitation (e.g. Wagenius, 2005) but masting may be a strategy that has evolved to mitigate the effects of this. During this high-flowering year, there were no effects of resource limitation seen, while the effects of pollen limitation were small. (Brookes & Jesson, 2006) found similar patterns for *Aciphylla squarrosa*, another large-leaved montane relative. At the same time, results from the isolated female experiment suggested that when female plants are flowering out of synchrony or are isolated, the effects on reproductive success are negative. If reproductive effort is concentrated into certain years, at the expense of sacrificing reproductive opportunities during others, there should theoretically be plentiful resources allocated to pollinator attraction, pollen dissemination (in males) and offspring provisioning (in females) that particular year, and the effects of resource and/or pollen limitation should be negligible.

It was anticipated that in this study, space would be a good analogue for time, in order to observe fitness gains to individuals in a 'mast' (high flowering density) situation, compared with a 'non-mast' situation (asynchronous/isolated flowering female plants), but the overall effects were generally very small. For example, at Burkes Pass, for the *A. aurea* isolated female experiment, the predicted seed set decreased from 59% to 48% over the range of experimentally induced low-flowering distances (to the nearest male) of 2 to 55 metres. When this is compared to the size of the huge benefits from masting known to occur in other systems, through predator satiation effects (e.g. seed set in *Chionochloa pallens* at Mount Hutt increased from 4% to 94 % during mast years (Kelly & Sullivan, 1997)), or wind pollination (e.g. in *Nothofagus solandri*, seed set is known to increase from <10% to 60% during mast years (Kelly *et al.*, 2001)), it is harder to see the observed increases in seed set through pollination economies of scale as being large enough to offset the potential costs of masting in the *Aciphylla* populations studied here. However, one limitation is that the range of variation in seed set could only be measured over the range of naturally produced inflorescence heights (as inflorescence height could not be manipulated). I predict that we might see more pronounced negative effects in these larger montane *Aciphylla* species if inflorescences were significantly smaller (through decreased pollinator

attraction), or more isolated (through decreased pollinator effectiveness) in space and time, than the experimental ranges in this study.

### **Chapter 3 - The effects of floral display and population structure on insect visitation rates and how this relates to reproductive success**

#### **3.1 INTRODUCTION**

The reproductive biology of the New Zealand flora has many unusual features: flowers are often small, structurally simple and not showy. Separate sexes (dioecy and monoecy), fleshy fruit and mast seeding are unusually common (Webb & Kelly, 1993), and pollination systems have been characterised as unspecialised, imprecise and entomophilous (Newstrom & Robertson, 2005). This leads to combinations of reproductive strategies that are unusual and uncommon worldwide (Newstrom & Robertson, 2005) such as *Aciphylla*, which has a mast seeding (Webb & Kelly, 1993), largely dioecious and sexually dimorphic (Webb *et al.*, 1999), insect-pollinated reproductive system. This is an unusual and interesting system that on the face seems it would not work well. For a masting species to be dioecious imposes important constraints on minimum levels of synchrony among the sexes. A masting species also provides a clumping of rewards in space and time with large flower/seed crops some years but little or none during other years. This presents an unpredictable and patchy food supply for the insect pollinators that rely on *Aciphylla* as a resource.

For a masting plant population to be successful, individuals need to attain sufficiently high seed set rates during a mast year. To achieve this requires a pollination system that is resistant to fluctuations in flower production, and this is usually achieved by wind pollination (Kelly, 1994). Insect pollinators must be either: a) generalists that are not solely reliant on *Aciphylla* for food each year and can forage on a range of other plant species but are still attracted to *Aciphylla* when it flowers; or b) specialised insect pollinator syndromes that have an emergence pattern that coincides with the flowering of *Aciphylla*. Little is known about the pollinator guild of *Aciphylla* and the degree of specialist or generalist plant-pollinator interactions. Insect visitation rates can be correlated with pollination success (Proctor *et al.*, 1996). If a large floral display size gives a disproportionate increase

in pollinator attraction, occasional large flowering years will lead to a higher average visitation rate (and pollination success), than regular small ones.

Masting species that can attract more insects during a high-flowering year (e.g. through increasing the size of floral display) would theoretically gain a selective advantage in doing so through economies of scale. To better understand the causes and consequences of mast seeding in *Aciphylla*, we need to know more about the pollination system and the importance (if any) of having a large floral display.

### 3.1.1 Aims

Overall, this chapter investigates the relationship among insect visitation rates, floral display size and seed set rates. It endeavours to elucidate the pollination system in large montane *Aciphylla* species. Specific questions are:

- What types of insects are visiting *Aciphylla* and are they potential pollinators?
- Are insects pollinators attracted to larger floral displays? Is this correlated with any variation in seed set rate in response to floral display size?
- Does the removal of floral bracts affect visitation rates?
- How does plant-level floral display size (i.e. number of inflorescences per plant) affect visitation rates?
- Are insects attracted to isolated female inflorescences? Is insect visitation rate higher when males and females are flowering in closer proximity to each other, and is this related to plant reproductive success?
- Does insect visitation rate differ among male and female inflorescences?
- How do results from the above questions relate to seed set rate (reproductive success) in *Aciphylla*? (Chapter 2).

## **3.2 METHODS**

### **3.2.1 Collecting insects**

Insects were collected from male and female *Aciphylla* plants at all sites in order to obtain information about what some of the insect visitors and potential pollinators to *Aciphylla* were. This was carried out during peak flowering time (December and January 2004/2005) and collections were made on two to four days throughout this period. Insects collected were those seen either on male or female flowers, yet it was difficult to determine *a priori* whether these insects were pollinators or not. Insects observed on flowers were caught either by direct trapping into glass vials or with a pooter catcher. Samples were preserved in 70% ethanol solution for subsequent photography, labelled and kept separate by site. Identification to the lowest possible taxonomic level was carried out.

### **3.2.2 Insect visitation surveys**

Insect observations were conducted on all female plants within the floral display size, bract removal, and female ‘isolation’ experiments performed in Chapter 2 (see Chapter 2 for full details of the experimental design). To record insect visits to inflorescences, visitors that were potential pollinators were grouped into 6 taxonomic categories that could be distinguished visually from a distance: big flies, hoverflies, small flies, beetles, weevils, and moths. This was done for practicality and ease of analysis, but does not necessarily depict accurately the relative roles or efficiencies of the different pollinator groups. Other visitors including ladybirds and spiders were also recorded. For all experiments at every site (excluding Lewis Pass due to weather), insect visits to each inflorescence were recorded.

Insect visitation is very difficult to measure on inflorescences with large numbers of small flowers such as Apiaceae (Dafni, 1992). For the purposes of this study, an attractiveness index (see Dafni, 1992) was employed to measure insect visitation to all the treatment

inflorescences specified in Chapter 2. For each inflorescence, an ‘instantaneous’ insect count was used to measure the degree of ‘attractiveness’ to insects. This involved an observer and a recorder moving between inflorescences, stopping near each only for long enough at each to record all the insects within each of the six taxonomic categories on that inflorescence at that particular point in time. Because of the large sample size of plants in each experiment, and the amount of time taken to move between each one, this method was preferred above measures of insect visitation rates such as ten-minute counts. Start and finish times of the instantaneous visitation counts were noted, as well as the date, weather conditions and any other relevant information such as other plants flowering at the time. Instantaneous insect visitation observations were performed 3-4 times at each site over a 3 week period during peak flowering season in late December/early January 2004/2005.

### **3.2.3 Insect visitation to male inflorescences**

Male reproductive success can be difficult to measure, especially for plants such as *Aciphylla* where each inflorescence can have tens of thousands of flowers. At Burkes and Hakataramea Pass for *A. aurea*, and Hakataramea Pass for *A. scott-thomsonii*, an average of 8 male inflorescences were randomly selected and tagged. Using the instantaneous insect visitation method outlined above, 3-4 observations were recorded for the male inflorescences over a 3 week period during peak flowering. These data were compared to control treatment female inflorescences within the same sites to see whether insect visits differed between male and female inflorescences.

### **3.2.4 Insect visitation rates in response to floral display**

This insect visitation information was used to test whether differences in pollinator attraction (i.e. number of insect visits per inflorescence per instantaneous count) were related to variation in floral display size and flowering densities in *Aciphylla*. As in Chapter 2, the effect of floral display was assessed at both the individual and the population level. Specifically, the following comparisons were made:

- 1) Total insect visitation rate per inflorescence for female inflorescences vs. flowering length, where the number of flowers was manipulated in three treatment levels (0, 40 and 80 % removal).
- 2) Relative insect visitation rate (adjusted to reflect insects per flower) for female inflorescences vs. flowering length, where the number of flowers was manipulated in three treatment levels (0, 40 and 80 % removal).
- 3) Insect visitation rate vs. flowering length (as a continuous variable) for female inflorescences, including both un-manipulated plants and the manipulated plants used in (1).
- 4) Insect visitation rate to female inflorescences vs. height of inflorescence, using all plants as in (3).
- 5) Insect visitation rates to female inflorescences in response to bract removal using a paired design (one inflorescences with all bracts removed and one un-manipulated inflorescence per plant).
- 6) Insect visitation rates to female inflorescences in response to the total number of inflorescences on a plant (as a measure of plant level floral display), using all plants as in 3.
- 7) Insect visitation rate to female inflorescences as a function of the distance to nearest males (a measure of population level floral display) using un-manipulated areas.
- 8) Insect visitation rate to female inflorescences as a function of the distance to nearest males (a measure of population level floral display) using areas where male spikes were removed to create low male density areas.
- 9) Differences in insect visitation rates to male vs. female inflorescences using unmanipulated male and female plants



### 3.2.5 Statistical Analyses

The insect count data were analysed using single-factor Generalised Linear Models (GLMs) with a poisson error distribution and a log link function (in R version 1.5.1). For comparison (2) above, relative insect visitation rates were calculated by multiplying the number of insect visits to 0, 40 and 80 percent flower removal by 2, 5 and 10 respectively. These values were chosen to retain integer values for poisson analysis. For all comparisons, a repeated measures design was used in order to avoid pseudo-replication on the inflorescence level and from the multiple observation dates. This required F and P values to be recalculated using inflorescence deviance as the residual deviance, in order to obtain the appropriate degrees of freedom. To calculate percentage deviance explained (as an indicator of the biological significance, equivalent to r-squared) the predictor deviance was divided by the null residual deviance then multiplied by 100.

Local availability of male inflorescences (measured using the Distance Weighted local Pollen Index (DWPI), see Chapter 2 methods) was tested as a predictor for variation in insect visitation rates to females in response to female plant-distance to flowering male plants. Secondly, data from isolated female manipulative experiments were also tested to see how insect visitation rates varied when female inflorescences were relatively far (isolated) from the closest flowering males. This was tested using distance to nearest flowering male. Inflorescence effect and insect visitation round were also considered in the model therefore, a nested design was employed in this analysis.

Each of the variables were tested using their own individual GLMs (as opposed to large models including multiple predictive terms in Chapter 2) due to the necessity for the nested repeated measures analyses to account for variation between each insect observation round.

## 3.3 RESULTS

### 3.3.1 What insects are visiting *Aciphylla* inflorescences?

A large and diverse insect fauna was associated with *Aciphylla* during flowering. A summary of all taxa collected from inflorescences during December 2004 and January

2005 (peak flowering time) is presented in Appendix 2. At all three sites, there were seven orders of insects found including many species of fly (Diptera) from thirteen families, several moth (Lepidoptera) species from at least three families, at least two species of crane fly (Plecoptera) from two families, native solitary bee and wasp species (Hymenoptera), a number of Hemipterans and a large number of beetles (Coleoptera) from at least eleven families. Many of these insect taxa could potentially act as pollen vectors in *Aciphylla*.

Other insects of significant interest (although possibly not contributing to pollination due to different life history strategies and morphological structures) were shield bugs (Heteroptera), at least four aphid species (Homoptera), which are sap-suckers and potentially seed predators, and four ladybird (Coccinellid) species.

Two giant weevil species (Coleoptera: Curculionidae) were also identified, one being the endangered speargrass weevil *Lyperobius huttoni* and the other being an entomologically significant rediscovery of the “extinct” (category ‘X’ - IUCN endangered species list) Canterbury knobbed weevil *Hadramphus tuberculatus*. This was the first sighting of this species since 1922.

### **3.3.2 Are insects attracted to larger floral displays?**

#### ***3.3.2.1 Insect visitation rates and flower removal manipulation***

In *A. aurea* at Burkes Pass and *A. scott-thomsonii*, insect visitation rate varied significantly between flower removal treatments (Table 3.1). Un-manipulated inflorescences received at least 12 times more visitors than inflorescences with fewer flowers in *A. aurea* at Burkes Pass, with flower removal explaining over 36% of the variation observed in insect visitation, suggesting there was a large biological effect. Although there was significant variation between the 3 levels of flower removal in *A. scott-thomsonii* at Hakataramea, the inflorescences with 40% of flowers removed had unexpectedly more insect visitors than those with no flowers removed. Experimental manipulation of floral display size (0, 40 & 80% removal) had no significant effect on insect visitor numbers in *A. aurea* at

Hakataramea Pass. In all three sites, the 80% flower removal treatment consistently received the least number of insect visitors.

Inflorescences of *A. scott-thomsonii* consistently had at least five times as many insect visitors as *A. aurea*, both on average and in total (Table 3.1). Inflorescences of *A. scott-thomsonii* are much larger than those of *A. aurea* (see Chapter 2) and consequently require more visits to fertilise the greater number of flowers, therefore it makes sense that this species receives more insect visits.



Table 3.1 Mean insect visitation to female *Aciphylla* inflorescences with 0, 40 and 80 per cent of flowers removed (in units of insects per inflorescence per instantaneous count). The total number of insects seen in each category over the total sampling period is given in parentheses. Statistical values given are for flower removal effects on insect visitor numbers from poisson GLMs with flower removal as a factor (percent variance explained, F statistic, (d.f.), and *P* value). (Abbreviations: BP = Burkes Pass, HP = Hakataramea Pass and *A. scott-th.* refers to *A. scott-thomsonii*).

Study species and site	Percent Flower Removal			% Deviance explained	F (d.f.)	P-value
	0%	40%	80%			
<i>A. aurea</i> BP	0.64 (27)	0.04 (2)	0.02 (1)	36.73	27.77 (2,43)	<0.0001
<i>A. aurea</i> HP	0.41 (16)	0.42 (19)	0.12 (5)	7.107	2.436 (2,39)	0.1007
<i>A. scott-th.</i> HP	5.97 (167)	9.58 (230)	3 (72)	18.7	10.985 (2,35)	0.0002

Even after the data were adjusted for flower removal to obtain relative visitation rates (~visits per flower), mean seed set still remained significantly different among the three levels of flower removal for *A. aurea* at Burkes Pass and *A. scott-thomsonii* at Hakataramea Pass (Table 3.2). Insect visits to *A. aurea* at Hakataramea Pass remained non-significant between flower removal categories. For *A. aurea* at Burkes Pass, there was still a significant positive relationship, with more insect visitors to inflorescences with the most flowers, however percent deviance explained decreased, suggesting the biological significance of this was slightly weaker. For *A. scott-thomsonii* differences became more significant and percent deviance explained increased relative to un-adjusted values (Table 3.1) to over 25%. There was an interesting variation in the proportional insect visitation

values in response to flower removal treatments for *A. scott-thomsonii* and *A. aurea* at Hakataramea pass, with the highest visitor numbers being observed on the 40% removal treatment. This trend was not observed for *A. aurea* at Burkes Pass.

Table 3.2 Mean relative insect visitation to female *Aciphylla* inflorescences with 0, 40 and 80 per cent of flowers removed, (scaled for the percentage of flowers remaining on the inflorescence). For raw insect counts see Table 3.1. Statistical values given are for relative flower removal effects on insect visitor numbers from poisson GLMs with flower removal as a factor (percent variance explained, F statistic, (d.f.), and *P* value). (Abbreviations: BP = Burkes Pass, HP = Hakataramea Pass and *A. scott-th.* refers to *A. scott-thomsonii*).

Study species and sites	Percent Flower Removal			% Deviance explained	F (d.f.)	P-value
	0%	40%	80%			
<i>A. aurea</i> BP	1.29	0.21	0.21	17.72	8.55 (2,43)	<b>0.00075</b>
<i>A. aurea</i> HP	0.82	2.11	1.19	4.08	1.30 (2,39)	0.28
<i>A. scott-th.</i> HP	11.93	47.92	30	25.18	16.66 (2,35)	<b>0.00001</b>

### 3.3.2.2 Insect visitation and inflorescence height

Inflorescence height significantly influenced the number of insect visitors to *A. aurea* at Burkes Pass and to *A. scott-thomsonii* at Hakataramea Pass (Table 3.3). The effect was not significant for *A. aurea* at Hakataramea. Taller inflorescences tended to attract higher visitor numbers to both species at all sites, although this was more distinct in *A. scott-thomsonii* (Fig. 3.1).

Table 3.3 Insect visitor numbers to female *Aciphylla* inflorescences in response to variation in inflorescence height (cm). Statistical values given are for the effects of inflorescence height on insect visitor numbers from single-factor poisson regressions with inflorescence height as the sole explanatory variable (percent deviance explained, F statistic, (d.f.), and *P* value). Slope and intercept values from the model are given in log<sub>e</sub> units, with the fitted model being in the form of insect numbers = e<sup>(slope\*inflorescence height + intercept)</sup>. (Abbreviations: BP = Burkes Pass, HP = Hakataramea Pass and *A. scott-th.* refers to *A. scott-thomsonii*).

Study species and sites	Mean inflorescence height ± 1 standard dev.	Slope		% Deviance explained	F (df)	<i>P</i> -value
		Slope	Intercept			
<i>A. aurea</i> BP	120.52 +/- 18.6	0.029	-4.85	6.83	8.46 (1,64)	<b>0.005</b>
<i>A. aurea</i> HP	139.56 +/- 25.52	0.012	-2.92	2.24	2.14 (1,57)	0.149
<i>A. scott-th.</i> HP	209.79 +/- 38.63	0.010	-0.50	10.34	11.31 (1,51)	<b>0.001</b>

*Aciphylla scott-thomsonii* overall received higher visitor numbers than *A. aurea*. Because the effect inflorescence height played on visitor numbers in *A. scott-thomsonii* and *A. aurea* at Burkes Pass was highly significant, it is possible to estimate the mean number of insects likely be present on an inflorescence of a particular height during any particular instantaneous observation (from the equations in Table 3.3). For the mean *A. scott-thomsonii* inflorescence height of 209.8 cm, an average of 4.9 visitors could be expected per instantaneous observation. For an inflorescence of 248.4 cm (+ 1 standard deviation of the mean), we would expect 7.2 insects, and a shorter inflorescence of 171.2 cm (- 1 std. dev.), around 3.4 insect visitors. For the mean *A. aurea* (Burkes Pass) inflorescence height of 120.5 cm, an average of 0.26 visitors could be expected per instantaneous observation. For an inflorescence of 139.1 cm (+ 1 std. dev. of the mean), we would expect 0.44 insects, and a shorter inflorescence of 101.9 cm (- 1 std. dev.), around 0.15 insect visitors.

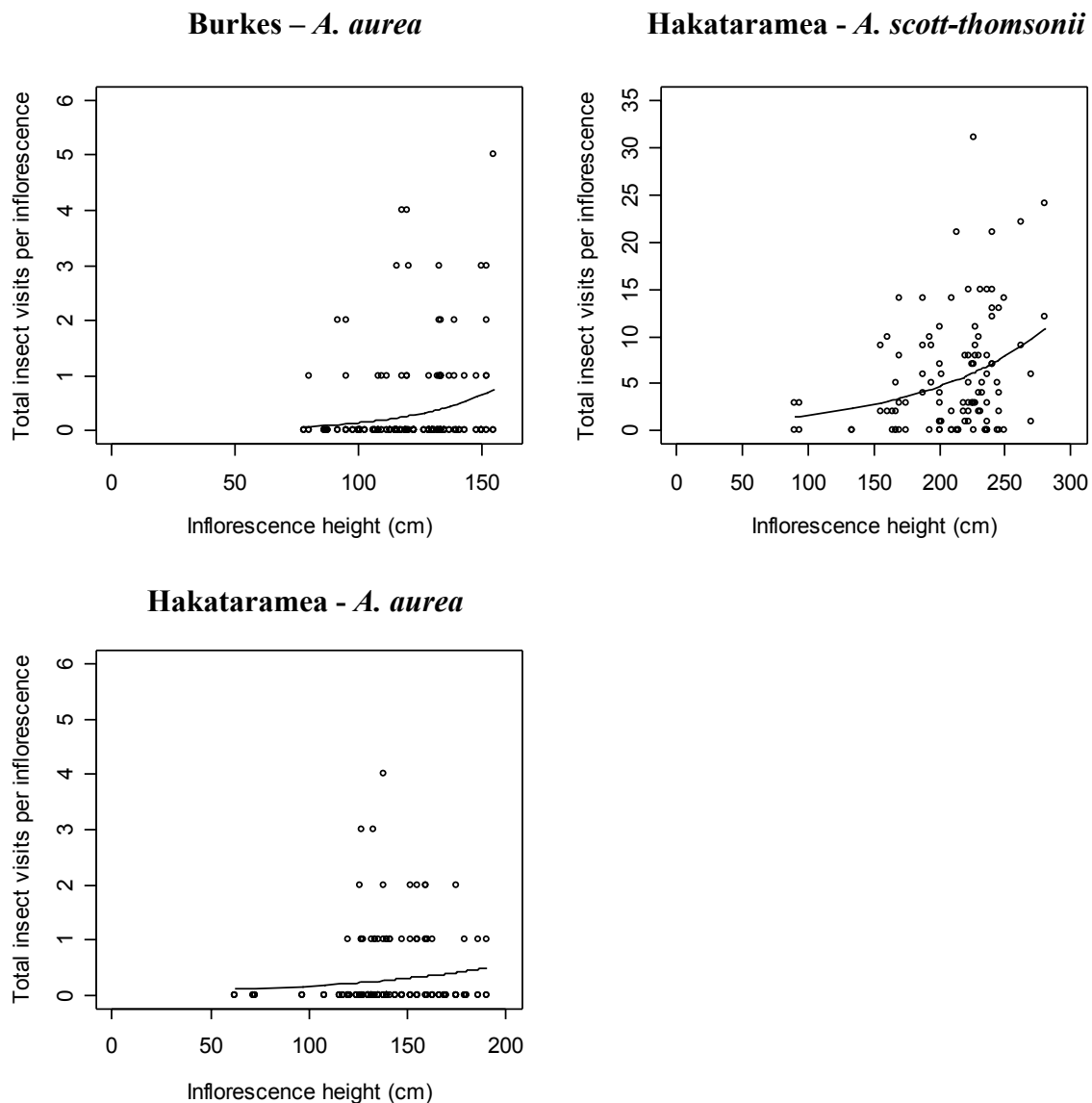


Fig. 3.1 Total number of insect visitors to female *Aciphylla* inflorescences (summed over all observation days) in response to inflorescence height. The relationship was significantly positive for *A. aurea* at Burkes Pass and *A. scott-thomsonii* at Hakataramea Pass but non-significant for *A. aurea* at Hakataramea Pass, although the direction of the relationship remained positive (see table 3.3).

### 3.3.2.3 Insect visitation and inflorescence flowering length

Flowering length (the length of the inflorescence stalk with flowers present) was also a strong predictor of insect visitor numbers to *A. aurea* at Burkes Pass and *A. scott-thomsonii*

at Hakataramea Pass (Table 3.4). The greater the length of the stem with flowers present, the more visitors there were in total. For *A. aurea* at Hakataramea, results were non significant, but at Burkes Pass, flowering length was a stronger predictor of insect visits than inflorescence height as it had the larger percentage deviance explained (Tables 3.3, 3.4). Figure 3.2 graphically demonstrates the positive relationship between length of inflorescence with flowers and number of insect visitors.

Table 3.4 Insect visitor numbers in response to variation in flowering length (cm). Statistical values given are for the effects of length of inflorescence with flowers on insect visitor numbers from poisson GLMs with length of inflorescence with flowers as a variable (percent deviance explained, F statistic, (d.f.), and *P* value). Slope and intercept values from the model are given in  $\log_e$  units, with the fitted model being in the form of insect numbers =  $e^{(\text{slope} * \text{flowering length} + \text{intercept})}$ . (Abbreviations: BP = Burkes Pass, HP = Hakataramea Pass and *A. scott-th.* refers to *A. scott-thomsonii*).

Study species and sites	Mean flowering length $\pm 1$ standard dev.	% Deviance				
		Slope	Intercept	explained	F (df)	<i>P</i> -value
<i>A. aurea</i> BP	43.63 +/- 21.95	0.048	-3.74	23.66	43.47 (1,64)	<0.0001
<i>A. aurea</i> HP	51.56 +/- 23.41	0.011	-1.89	2.1	2.01 (1,57)	0.162
<i>A. scott-th.</i> HP	87.42 +/- 34.51	0.008	0.95	6.64	6.72 (1,51)	0.012

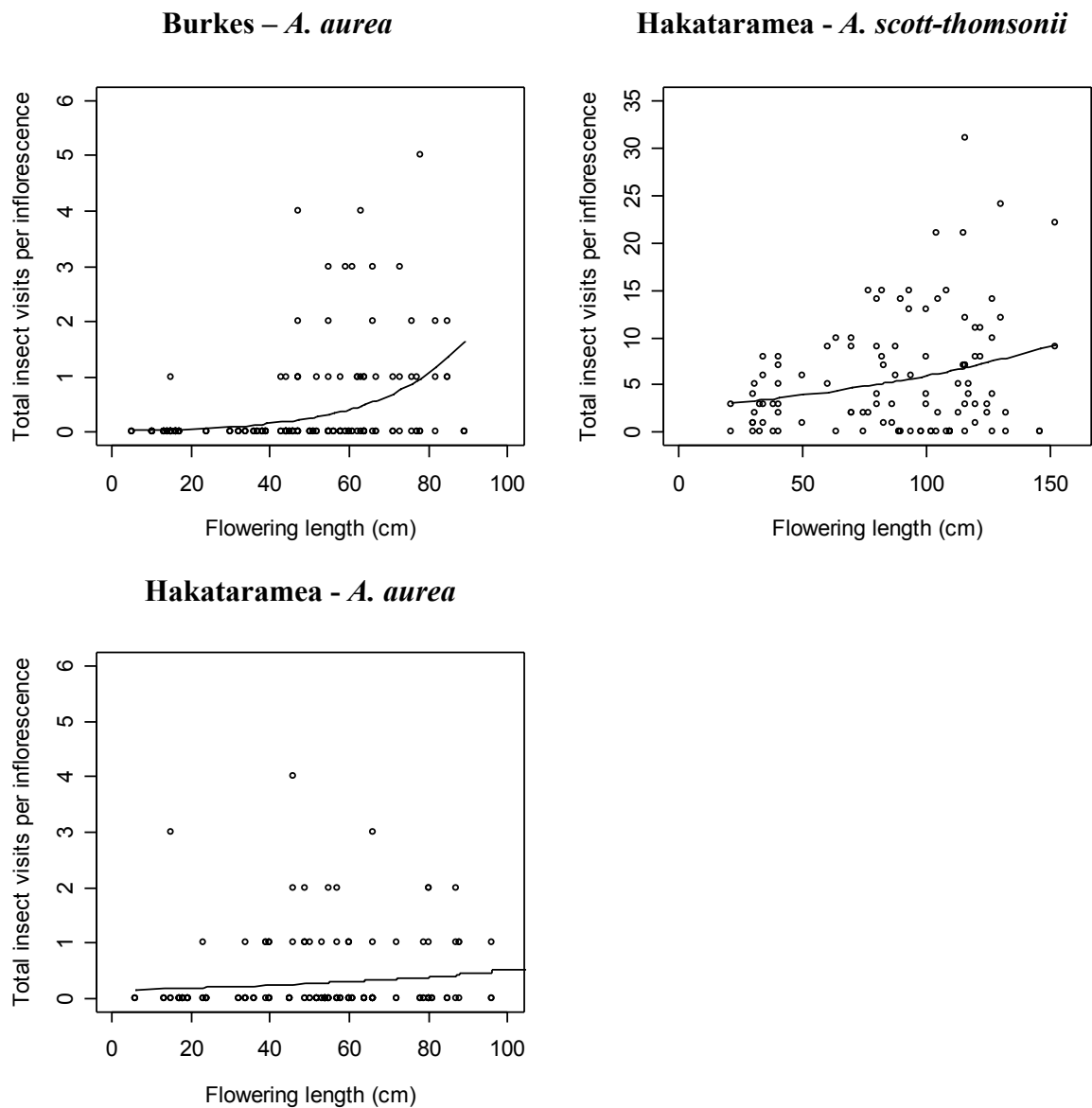


Fig. 3.2 Total number of insect visitors to female *Aciphylla* inflorescences (summed over all observation days) in response to flowering length (length of inflorescence with flowers on). The relationship was significantly positive for *A. aurea* at Burkes Pass and *A. scott-thomsonii* at Hakataramea Pass but non-significant for *A. aurea* at Hakataramea Pass (see table 3.5).

### 3.3.3.4 Does bract removal affect insect visitation rates?

Bract removal had no significant effect on insect visitor numbers to female inflorescences at any site (Table 3.5). The trend for *A. aurea* at Hakataramea Pass ( $P = 0.093$ ) was negative – bract removal resulted in a (non-significant) decrease in the numbers of insect



visits. Although non significant, this trend was supported in *A. aurea* at Burkes Pass but not in *A. scott-thomsonii*.

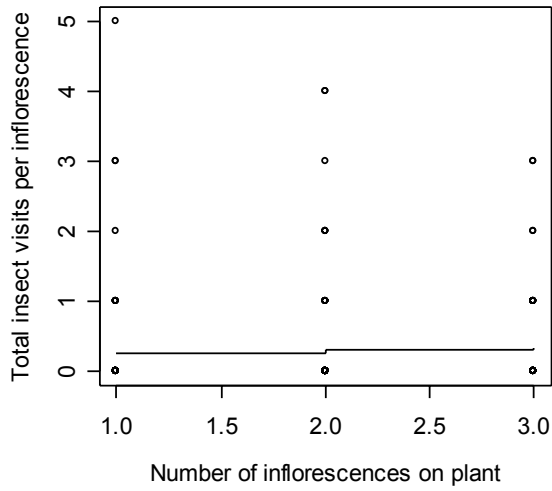
Table 3.5 Mean insect visitor numbers per female inflorescence per instantaneous observation in response to bract removal. Statistical values (percent deviance explained, F statistic, (d.f.), and *P* value) are from poisson GLMs comparing insect visitor numbers between bract removal treatments and control inflorescences. (Abbreviations: BP = Burkes Pass, HP = Hakataramea Pass and *A. scott-th.* refers to *A. scott-thomsonii*).

Study species and site	Bract removal		% Deviance explained	F (df)	P-value
	Control	No bract			
<i>A. aurea</i> BP	0.81	0.56	1.48	0.66 (1,14)	0.431
<i>A. aurea</i> HP	0.39	0.095	11.79	3.39 (1,11)	0.093
<i>A. scott-th.</i> HP	5.75	6.58	0.55	0.098 (1,8)	0.763

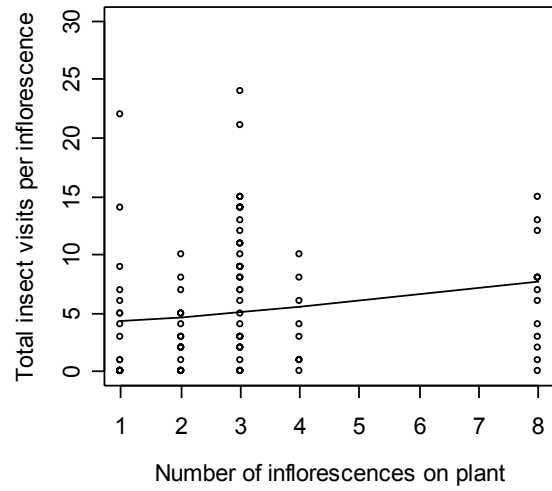
### 3.3.3.5 Insect visitation and number of inflorescences per plant

There was no significant difference in mean visitation rates to inflorescences situated on plants varying in inflorescence number, although an upward trend was apparent for *A. aurea* at Hakataramea Pass and *A. scott-thomsonii* (Table 3.6, Figure 3.3). *Aciphylla scott-thomsonii* inflorescences had a greater mean number of visitors in every category than *A. aurea* (Table 3.6).

**Burkes – *A. aurea***



**Hakataramea - *A. scott-thomsonii***



**Hakataramea - *A. aurea***

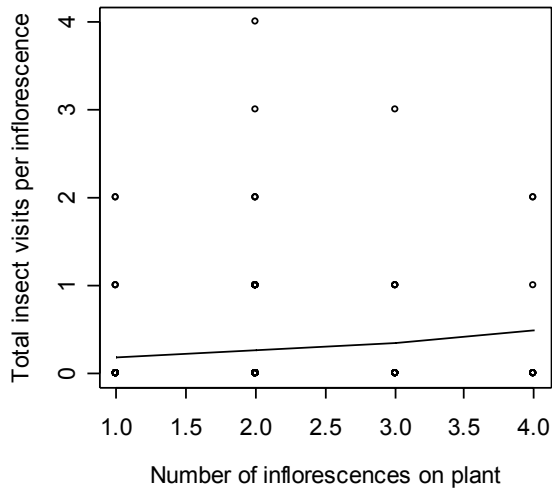


Figure 3.3 Total insect visitor numbers (per instantaneous observation) on single female inflorescence on plants with 1,2,3,4 or 8 inflorescences. Lines are fitted from the single factor poisson GLMs in Table 3.6, with inflorescence number as a variable. All the relationships are not significant (Table 3.6).

Table 3.6 Mean insect visitor numbers (per instantaneous observation) on single female inflorescence on plants with 1,2,3,4 or 8 inflorescences. The sample size for each category (i.e. the total number of instantaneous observations) is listed adjacently in parentheses. Statistical values presented are the output from single factor poisson GLMs with inflorescence number as a variable (percent deviance explained, F statistic, (d.f.), and *P* value). (Abbreviations: BP = Burkes Pass, HP = Hakataramea Pass and *A. scott-th.* refers to *A. scott-thomsonii*).

Species and sites	Number of inflorescences on plant					% Dev.		
	1	2	3	4	8	explained	F (df)	<i>P</i> -value
<i>A. aurea</i> BP	0.38 (60)	0.20 (148)	0.33 (64)	NA	NA	0.051	0.062 (1,65)	0.804
<i>A. aurea</i> HP	0.14 (48)	0.29 (108)	0.33 (21)	0.42 (12)	NA	2.031	1.982 (1,61)	0.164
<i>A.scott-th.</i> HP	3.5 (22)	2.68 (22)	7.79 (34)	4.13 (8)	6.58 (12)	3.234	2.458 (1,47)	0.124

### 3.3.4 Insect visitation to isolated flowering female plants

Unexpectedly, there was no significant difference in insect visitor numbers to female inflorescences at varying distances from flowering males (using the Distance Weighted local Pollen Index (DWPI)) for unmanipulated male flowering densities in *A. aurea* and *A. scott-thomsonii* at both study sites. *A. aurea* – Burkes Pass poisson GLM:  $F_{(1,33)} = 0.03$ ,  $P = 0.870$ , *A. aurea* – Hakataramea Pass poisson GLM:  $F_{(1,34)} = 0.3$ ,  $P = 0.59$  and *A. scott-thomsonii* – Hakataramea Pass poisson GLM:  $F_{(1,21)} = 0.12$ ,  $p = 0.74$ .

Where flowering plant distributions were manipulated by removing males and effectively isolating female plants to represent a ‘non-mast’ year, results were also unexpectedly non significant. *A. aurea* – Burkes Pass, poisson GLM:  $F_{(1,39)} = 0.149$ ,  $P = 0.701$ , and *A. aurea* – Hakataramea Pass, poisson GLM:  $F_{(1,34)} = 0.139$ ,  $P = 0.711$ .

### 3.3.5 Insect visits to male vs. female inflorescences

At all sites there were significant differences between insect visits to male compared with female inflorescences, with males receiving a significantly greater average number of visits

per inflorescence per observation. The most common insect visitors to *A. aurea* at both sites were big flies, small flies and weevils and these groups were almost always significantly more common on male inflorescences (Table 3.7a and 3.7b). Total insect numbers were particularly high on *A. scott-thomsonii* males, especially big flies and hoverflies (Table 3.7c).

Table 3.7 The mean number of insects per inflorescence per observation in each taxonomic category on male and female *Aciphylla* inflorescences according to site and species. The total number of insects seen in each category over the entire sampling period is given in parentheses. Statistical values given are for plant sex effects within each taxonomic group (as well as the total number of insect pollinators) from single-factor poisson GLMs (percent variance explained, F statistic (d.f.), and *P* value). NA values indicate too few data to run test.

a) Burkes Pass – *Aciphylla aurea*

<b>Plant sex</b>	<b>Big flies</b>	<b>Hoverflies</b>	<b>Small flies</b>	<b>Beetles</b>	<b>Weevils</b>	<b>Moths</b>	<b>Total</b>
Male	0.83 (40)	0 (0)	1.13 (54)	0.02 (1)	2.75 (132)	0.06 (3)	19.17 (230)
Female	0.69 (33)	0 (0)	0.17 (8)	0.08 (4)	0 (0)	0.04 (2)	3.92 (47)
% var. expl.	0.4	NA	16.73	NA	38.56	NA	24.9
F (d.f. 1,22)	0.27	NA	11.16	NA	32.28	NA	9.08
<i>P</i> -value	0.61	NA	<b>0.003</b>	NA	<b>&lt;0.001</b>	NA	<b>&lt;0.001</b>

b) Hakataramea Pass – *Aciphylla aurea*

<b>Plant sex</b>	<b>Big flies</b>	<b>Hoverflies</b>	<b>Small flies</b>	<b>Beetles</b>	<b>Weevils</b>	<b>Moths</b>	<b>Total</b>
Male	2.5 (75)	0.3 (9)	0.47 (14)	0.1 (3)	13.07 (392)	0 (0)	16.43 (493)
Female	0.1 (3)	0 (0)	0.1 (3)	0.03 (1)	0.03 (1)	0 (0)	0.27 (8)
% var. expl.	23.39	26.95	8.37	4.28	3.43	NA	38.53
F (d.f. 1,18)	9.03	9.54	2.95	1.29	15.67	NA	15.54
<i>P</i> -value	<b>0.008</b>	<b>0.006</b>	0.1	0.27	<b>0.009</b>	NA	<b>&lt;0.001</b>

c) Hakataramea Pass – *Aciphylla scott-thomsonii*

<b>Plant sex</b>	<b>Big flies</b>	<b>Hoverflies</b>	<b>Small flies</b>	<b>Beetles</b>	<b>Weevils</b>	<b>Moths</b>	<b>Total</b>
Male	31.56 (568)	24.11 (434)	0.28 (5)	0.11 (2)	5.56 (100)	0 (0)	61.6 (1109)
Female	3.45 (76)	1.77 (39)	0.45 (10)	0.09 (2)	0.05 (1)	0 (0)	5.8 (128)
% var. expl.	65.98	56.1	1.4	0.19	22.31	NA	65.16
F (d.f. 1,18)	45.51	29.27	0.39	0.05	37.48	NA	39.98
<i>P</i> -value	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.01</b>	0.83	<b>&lt;0.001</b>	NA	<b>&lt;0.001</b>

The ratio of mean number of insect visitors per inflorescence per observation for males, divided by the mean number of insect visitors per inflorescence per observation for females (Table 3.7) was varied considerably between sites. *A. aurea* at Burkes Pass had the lowest ratio (4.9 visitors per male for every one visitor per female), followed by *A. scott-thomsonii* (10.6) and *A. aurea* at Hakataramea (60.9).

### 3.3.6 How do these results relate to results on seed set rate in chapter 2?

Overall, Table 3.8 shows some significantly similar trends occurring between a number of variables/factors representing floral display and both reproductive success (seed set) and insect visitation rates. In general, seed set rates showed similar patterns to that of insect visitation rates at the majority of sites (for floral display size), in terms of the direction and significance of the relationships in question (Table 3.8). For instance, a decrease in the level of floral display (0, 40, 80% flower removal experiment) elicited a decrease in both insect visitation and seed set rates (to varying extents), while taller inflorescences had a significant positive effect on both visitation rates and seed set, as did greater flowering length. There was no significant effect of female isolation on insect visitation rates, but there was a significant negative effect on seed set rates at most sites (Table 3.8). These results will be discussed further in Chapter 5.

Table 3.8 Summary of the results from Chapters 2 and 3 showing the effects of floral display at both the individual (flower removal experiment) and population (isolated female experiment) level on seed set rate (Chapter 2) and insect visitation rates (this Chapter) at all sites during the summer of 2004/2005. There are six predictive terms. Where there are gaps, this means that a particular term does not apply to a certain species and/or site. BP refers to Burkes Pass, HP to Hakataramea Pass and LP to Lewis Pass. Positive and negative signs indicate the direction of the relationship between each predictor and response, with expected direction provided in the first column based on initial hypotheses. A zero refers to a statistically non-significant result. For percent flower removal, signs on the left side of the dash explain the direction of the relationship from 0 to 40% and the right side is from 0 to 80% removal. A double sign on one side of the dash refers to the flower removal treatment that shows that strongest effect.

Predictive terms	Expected direction of relationship	Flower removal experiment													
								'Isolated' female experiment							
		<i>A. aurea</i> - BP		<i>A. aurea</i> - HP		<i>A. scott-thom.</i> - HP		<i>A.</i> 'lewis' - LP		<i>A. aurea</i> - BP		<i>A. aurea</i> - HP			
Seed set rate	Visitation rate	Seed set rate	Visitation rate	Seed set rate	Visitation rate	Seed set rate	Visitation rate	Seed set rate	Visitation rate	Seed set rate	Visitation rate	Seed set rate	Visitation rate		
Hand pollination	+	+		+		+				+		+			
Pollen index	+	+	0	+	0	-	0								
Nearest male	-							-		-	0	-	0		
% Flwr removal	- / - -	- / - -	- / -	- / - -	0	- / - -	+ / -	- / +							
Flowering length	+	-	+	0	0	-	+	+		+	+	+	+		
Inflor. height	+	+	+	-	0	+	+	+		+	0	-	0		

### 3.4 DISCUSSION

#### 3.4.1 The insect fauna of *Aciphylla*: a generalist or specialist pollination system?

A large number and diversity of insects were collected on flowering *Aciphylla*, yet without further study, it is difficult to ascertain the relative roles and pollination efficiencies of the different groups. Pollinator spectra for many plant species range across orders of insects (beetles, flies, bees, moths and butterflies) classes of animal (insects, birds and mammals) and pollinations agents (wind, water and animal) (Herrera, 1996; Ollerton, 1996). Based on the ready availability of nectar and pollen, and on the large numbers and great diversity of insect visitors, species in the Apiaceae have been labelled promiscuous (Lindsey, 1984), which also seemed to hold true for *Aciphylla aurea* and *A. scott-thomsonii* in this study. The distinction between floral visitors and effective pollinators, however, is extremely important and is rarely discerned (Lindsey, 1984; Newstrom & Robertson, 2005). Although it appears that a range of insects may pollinate New Zealand Apiaceae members such as *Aciphylla*, this may not be the case. Some taxonomic or functional groups may serve to be more effective and efficient than others.

This study provides the first recorded comprehensive description of the insect fauna associated with flowers of lower montane *Aciphylla* species (but see Primack, 1983), but is not an exhaustive list as collections were limited to diurnal visitors over a short period during only one season, at three sites, using only one trapping technique.

At all three sites, there were seven orders of insects found. By far the most abundant and ubiquitous of these were the Dipterans (flies) with a range of species belonging to thirteen different families collected. This was probably the most significant pollinator group due to their extremely large numbers at each site, and because many flies are known to be opportunistic feeders on pollen and/or nectar and are important pollinators worldwide (Newstrom & Robertson, 2005). Important pollinator families in New Zealand are bristle flies (Tachinidae) and hoverflies (Syrphidae) (Newstrom & Robertson, 2005), which can carry significant pollen loads on their gena (or cheeks) and perhaps other families such as Bibionidae and Calliphoridae (Primack, 1983), all of which were collected on *Aciphylla* during this study. Among other insect groups found that are likely to contribute to pollen

movement in *Aciphylla* were: moths, craneflies, native solitary bee species, native wasps, and a large number of beetles, although there have been very few studies of the effectiveness of beetles as pollinators. They are less mobile and may not have as important a role as bees, flies and moths. Godley (1979) listed weevils as pollinators of *Aciphylla* but the effectiveness of other small beetles has not been investigated (Newstrom & Robertson, 2005).

Although many of these insects are potential pollinators, it is possible that only a small proportion of these species are significantly contributing to the movement of pollen from male to female plants. Lindsey (1984) tested insect floral visitation for plants of nine populations in a comparative study of three species of the closely related Apioideae genera, *Thaspium* and *Zizia*. Results showed that, despite a high diversity of insect visitors, generally 1-4 species accounted for at least 74% of the pollinations in all populations.

The wide variety of potential pollinators found on *Aciphylla* in this study suggests that these species are adapted to pollination by a suite of generalists. However, further work should be done to test the relative importance of insect pollinators in *Aciphylla*, as the presence of specialist pollinator(s) cannot be ruled out from the findings in this study.

### **3.4.2 Why are insects more abundant on male inflorescences, and are females receiving sufficient visits?**

All experiments so far have been largely concerned with female reproductive success, i.e. proportion of seed set. “Success” in males however can be difficult to define and measure (Sutherland & Delph, 1984). Methods of measuring male reproductive success include measuring the amount of pollen being carried away from the flowers, or frequency and duration of pollinator visits to flowers. On species such as *Aciphylla* with tens of thousands of flowers on a single male inflorescence, this can be difficult therefore a rough measure of insect visitation to male flowers was employed here. One thing that did become evident however was that on average male inflorescences received a much greater number of insect visitors during the survey than females.



So why is there an apparent bias towards insects visiting male rather than female inflorescences? Male bias in visitation has been reported for several dioecious and monoecious species (Bierzychudek, 1987; Le Corff *et al.*, 1998). The ability of pollinators to discriminate between male and female flowers has been observed by many, (e.g. Bawa, 1977; Bierzychudek, 1987; Armstrong, 1997; Le Corff *et al.*, 1998). Baker (1976) suggests that visits to female flowers are often made only by mistake, and predicted that discrimination against female flowers should be common amongst species with unisexual flowers. In this case, female flowers presumably offer less reward to insects than do males, because they are usually incapable of producing pollen. Male flowers in this case would produce not only copious quantities of pollen, but may also produce nectar, offering twice the reward (Holland & Fleming, 2002; Orellana *et al.*, 2005) to species that collect pollen. Therefore, under these circumstances, it seems logical to assume that insects may be more attracted to male inflorescences, especially if their floral display is generally larger, showier, and more rewarding. Realistically however, insect visitation rates between male and female flowers may depend on the quality of the rewards offered and on the insect's preferences which varies greatly between plant species and insect species. Some species produce different volumes of nectar by sex, e.g. *Fuchsia excorticata* (Delph & Lively, 1989) and consequently that sex would ultimately be more attractive to nectar feeders but not pollen feeders.

Possible reasons for the discrepancy between insect visitor numbers to male versus female inflorescences in *Aciphylla* could be attributed to the female floral reward. Primack (1983) reported that insects gather nectar from staminate (male) flowers, as well as pollen in *A. scott-thomsonii*, however whether pistillate (female) flowers in *Aciphylla* offer nectar rewards remains to be testified. Presuming pistillate flowers of *Aciphylla* do offer nectar rewards, the timing and availability of nectar production and quality are not known. Nectar is not a product of a plants sexual system but a reward offered to a foraging agent (Dafni, 1992), i.e. a secondary sex characteristic (Delph, 1999). There may be daily variation in nectar production, due to weather or temperature changes, or production at a certain time of the day such as morning (Proctor *et al.*, 1996). If in *Aciphylla*, nectar is being produced by the stylopodium only at a certain time of day, for example, during early morning, nectar-feeding insects are likely to be more attracted to these flowers during peak production times (Vaughton & Ramsey, 1998; Kudo & Harder, 2005). It is possible that the timing of insect observations conducted during this study (which were usually between 10 am and 2

pm) may not have encompassed peak nectar production times, and hence attracted fewer insects, potentially causing the male-bias in visitation rates. Incorporating temporal variation in observation times should be an important point of consideration for future research in this area.

Overall, it seems that in *Aciphylla*, males probably offer both pollen and nectar and females are presumed to only produce nectar. This suggests that because males offer a larger variety of rewards to pollinators, they consequently attract a larger diversity of insect feeders that have different feeding preferences, and this could explain the male-bias insect visitation rates obtained in this study.

### **3.4.3 How do the effects of floral display on insect visitation rates relate to seed set rates?**

#### **3.4.3.1 *Insect visitation and floral display***

Although insect visitation rates were significantly influenced by floral display size, it depended on the measure of floral display and methodology used to measure visitation. In both *A. aurea* (Burkes Pass) and *A. scott-thomsonii* (Hakataramea Pass), floral display affected insect visitation rates when it was measured using inflorescence height, flowering length (the length of stem with flowers on), and flower removal (0, 40 & 80 % flower removal experiment). These significant outcomes did not always conform to the expected direction of the relationship however. At Hakataramea, visitation rates were significantly higher for 40% flower removal – an inflorescence of intermediate floral display. At this site, insect observations were only performed twice due to weather and time limitations. The most statistically significant outcome was for *A. aurea* at Burkes Pass where visitor numbers significantly increased with increasing floral display size. Observations were carried out four times at this site, and results suggest that at Burkes Pass, floral display plays an important role in attracting pollinators, and this subsequently affected seed set. Studies supporting an increased floral display size increases pollinator attraction are many (e.g. Donnelly *et al.*, 1998; Kawarasaki & Hori, 1999; Lortie & Aarsen, 1999; Abe, 2000; Grindeland *et al.*, 2005). The magnitude of this effect is relatively small in this study

however, and further research is needed to test visitation rates in more detail to see if the effect of inflorescence height on pollinator visits per flower is evident.

Visitation rates did not vary significantly when floral display was represented by number of inflorescences per plant, although there was a suggestive trend towards an increase in inflorescence number attracting more insects. If insects were visiting taller flowers significantly more often, one would also expect insects to be more attracted to plants with many inflorescences due to the benefits gained from minimising foraging efforts for a concentrated food source (Collevatti *et al.*, 1998). This was not found during this study, as insect visitors were generally few and inflorescences were usually growing in dense clumps, probably not limiting insect preferences for localised resources.

#### **3.4.3.2 *Are isolated flowering females reproductively successful?***

For animal pollinated species, relationships between plant density and pollination rates depend on the functional and behavioural responses of pollinator visitation to plant density (Kelly, 1994). Results showed that the density of male inflorescences around females played an important role in determining seed set rate, with the isolated females receiving significantly lower levels of seed set than those flowering closer to males. However, the frequency of insect visits to inflorescences at varying distances to males was not significantly different. A number of studies show similar findings (e.g. Roberts & Boller, 1948; Grindeland *et al.*, 2005). This would suggest that the reduction in seed set with increasing distance to nearest male is a result of a decline in the efficiency of the pollinators (Robertson, 1992; Grindeland *et al.*, 2005) in successfully cross-pollinating *Aciphylla*, rather than a decline in the numbers of pollinator visits as such (see Chapter 5 for further discussion).

#### **3.4.4 Conclusions**

Overall, seed set rates were high and insect visitation rates were low in this study. However, having for instance, one insect on the inflorescence at any one time could be enough if the flowers have a 2 week life and each insect crawls over 25 flowers. Therefore,

the numbers look low but may be sufficient for pollination. This remains to be examined in detail. In many natural populations, seed set can be limited by the effectiveness of pollinators (Bierzychudek, 1987). It seems unlikely, given the naturally high seed set rates found in this study, and only minor or no increases in seed set when provisioned with supplementary pollen, that pollinators are ineffective. This is of course, if the reproductive system in *Aciphylla* is solely dependant on insects as pollinators. If there are other methods by which *Aciphylla* are pollinated and can set seed, then this may help explain why when visitor numbers are low, seed set can still be high. The questions remaining to be answered therefore, are: how do *Aciphylla* manage to achieve such a high seed set rate when insect visits appear to be low, and why do inflorescences in bags still have a greater than zero seed set? Further research should be directed towards understanding whether seeds can still be made without fertilisation, i.e. by apomixis, and should test for the effectiveness of wind pollination. The implications of this will be discussed further in chapter 5.

## CHAPTER 4 – Sex-ratios, dioecy and sexual dimorphism in *Aciphylla*

### 4.1 INTRODUCTION

Dioecy exists in about 6% of the world's angiosperm flora and has arisen independently in many families (Sakai & Weller, 1999). In the Apiaceae, almost all genera are hermaphroditic (Webb, 1981) and dioecy is unusual for the family; yet some New Zealand Apioideae genera – *Aciphylla*, *Anisotome* and *Lignocarpa* - have developed separate sex plants (Webb, 1979) (along with two Australian *Aciphylla* species (Pickering, 2000)). There are a number of hypotheses that exist to explain why dioecy evolved from hermaphroditism, (not the focus of this research but see Charlesworth, 1999; Sakai & Weller, 1999). Dioecious breeding systems usually exhibit sexual dimorphism (Delph, 1996; Eckhart, 1999). New Zealand possesses a high number of sexually dimorphic taxa, along with Hawaii, (Webb & Kelly, 1993) - yet another plant trait that appears particularly prevalent in island floras (Webb *et al.*, 1999). The frequency of sexually dimorphic genera is estimated at 18%, higher than for most continental areas (Lloyd, 1985; Renner & Ricklefs, 1995).

Sexual dimorphism in plants refers to differences in primary and secondary sex characteristics among plants that are dimorphic in gender (Delph, 1999) such as dioecious species. Primary sex characteristics are traits such as gonads and copulatory organs that are essential for reproduction (Darwin, 1877), (which are necessarily dimorphic), while secondary sex characters are traits that are not directly connected with reproduction (Geber, 1999). The latter include reproductive sex characters such as features of flowers or inflorescences (e.g. size and number) that affect male and female mating success or fertility (Eckhart, 1999), as well as ecological life history traits (e.g. flowering phenology, age at first flowering, and mortality) (Delph, 1999), and vegetative morphology, physiology and competition (Lloyd & Webb, 1977; Bawa, 1980; Dawson & Geber, 1999; Delph, 1999; Geber, 1999). Variation in any of the aforementioned traits between male and female plants can be considered as sexual dimorphism, and there is considerable evidence for this in dioecious plants in both reproductive and ecological secondary sex characters (Lloyd & Webb, 1977). Sexually dimorphic traits have been reported for *Aciphylla* species (e.g. Godley, 1964; Lloyd & Webb, 1977; Dawson & Le Comte, 1978; Webb, 1979; Webb & Lloyd, 1980; Pickering, 2000, 2001; Pickering & Hill, 2002; Pickering & Arthur, 2003).

See Figure 4.1 for morphological variation in floral display between male and female *Aciphylla aurea* plants.



Fig. 4.1 Male (left) and female (right) plants of *Aciphylla aurea* showing typical variation in display size, inflorescence number and showiness of males and females present within a population. This represents an example of sexual dimorphism in floral display between the sexes, observed in most species of this dioecious genus *Aciphylla*.

Sexual dimorphism can arise and be maintained in populations through natural selection and sexual selection (Darwin, 1877; Lloyd & Webb, 1977; Bawa, 1980; Delph, 1999). Sexual selection involves the differential effect on individuals of selection for male function (pollen production and dissemination) and female function (seed production and dissemination) (Bateman, 1948; Lloyd & Webb, 1977; Bawa, 1980; Delph, 1999). Sexual selection theory proposes that males of dioecious plants are limited by the number of mates (Bateman, 1948) resulting in intra-sexual competition among males (Eckhart, 1999), while for females there is a trade-off between allocating resources to attracting mates and seed production (Haig & Westoby, 1988) (see Chapter 2). This is thought to lead to sexual dimorphism in secondary sex characters such as floral display and other life history traits (Lloyd & Webb, 1977; Dawson & Geber, 1999).

Pickering (2000) suggests specific examples of traits thought to be the result of sexual selection: male-biased sex ratio, younger flowering of male plants, earlier flowering of male plants, larger floral display in males, and allocation of more resources to reproduction at the time of flowering in males, and the overall allocation of more resources to reproduction by females (see Pickering, (2000) and references therein).

In sexually dimorphic dioecious flowering plants, male-biased sex ratios have been reported for a wide variety of species (Darwin, 1877; Godley, 1964; Lloyd & Webb, 1977). There have also been a number of hypotheses proposed to explain this deviation from equality. These include gender-specific patterns of mortality due to different costs of reproduction in females versus males (Lloyd & Webb, 1977), local mate competition (de Jong *et al.*, 2002), differential herbivory of the sexes (Ågren *et al.*, 1999), selfish genetic elements, (Delph, 1999), site productivity and environmental gradients (Pickering & Hill, 2002), among others (see Lloyd & Webb, 1977; Delph, 1999; Pickering & Hill, 2002).

It is thought that as a result of resource limitation, alpine dioecious species may exhibit sexual dimorphism in life history traits, and as a result of this, populations of alpine dioecious species may display a male-biased sex ratio (Pickering, 2000). As mentioned above, *Aciphylla* populations have been previously reported to exhibit sexual dimorphism in the floral display of male and female inflorescences in New Zealand (Webb & Lloyd, 1980) and Australia (Pickering, 2000). Webb and Lloyd (1980) measured 53 populations of 20 predominantly-alpine New Zealand apioid Apiaceae species, and reported that of the six species of *Aciphylla* studied, the larger species *A. scott-thomsonii* and *A. aurea* exhibited a significantly male-biased sex ratio and had significantly more inflorescences per plant. This chapter further explores work carried out by Webb & Lloyd (1980) on the sex ratios of *Aciphylla*, and aims to quantify some of the sexually dimorphic characters of three New Zealand large-leaved montane *Aciphylla* species, focussing on four specific sites at similar altitudes within the wider Canterbury region of New Zealand.

### 4.1.1 Objectives

The previous two chapters of this study investigated the ecological intricacies of a large floral display in *Aciphylla* and the paradoxical combination of mast-seeding, dioecy and insect-pollination, with particular focus on female fecundity. This chapter aims to explore the evolutionary impetus for sexual dimorphism and floral display in *Aciphylla*. Specifically the aims of this study were:

- 1) To determine the plant- and inflorescence- sex ratio of dioecious *Aciphylla* populations during a high-flowering year for multiple sites and species
- 2) To investigate and quantify some of the sexually dimorphic characteristics present in *Aciphylla* by investigating whether certain plant or inflorescence traits varied with plant gender.
- 3) The above measurements are made during only a single flowering season, capturing a snap-shot of population sex ratios and sexually dimorphic traits. It has been hypothesised that costs of reproduction are greater for females (Webb & Lloyd, 1980), therefore if male plants can flower more often than females, as this hypothesis suggests, we are more likely to overestimate the number of males in a population. The effects of single-year versus long-term sampling to obtain population estimates of sex ratio will be discussed.

## 4.2 METHODS

### 4.2.1 Measuring population plant and inflorescence sex ratios

Sex ratios were recorded in a single year for four populations of three large-leaved montane *Aciphylla* species at three different sites in Canterbury, New Zealand (see Chapter 2 for details on site and species information). Counting was carried out during the summer of 2004/2005 during a relatively high-flowering year (except for Lewis Pass). Twenty-by-twenty metre plots were set out at 2 or 3 random locations within each population to obtain



sample sizes of at least 200 plants within each site. Only flowering individuals were counted, because the sex-ratio of the remaining plants at each site without inflorescences could not be determined (as non-flowering *Aciphylla* plants are impossible to sex in the field). The proportion of flowering to non-flowering plants was not counted. Determining the sex of each flowering plant was not difficult during flowering or fruiting in dioecious *Aciphylla* populations, therefore counts were made later in the season to include all plants that flowered that summer. The methods outlined above were carried out at all sites except for Lewis Pass, where the population was naturally sparser with fewer plants. Instead, the sex ratio of all flowering plants of *A.* 'lewis' in the entire Lewis Pass population was obtained.

In addition to the sex of the plants, the number of inflorescences per plant was counted. From these data, the average number of inflorescences per plant (un-weighted mean of male and female averages) was obtained, along with the inflorescence sex ratio of the population. Population plant and inflorescence sex ratios were obtained by calculating the percentage of males and the percentage of females in the population (of the plants that flowered). Chi-squared tests of equality were performed to examine whether sex ratios deviated significantly from the expected ratio of 50:50.

#### **4.2.2 Measuring sexual dimorphism in plant and inflorescence traits**

Approximately eight individual plants from both sexes were selected randomly from each site (excluding Lewis Pass), and plant and inflorescence measurements were made. These included plant height, plant area, rosette number per plant, inflorescence height (total length of inflorescence from base to apex) and flowering length (length of inflorescence with flowers on). These data were used to calculate whether there were differences in plant-level traits (sexual dimorphism) between sexes, species and sites. Analysis of Variance tests (ANOVA) were used to compare mean plant trait values between male and female plants. Inflorescence traits such as flower number per inflorescence and perianth size were not measured quantitatively during this study, but general observations of these were made and an extensive literature search was carried out in order to examine whether there was evidence for sexual dimorphism in other *Aciphylla* species.

## 4.3 RESULTS

### 4.3.1 Sex ratios

There was a preponderance of flowering male plants in all populations studied (Fig. 4.2), resulting in male-skewed plant sex ratios. For *Aciphylla scott-thomsonii* at Hakataramea Pass, the plant sex ratio was significantly more male-biased than the other sites (Table 4.1), with almost 68% of the flowering plants being male and 32% female. There were also significantly more male than female plants in the *A. aurea* population at Hakataramea. The sex ratios for *A. 'lewis'* at Lewis Pass and *A. aurea* at Burkes Pass were not significantly different from 1:1 at these sites (Fig. 4.2 and Table 4.1), however, the trend was still towards male-bias.

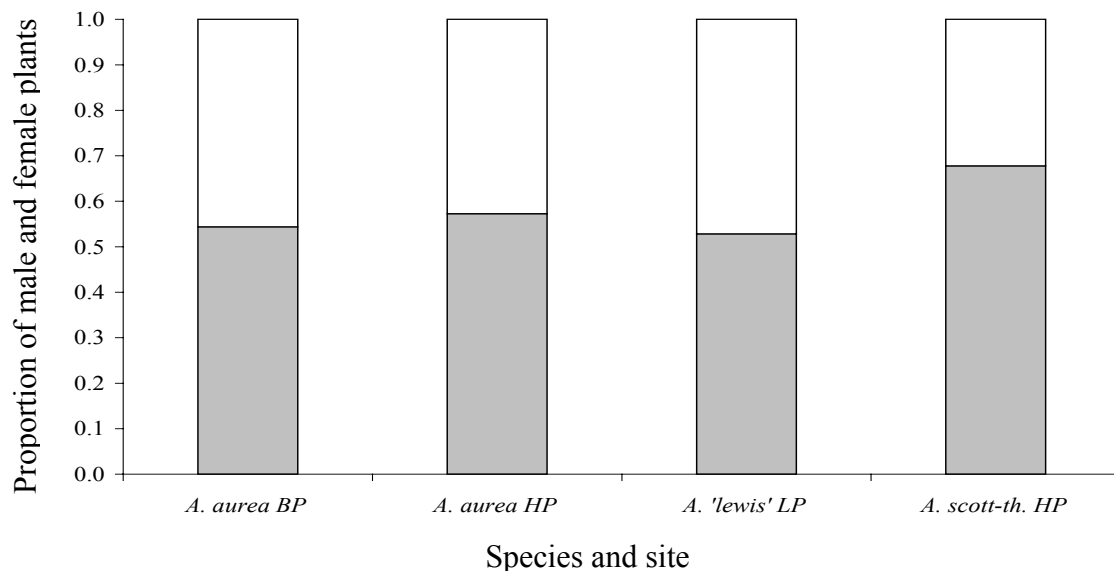


Fig. 4.2 Proportion of male (■) and female (□) flowering plants in each population of *Aciphylla* species. BP = Burkes Pass, HP = Hakataramea Pass and LP = Lewis Pass, and *A. scott-th.* refers to *A. scott-thomsonii*. (n = total number of plants counted at each site).

The total inflorescence sex ratio for most populations was even more male-biased than for plants (Fig. 4.3 and Table 4.1). Again, this was most prominent in *A. scott-thomsonii* at

Hakataramea Pass with over 75% of the inflorescences sampled within the population being male. This was also particularly significant for *A. aurea* at Hakataramea Pass, with more than 63% male inflorescences and was also significant for *A. aurea* at Burkes Pass. Again, the trend towards male-bias was present but not significant at Lewis Pass.

Table 4.1 Percentage of males for four populations of flowering *Aciphylla* in Canterbury. Population-level plant and inflorescence sex ratios are shown for each site, with sample size, percentage of males, Chi-square statistic and *P*-values showing significant deviation from 50:50 sex ratios. BP = Burkes Pass, HP = Hakataramea Pass and LP = Lewis Pass, and *A. scott-th.* refers to *A. scott-thomsonii*.

Species and site	Plant counts				Inflorescence counts			
	Sample size	% Males	$\chi^2$	<i>P</i> -value	Sample size	% Males	$\chi^2$	<i>P</i> -value
<i>A. aurea</i> BP	252	54.37	1.92	0.17	362	57.73	8.66	0.003
<i>A. aurea</i> HP	213	57.28	4.51	0.03	357	63.03	24.23	<0.0001
<i>A. scott-th.</i> HP	236	67.8	29.9	<0.0001	503	75.35	129.27	<0.0001
<i>A. 'lewis'</i> LP	53	52.83	0.08	0.77	77	55.41	0.86	0.35

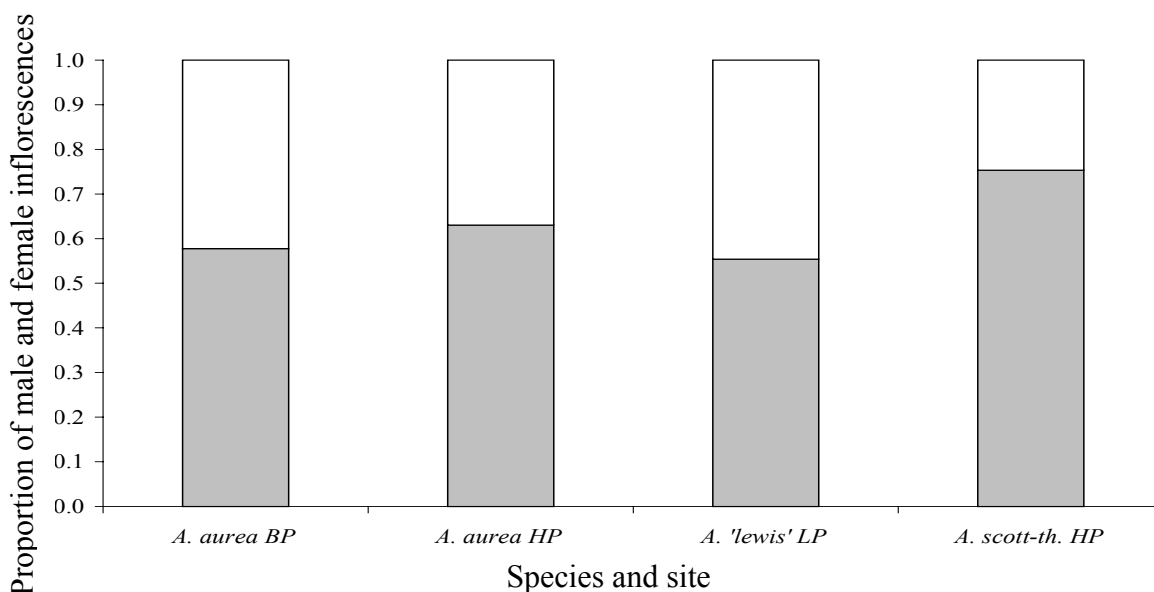


Fig. 4.3 Proportion of male (■) and female (□) inflorescences, measured as the percentage of male and percentage of female inflorescences (out of a total of n inflorescences). BP = Burkes Pass, HP = Hakataramea Pass and LP = Lewis Pass, and *A. scott-th.* refers to *A. scott-thomsonii*.

There were a significantly greater number of inflorescences per male plant than for females for *A. aurea* at Burkes Pass, (ANOVA:  $F_{(1,250)} = 4.39$ ,  $P = 0.04$ ) and at Hakataramea (ANOVA:  $F_{(1,211)} = 6.35$ ,  $P = 0.01$ ), and for *A. scott-thomsonii* at Hakataramea Pass (ANOVA:  $F_{(1,234)} = 10.72$ ,  $P = 0.001$ ) (Fig. 4.4). At Lewis Pass, inflorescence number was not significantly different between sexes (ANOVA:  $F_{(1,51)} = 0.43$ ,  $P = 0.51$ ).

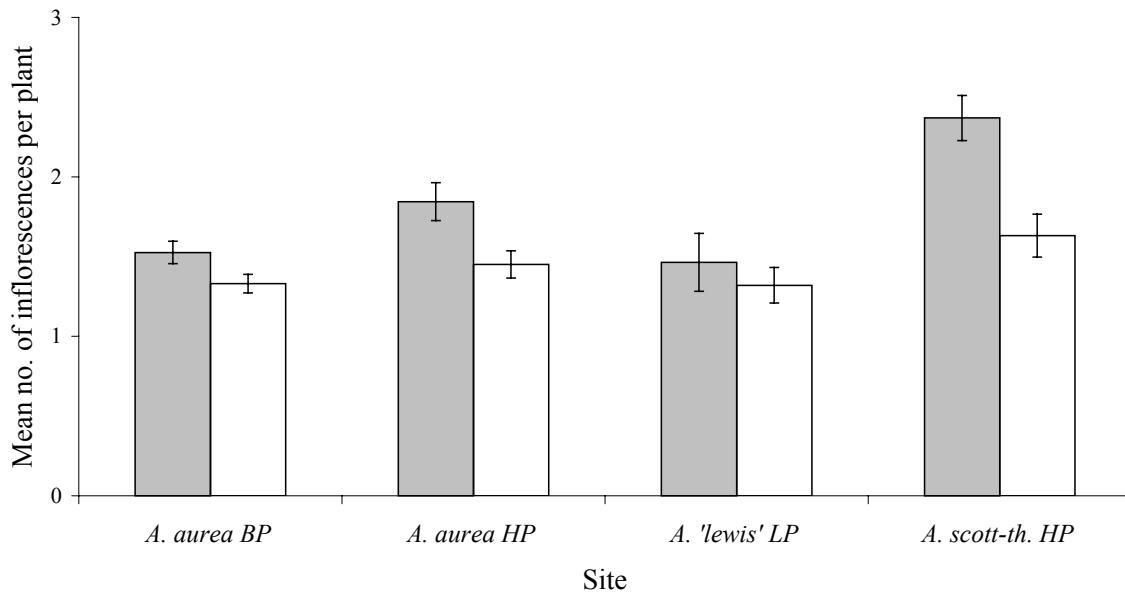


Fig. 4.4 Mean number of inflorescences per male (■) and female (□) plant ( $\pm 1$  SE) for all flowering plants counted within each population ( $\pm 1$  SE). BP = Burkes Pass, HP = Hakataramea Pass and LP = Lewis Pass, and *A. scott-th.* refers to *A. scott-thomsonii*.

### 4.3.2 Sexual dimorphism in plant and inflorescence traits

There were almost no significant differences between mean male and female plant dimensions, including plant height, plant area, and the number of rosettes per plant at any site (Table 4.2). There was one significant but weak exception, with female plant area being greater than males for *A. aurea* at Hakataramea Pass.

Table 4.2 Mean plant dimensions for male and female *Aciphylla* plants at three populations in Canterbury, including mean plant height (cm), mean plant area (cm<sup>2</sup>) and number of rosettes per plant. BP = Burkes Pass, HP = Hakataramea Pass and LP = Lewis Pass, and *A. scott-th.* refers to *A. scott-thomsonii*. Summary statistics from Analysis of Variance (ANOVA's) performed for each site and each variable. Significant *P*-values (at 0.05) are highlighted in bold.

Plant sex	Plant height (cm)			Plant area (cm <sup>2</sup> )			Rosettes per plant		
	<i>A. aurea</i> BP	<i>A. aurea</i> HP	<i>A. scott</i> HP	<i>A. aurea</i> BP	<i>A. aurea</i> HP	<i>A. scott</i> HP	<i>A. aurea</i> BP	<i>A. aurea</i> HP	<i>A. scott</i> HP
Female	45.8	45.23	79.71	5654	6358	18123	2.23	2.63	3.64
Male	47.75	42.67	77.5	7847	3946	17102	3.75	1.67	4.25
% var. expl.	0.4	1.4	0.3	5.45	8.1	0.1	7.68	0.06	0.76
F (d.f.)	0.18 <sub>(1,43)</sub>	0.61 <sub>(1,44)</sub>	0.09 <sub>(1,30)</sub>	2.47 <sub>(1,46)</sub>	4.04 <sub>(1,43)</sub>	0.03 <sub>(1,30)</sub>	3.49 <sub>(1,42)</sub>	2.61 <sub>(1,44)</sub>	0.21 <sub>(1,27)</sub>
<i>P</i> -value	0.67	0.44	0.76	0.12	<b>0.05</b>	0.86	0.07	0.11	0.65

There were no significant differences in mean inflorescence height between male and female plants at all sites, although male inflorescences were consistently taller on average than females (Table 4.3). Male plants on average did however have significantly greater flowering length than females, with the effect being strongest for *A. scott-thomsonii* at Hakataramea Pass.

Table 4.3 Mean inflorescence height (cm) and flowering length (cm) of male and female *Aciphylla* plants at three sites in Canterbury, BP = Burkes Pass, HP = Hakataramea Pass and LP = Lewis Pass, and *A. scott-th.* refers to *A. scott-thomsonii*. Summary statistics from Analysis of Variance (ANOVA's) performed for each site and each variable. Significant *P*-values (at 0.05) are highlighted in bold.

Plant sex	Inflorescence height (cm)			Flowering length (cm)		
	<i>A. aurea</i> BP	<i>A. aurea</i> HP	<i>A. scott-th</i> HP	<i>A. aurea</i> BP	<i>A. aurea</i> HP	<i>A. scott-th</i> HP
Female	74.11	84.19	128.78	56.5	60.12	69.08
Male	80.78	94.33	141.5	73.22	73.58	92.63
% var. expl.	0.85	3.88	4.03	9.85	11.93	26.61
F (d.f.)	0.66 <sub>(1,78)</sub>	3.15 <sub>(1,78)</sub>	1.39 <sub>(1,33)</sub>	8.2 <sub>(1,75)</sub>	8.13 <sub>(1,60)</sub>	11.60 <sub>(1,32)</sub>
<i>P</i> -value	0.42	0.08	0.25	<b>0.005</b>	<b>0.006</b>	<b>0.002</b>

Table 4.4 compares plant sex ratios found during this study to Lloyd and Webb's (1980) results from one-year sex ratio data from six populations of dioecious *Aciphylla* species in the South Island of New Zealand. All species except *A. pinnatifida* exhibit a preponderance of male plants, while it is mostly the larger species: *A. aurea* and *A. scott-thomsonii* which exhibit a significantly male-biased sex ratio (Table 4.4).

Table 4.4 A comparison of single-year plant sex-ratio data for six species of dioecious *Aciphylla* in the South Island of New Zealand between this study and data published in Webb and Lloyd, (1980). Only plants flowering were counted due to the inability to sex non-flowering plants. \*(P<0.05), \*\*(P<0.01), \*\*\*(P<0.001), calculated using Chi-square tests on hypothesis of equality.

Species	Site	Sample size	% Males	Source
<i>A. monroi</i>	Robert Ridge, Nelson	117	61.8**	Webb & Lloyd, 1980
<i>A. poppelwellii</i>	Rock & Pillar Range, Otago	103	55.3	Webb & Lloyd, 1980
<i>A. pinnatifida</i>	Old Man Range, Otago	202	49.5	Webb & Lloyd, 1980
<i>A. aurea</i>	Mt. St. Patrick, Canterbury	152	55.6*	Webb & Lloyd, 1980
	Mt. St. Patrick, Canterbury	135	51.9	Webb & Lloyd, 1980
	Burkes Pass, Canterbury	252	54.8	This study
	Hakataramea Pass, Canterbury	213	57.3*	This study
<i>A. scott-thomsonii</i>	Old Man Range, Otago	157	61.8**	Webb & Lloyd, 1980
	Hakataramea Pass, Canterbury	236	67.8***	This study
<i>A. 'lewis'</i>	Lewis Pass, Canterbury	53	52.8	This study

Further non-quantitative observations made during the flowering season suggested that male flowers had markedly whiter and larger petals than female flowers. Male flowers were 'showy' and more apparent from a distance than the more drab-coloured females (see Fig. 4.1), a characteristic especially prominent in *A. aurea*. It was also observed during the summer field season of 2004/2005 that male plants commenced and completed flowering mostly before female plants suggesting that flowering phenologies are also different between the sexes.

## 4.4 DISCUSSION

### 4.4.1 Male-biased sex ratio in *Aciphylla*

There was a significant male bias in plant sex ratio in *A. aurea* and *A. scott-thomsonii* populations at Hakataramea Pass, and a similar (though non-significant) trend towards this at Burkes Pass and Lewis Pass. Population inflorescence sex ratios were also significantly male-biased at all sites apart from Lewis Pass. *Aciphylla scott-thomsonii* showed the most marked predominance of males compared to females for both plant and inflorescence sex ratio within the Hakataramea Pass population, while *A. 'lewis'* exhibited a sex ratio closer to 50:50. There is much evidence for male-biased sex ratios in dioecious species and it is significantly more common than equal or female-biased ratios (of the species for which sex ratio data is available) (Delph, 1999).

### 4.4.2 Apparent vs. real sex ratio

Is this simply an apparent sex ratio that we observe during one flowering year amongst only the flowering individuals, or does this reflect the real sex ratio of all plants in a population? Other researchers have attempted to determine sex ratios in *Aciphylla* in the past and the answer to this question still remains unclear. Some researchers may have underestimated the frequency of male plants within *Aciphylla* populations; e.g. Godley (1964) reported a female-biased sex ratio during one summer for *A. aurea*. After anthesis in large *Aciphylla* species, male inflorescences fall over soon after flowering whereas female inflorescences remain erect and are more easily observed (Webb & Lloyd, 1980), and when sex-ratio counts are performed late in the season, it would be easy to underestimate the number of males. Others however, have similarly reported significant male-biased sex ratios for *Aciphylla* populations (e.g. Lloyd & Webb, 1977; Webb & Lloyd, 1980). Under the “higher costs of reproduction for females” hypothesis, it is easy to predict that males should be more “optimistic” about flowering than females, because males can take a chance of making some flowers in a low year in case there is a female to pollinate. Females should be selected to more strongly concentrate their flowering in high years when pollination is assured. Therefore, it can be predicted that low-flowering years should have strong male-bias, but the highest flowering years should mirror the actual sex ratio of plants

on site, or even show an apparent female bias. Hence why single-season counting techniques should be replaced with long-term studies to map and eventually sex all individual plants within a population to test whether the male bias in sex ratio is apparent or real.

A number of researchers have shown from one-year experiments, strongly male-biased sex ratios for American Holly *Ilex opaca* (Colville, 1932; Barton & Thornton, 1947; Ackerman & Creech, 1965). These were largely in populations where less than 50% of the plants were flowering. Nearing (1947), from observation, suggested that the preponderance of male plants probably originated from studies of young seedling populations of which many of the female plants had not yet flowered. Clark & Orton (1967) demonstrated with a seven year study that in fact, male American Holly plants did flower at a younger age than females, and as seedling age increased, flowering male and female plants occurred at a more equal frequency. This may be true for a cohort of even-age young plants reaching first-flowering age, however, in mixed-age populations in the field, the possible higher mortality of female plants may come to give an increasing (and real) skew to the population. This example demonstrates the importance of long-term studies, waiting for all plants within the population to flower, in order to determine actual population structure and real sex ratios. A similar situation is likely to be the case for *Aciphylla* as male plants flower more often (Webb & Lloyd, 1980) due to the lower costs of reproduction. The higher costs of reproduction in female *Aciphylla*, compared with males, was not directly investigated in this study, however the fact that female inflorescences tended to have fewer flowers (for a given height) suggests that the costs of reproduction was higher for females than for males (discussed further in Chapter 5).

Lloyd & Webb (1977) provided evidence for different survival of the sexes for *A. scott-thomsonii* with a male-biased sex ratio, where - among smaller plants the sex ratio was close to 50:50, and that there were many small plants that did not flower that year. Webb & Lloyd (1980) suggest that these non-flowering plants may account for part of the male-biased ratio in the whole population, because more of them are likely to be females than males since females flower less often and are usually larger at first flowering (Lloyd & Webb, 1977). Furthermore, among larger plants that all flower, there is a distinct male-bias - either indicating that males grow more quickly than females, or they survive longer (Webb & Lloyd, 1980).



There is much evidence for real male-biased sex ratios in dioecious species and it is significantly more common than equal- or female-biased ratios (of the species for which long-term sex ratio data is available) (Delph, 1999). There have been a number of hypotheses proposed to explain this deviation from equality. A few of them are briefly described below.

#### *Local mate competition*

Sexual dimorphism can evolve through intersexual interactions such as competition or mate choice in dioecious taxa, e.g. competition for limiting resources within and between the sexes can cause the sexes to specialise on different forms or habitats. Some empiricists have suggested that this form of intersexual character displacement explains the widespread occurrence of spatial segregation of the sexes and of sex ratio biases by habitat in dioecious plants (see review in Geber, 1999).

#### *Effective pollination*

Godley (1964) suggested that male-biased sex ratios may have arisen to compensate for inefficiencies of pollination in situations where seed production is pollen-limited. However, Webb (1979), Lloyd *et al.*, (1980) and Webb & Lloyd (1980) provide evidence for female plants of dimorphic Apiaceae being very effectively pollinated even when sex ratios show little or no male bias. This study did however demonstrate significant evidence for (weak) pollen limitation at most sites. This will be discussed further in Chapter 5.

#### *Site productivity*

Some researchers suggest that where recognisable habitats are found, sex ratios are often more male-biased in stressful habitats and female-biased in favourable habitats (Geber,

1999). For example, productivity declines with altitude and it has been shown that within a species, male-bias in sex ratio becomes more pronounced at higher altitudes, and it is hypothesised that males may gain a reproductive advantage in low resource environments (Geber, 1999; Pickering & Hill, 2002). Pickering & Hill's (2002) findings arose from only single-year sex ratio, and may be simply exhibiting an apparent sex ratio bias.

### *Different costs of reproduction between males and females*

Sex-specific life histories may be a consequence of sex-differential patterns of resource allocation, which are in turn caused by differences in requirements for disseminating pollen and maturing fruit (Delph, 1999). This may also include the timing of allocation to various activities as well as differences in the amount of resources allocated. In a seminal paper by Lloyd & Webb (1977), several predictions were made concerning how sexual dimorphism in reproductive effort would lead to life-history differences between the sexes. Most important to note is that the costs of reproduction between the sexes are significant evolutionary determinants of sexual dimorphism in life histories (Lloyd & Webb, 1977). They postulated that the energy cost to females of producing ovuliferous flowers and maturing fruit is higher than the cost to males of producing polliniferous flowers, and that many observed secondary sex characters in plants (including differential survival of the sexes), are probably a consequence of this difference in reproductive effort between males and females (Lloyd & Webb, 1977). It is important to consider sex ratio at other levels of floral display, beyond the plant-level. The average number of inflorescences per plant is usually higher among males than among females in dioecious species (Webb & Lloyd, 1980). This was also found for most species in this study. Webb & Lloyd (1980) propose that males invariably produce more flowers per plant on average than do females in dioecious species because the number of flowers per inflorescence is greater in males. The relative number of flowers per inflorescence is important in dioecious populations (Lloyd & Webb, 1977), and often contributes most to the relative number of pistillate (female) and staminate (male) flowers in any population, as Opler & Bawa (1978) found for dioecious tropical forest trees. Results from this study parallel the findings outlined above.

The greater resource allocation to reproduction by females can result in females needing to accumulate more resource reserves prior to flowering, affecting seasonal and lifetime

flowering phenologies (Lloyd & Webb, 1977; Delph, 1999). Because resource constraints involved with flowering are supposedly greater for females, female plants may be under-represented in populations because of factors such as age of first flowering and/or flowering less frequently (Lloyd & Webb, 1977; Meagher & Antonovics, 1982; Delph, 1999; Eckhart, 1999). Also the high costs to females of producing seed and fruit are likely to increase female mortality more than male, leading to male-biased sex ratios (Lloyd & Webb, 1977; Delph, 1999). In some species, females must de-fray the costs of reproduction by delaying reproductive maturity, or reproducing less frequently than males. Also, differences in the investment per inflorescence may make it advantageous for males to produce more inflorescences than do females (Pickering & Arthur, 2003), possibly contributing to a male-biased sex ratio. (The link between costs of reproduction, sex ratios and mast-seeding will be further discussed in Chapter 5).

#### **4.4.3 Other sexually dimorphic traits in *Aciphylla***

Pickering & Hill (2002) reported that for *A. simplicifolia*, male plants exhibited a significantly greater number of flowers per inflorescence, number of lateral umbels, number of umbellules per lateral umbel, number of flowers per umbellule on lateral umbels, number of umbellules on terminal umbel, and overall greater floral display area (cm<sup>2</sup>) than females. These dimensions were not quantified for *Aciphylla* species in this study, but similar results are expected judging from observation together with findings from the literature.

It was also observed during the summer field season of 2004/2005 that male *Aciphylla* plants commenced and completed flowering mostly before female plants finished. Clark & Clark (1987) also found in *Zamia skinneri*, a tropical rainforest cycad, that males began releasing pollen before the first female was receptive. This makes sense in that by flowering first, males encourage pollinators initially so they can then deliver pollen on to females (Proctor *et al.*, 1996). However, the viability of pollen grains after such a period of time may be affected, and in this case it would not make sense for the male plants in a population to have their flowers finish and die before female flowers are receptive. Clark & Clark (1987) also found for *Zamia*, that individual males were reproductively active for longer periods than individual females were receptive. It is possible that the female flowers

are able to collect (and store or use) pollen earlier than seems obvious from visual observation. This would make sense of the male timing, and also perhaps of the high seed set inside bags (Chapter 2). These findings have merely been observational in this study and should be explored further in future studies as this can have further consequences for both male and female reproductive success in terms of pollen wastage, and pollen limitation for females (Marden, 1984; Harder & Wilson, 1994).

#### **4.5 Conclusions**

Sexual dimorphism, specifically male-biased sex ratios, was found to occur in nearly all the *Aciphylla* populations studied here. The male biased sex ratio and male bias in other reproductive traits (such as inflorescence size) are entirely consistent with the literature that states that male-biased sex ratios should be more prominent in dioecious, alpine flora, and is most likely due to the differential costs of reproduction between male and female plants. Male bias during a high flowering year indicates that sexual dimorphism is an important feature of the reproductive ecology of *Aciphylla*. It still remains to be differentiated whether there is:

- 1) an apparent male bias in a single year because a higher percentage of males flower in any given year (or in all but the highest flowering years);
- 2) a real male bias in surviving plants due to slower growth and higher mortality of females, even though the seed sex ratio might be 50:50;
- 3) Real male bias at all stages due to other skewing factors which means more male seeds than female are produced.

## CHAPTER 5 – Discussion

### 5.1 Insect pollination, masting and reproductive success in *Aciphylla*

Previously little was known about the reproductive ecology of *Aciphylla*, although they are a ubiquitous and prominent component of the high country grassland flora. They have a unique breeding system that is characterised by being mast seeding, insect-pollinated, dioecious and sexually dimorphic in a number of traits. Together this results in a paradoxical reproductive system, because all these individual features are not usually associated together within one particular breeding system. Theoretically, insect-pollinated species should not provide a pulsed resource supply (a consequence of masting) if the pollination system is highly specialised, as this could lead to a break-down of the mutualism between the pollinator and the plant through pollinator satiation (Kelly, 1994). On the other hand, if the pollination system of a masting species is generalised, the plant may compete for pollinators with other flowering plants in the community (Rathcke & Lacey, 1985). Therefore, how does *Aciphylla* escape from the apparent disadvantages of being both masting and insect pollinated?

Chapter 3 showed that *Aciphylla* are most probably pollinated by a range of insect taxa from a large number of families and hence have a suite of generalised pollinators. Although this study did not test for the relative effectiveness of each taxon as pollinators, it is anticipated that by having more than one pollinator, *Aciphylla* have probably overcome the effect of satiating (and starving) pollinators with an unpredictable food supply, (an important corollary of mast seeding). However, as a consequence of being visited by a number of insects, *Aciphylla* must then have to compete for insect pollinators with a wide range of other plant species flowering at the same time (Rathcke & Lacey, 1985) (assuming pollinators are a limiting factor in reproductive success (Burd, 1994; Pias & Guitian, 2006)).

It is possible that masting behaviour in *Aciphylla* may serve to out-compete other plants for pollinators by providing the biggest and best rewards during high flowering years.

Evidence exists for some plant species when in full flower; insects may solely target that species, in preference to other flower species (Rathcke & Lacey, 1985). In oldfields in Michigan, honeybees visit *Solidago graminifolia* only after other plant species ceased flowering; consequently, earlier flowering clones set fewer seeds (Gross & Werner, 1983). Also in gardens, competition for honeybee visits commonly reduces seed set among crop varieties (Evans, 1980). Bees have been shown to search more efficiently for flowers that are similar to the flower they had just left, directing most of their foraging visits in a single trip to one flower species, ignoring other rewarding species that they encounter (Waser, 1986; Chittka *et al.*, 1999). Bees also show higher constancy to plants that are locally abundant (Kunin, 1993; Chittka *et al.*, 1997). It is hence possible, that some insect taxa that pollinate *Aciphylla*, are also demonstrating this preference for one species. Therefore it is likely that in *Aciphylla*, generalist insect pollinators feed on other (more constant) flowering species when *Aciphylla* provide few resources (i.e. during low-flowering years) and during high-flowering years, insects switch food resources to *Aciphylla*. Because of their large floral display size and the provision of huge quantities of pollen and probably nectar, with dense populations in localised patches (which is likely to be more efficient for insects in terms of foraging bouts), they offer a highly attractive food source during the years when they do flower. In this way they may be able to overcome the negative effects of offering an unpredictable food reward, (through masting) to their pollinators and still achieve high seed set as observed in this study.

## **5.2 The importance of synchronous flowering and display size**

The isolated female experiment tested for not only flowering asynchrony (i.e. individuals flowering in a ‘non-mast’ year), but for female asynchrony, because if in dioecious species, males and females do not flower in synchrony, this could have detrimental consequences for the reproductive success of both sexes (Crone *et al.*, 2005). This study focussed mostly on female fecundity, because it is more readily measurable than male success. In Chapter 2 it was demonstrated at some sites that, with increasing distance to flowering males, females experienced lower seed set rates. Although these effects were relatively small, they nevertheless demonstrated that even at a relatively small scale, the need for synchronous flowering between females and males is important. Also important was display size because individual inflorescences mostly showed increased benefits through having taller

inflorescences with more flowers (except *A. scott-thomsonii*). Larger floral displays were especially important when females were isolated. In a true non-mast year, when only a small fraction of the individuals are flowering, it is expected that the effects of pollen limitation will be considerably greater, and therefore there should be selection against very small population flowering efforts and small floral display size in *Aciphylla*. This could explain the emergence towards having such a large and spectacular floral display size in large montane *Aciphylla* species.

It was expected in this study that these negative effects of isolation seen in females (i.e. lower seed set) would correlate with a decrease in pollinator attraction (i.e. lower average insect visitation rates to isolated females); however, this was not the case. Isolated females experienced lower seed set rates than non-isolated females, but did not experience lower insect visitation rates. The most likely explanation for this is that in areas with only female inflorescences, insect pollinators move among female stalks just as frequently as in mixed-sex areas, but are less effective at delivering pollen as they have fewer opportunities to recharge their pollen loads on males. Another contribution to this effect could be the possible role of wind pollination, which should vary with distance to males independent of insect visitation rates. Mixed pollination systems, including generalist insect and wind, are not uncommon in plants (Mahy *et al.*, 1998). In areas with low insect abundance, such as alpine communities (Primack, 1983), predominantly insect-pollinated plant species can also be pollinated by wind, and this has been shown to provide reproductive assurance in some species, e.g. *Linanthus parviflorus* (Polemoniaceae), (Goodwillie, 1999). Wind pollination has been reported for *Aciphylla* by Dawson (1971) and Mitchell *et al.*, (1999), but they did not report any tests to confirm whether this was the case. This study points to wind pollination being possible, but probably not as important as insect pollination for *Aciphylla*.

### **5.3 Pollen- and resource-limitation and masting**

Pollen limitation is described as an inadequate quantity or quality of pollen that reduces plant reproductive success (Ashman *et al.*, 2004). Over the past 2 decades, determining whether seed production is pollen limited has been an area of intense empirical study (Burd, 1994), however there have only been weak attempts to explain the ecological and evolutionary causes and consequences of pollen limitation, or evaluate how this influences

plant species coexistence, community structure and ecosystem functioning. For example, plant life histories and mating systems may all influence or be associated with the probability or strength of pollen limitation (Kunin, 1993; Ashman *et al.*, 2004).

This study tested for pollen limitation to females in *Aciphylla* populations, using hand pollination techniques and assessed whether decreased flowering densities and inflorescence display size limited pollinators. Moreover, it also tested how severe the effects of pollen limitation were by simultaneously testing for resource limitation. Results demonstrated no evidence for resource limitation when measured by removing flowers (see Chapter 2), i.e. with increasing resources per seed to inflorescences with flowers removed. Brookes & Jesson (2006) tested for simultaneous pollen and resource limitation in *A. squarrosa* by removing flowers (comparable to methods used in this study) and obtained similar results. They also found that resource reduction (removing leaves) and fertiliser addition did not significantly influence fruit set. Both of these studies suggest that reproduction in these species was limited by other unknown factors. The effects may not be seen in a one-year study because masting involves switching resources from growth to reproduction during flowering years (Kelly & Sork, 2002; Monks & Kelly, 2006). Pollen was significantly limited however, implying that plants invested sufficient resources for maximum seed provisioning during this high-flowering year. However, inflorescences at Burkes and Lewis Passes had relatively lower seed set overall compared with Hakataramea Pass, leaving many ovules that didn't (or couldn't) make seed. This in itself suggests that there was some level of resource limitation occurring at the plant-level because otherwise supplemental pollination would have increased seed set rates in a more pronounced fashion, bringing it nearer to 100%. The effects of pollen and resource limitation were almost non-existent for *A. scott-thomsonii*, probably because natural seed set rates were extremely high (averaging around 90%). In saying that, tall inflorescences with greater flowering length had slightly negative effects on seed set rate, with more ovules probably representing a resource cost (Vaughton & Ramsey, 1998).

Some plants exhibit high flower to fruit ratios (Sutherland & Delph, 1984; Sutherland, 1987; Holland *et al.*, 2004), however, in female flowers, the potential advantage of increased gamete production is unclear. Extra ovules in large flowers are often not converted into seeds, and because they usurp resources that could be used to increase fecundity during current or future reproductive events, increased ovule production in



excess of what can be ripened may be disadvantageous (Vaughton & Ramsey, 1998). Newstrom and Robertson (2005) suggest that low fruit-to-flower ratios may be a normal part of several alternative plant strategies to maximise fitness from the excessive flowers or to allow a bet-hedging strategy that maximises fruit set when pollinator service is unreliable. Alternatively, increased ovule production may allow seeds of higher genetic quality to be produced without reducing seed production if inferior embryos are aborted, (Vaughton & Carthew, 1993; Newstrom & Robertson, 2005).

Examples of plants that exhibit high flower to fruit ratios are not uncommon (Sutherland, 1987) and include orchids which regularly have low capsule set, but compensate by producing thousand of tiny seeds per capsule (Nilsson, 1992). However, *Aciphylla* develop a maximum of 2 seeds in one schizocarp (fruit) and while thousands of flowers may be presented along an inflorescence, some are not converted into seed. It remains to be determined whether this occurs due to the ovule not being fertilised due to the lack of pollen delivery, or whether the plant was not capable of making seed even if the ovule was fertilised. However in this study, because supplemental pollen almost always increased seed set to some degree, (although at Burkes Pass it never increased as much as expected), this suggests that both of these factors (pollen and resources) play an important role in the reproductive success of *Aciphylla*, and their relative effects are probably site-specific.

Some plants may incorporate pollen limitation as an important mediator of selection (Ashman *et al.*, 2004). Bet-hedging (excess flower or ovule supply relative to the average pollen load received) may be a common strategy that is employed by plants to attract some pollen in a stochastic pollinator environment. The fitness gains from the conversion of occasional “jackpot” visits into extra seeds may outweigh the costs of unutilised ovules or flowers (Ashman *et al.*, 2004). This may be part of the reason for lower seed set rates in *A. aurea*, as extra resources (per flower) did not increase seed set as much as expected.

If reproduction is pollen limited in animal-pollinated, dioecious plants in a stochastic pollinator environment, it could be argued that under any of the following scenarios: (a) during a low-flowering year; (b) when females are isolated from males (i.e. pollen source); (c) when the flowering population is sparse, or; (d) when individual plants flower out of synchrony with the majority of the population (as shown by naturally isolated Lewis Pass individuals), that the effects of pollen limitation would be more severe (Kunin, 1993;

Forsyth, 2003). Therefore, one could expect selection for reproductive strategies that minimise the effect that factors such as pollen limitation have on reproductive success, (unless this is part of an alternative adaptive strategy that maximises fitness from excessive flowers (Newstrom & Robertson, 2005)). The best example of this is probably seen in *A. scott-thomsonii* where natural seed set rates are high, resources are not significantly limited, and excess flowers lead to a slight fitness loss (see Figure 2.10).

#### **5.4 The effects of sexual dimorphism on reproductive success in *Aciphylla***

Chapter 4 demonstrated that sexual dimorphism was evident in a number of traits in *Aciphylla*. Firstly, although largely observational in this study, it was found that life histories such as flowering phenology and inflorescence longevity differed between male and female plants. Male plants generally begin flowering before females and male flower stalks fell over soon after flowering ceased, while female stalks remain standing often for more than a year after flowering. (Primack, 1983) reported that individual male flowers are open for a longer period of time than females. Sexual dimorphism was also evident in a number of inflorescence level traits, with floral display in males being showier (see Fig. 4.1), with greater flowering length, and more inflorescences per plant. Similar findings have been reported for various New Zealand *Aciphylla* species (e.g. Dawson & Le Comte, 1978; Webb *et al.*, 1999) as well as for Australian *Aciphylla* species (Pickering, 2000, 2001). Additionally, inherent in primary sex characteristics, is that males produce pollen as well as nectar, offering twice the variety of reward than female flowers. In this study, most insect groups responded to this dimorphism in inflorescence traits and visited males more frequently than females. Males also received a greater diversity of insect visitors than females did. These sex-specific traits therefore, probably lead to the discrepancy in visits between male and female inflorescences via pollinator attraction.

The greater resource allocation to reproduction by females most likely results in female plants needing to accumulate more resources prior to flowering (Lloyd & Webb, 1977). This can sometimes take years in masting species, leading to the ability for males to flower more often than females (Meagher & Antonovics, 1982; Delph, 1999; Eckhart, 1999). Consequently, this is probably an important reason for the apparent male-biased sex ratios reported in this study, and elsewhere for *Aciphylla* (e.g. Pickering & Hill, 2002).

Nonetheless, during a high-flowering year (which is particularly important for the reproductive success of females), a male-biased sex ratio can pose a number of important consequences on a population. For instance, the sex ratio is indicative of local pollen densities. Having more male plants and/or inflorescences in a population may positively affect female fecundity, because as this study suggests, males attract significantly more insects overall. If *Aciphylla* do compete for generalist pollinators then males could provide the resource rewards, and visits to female flowers would also occur more often (even if this is by chance). On the other hand, male-biased sex ratios could have negative consequences for female visitation rates if insects consciously differentiate between male and female inflorescences and display a real preference for males. Overall, males appear to be attractive to insects regardless of size, while females generally benefit from being taller and/or having more flowers. Perhaps by being more “attractive” and more abundant, males are helping the females out by attracting more insects to the *Aciphylla* population (which indirectly benefits males by ensuring there will be more seeds produced which each male can potentially sire).

If reproduction costs relatively less for males in *Aciphylla*, the pressures towards needing to mast are not so great for males. If males do in fact make up the majority of the plants flowering during low-flowering years, whereas females only flower during high-flowering years (Hogan *et al.*, 1998) then maybe selection for masting has been stronger in females than males. Hence females may be driven to display masting behaviour as the costs of reproduction are so great that they can't do it every year and males may then be forced to follow suit (although they still flower more often in low-flowering years, presumably on the grounds that male prediction of when females will flower is imperfect so there is always a chance of siring some seeds in low-flowering years).

### **5.5 Further research on the reproductive ecology of *Aciphylla***

Also observed during this study was that seed predation levels were high on many inflorescences. Aphids were particularly abundant, and it was discovered that they also prey upon seeds (as well as sucking sap from foliage). Many plant species are known to show clear benefits from masting by satiating seed predators during high-flowering years, e.g. *Chionochloa* (Kelly & Sullivan, 1997; McKone *et al.*, 1998). Because *Chionochloa*

and *Aciphylla* species often coexist in high-country grasslands, it is possible they share some of the same seed predators. The effects of seed predation on reproductive success in *Aciphylla* should not be ruled out and remains to be tested in relation to masting.

Monitoring the effects of pollen limitation on naturally low-flowering years also remains to be tested as long-term studies would provide a better insight into whether this is an important selection pressure for masting in *Aciphylla* species. The pollination aspect of this system was not studied in great detail and it was not possible to gain a clear understanding of the movement of pollen, the relative effectiveness of the pollinator taxa, and the floral rewards offered. Obtaining such detailed information about the pollination biology of *Aciphylla* was beyond the scope of this research, but detailed analyses would greatly aid in the understanding of the pollination system of *Aciphylla* and the myriad interactions between the plant and its extensive suite of visitors. This is important, because in order to fully understand the reproductive system of these species, we need to know more about the role of floral attractiveness to pollinators.

The conservation implications of this research are also noteworthy. A decline in the diversity and abundance of insect pollinators, for example, caused through habitat fragmentation (McGlone & Moar, 1998), can lead to a decrease in pollination rate (Wilcock & Neiland, 2002) and have detrimental consequences on fecundity. This effect could be particularly threatening to plant species that are self-incompatible and rely on insects for pollination, such as *Aciphylla*. Moreover, if plant populations become fragmented, it becomes more important on a spatial scale as pollinator movement between more distant populations would be increasingly unlikely (Farwig *et al.*, 2004) and hence fail to promote genetic diversity within a species (Mitchell *et al.*, 1999). For species such as *Aciphylla subflabellata*, which is now becoming rare and sparse on the Canterbury plains and foothills due to habitat loss, small populations with isolated individuals flowering far from nearest conspecific neighbour could be affected by loss of pollinator movement and the likelihood of decreased transfer of conspecific pollen.

## 5.6 Conclusions

For *Aciphylla*, overall high seed set rates in the mass-flowering summer studied suggests that this is a reproductive strategy that works well for female plants. Although masting is probably a necessary outcome of having a large floral display size, benefits must be greater than the high costs of masting in order for this reproductive system to be successful. Results demonstrated that *Aciphylla* are generalist insect-pollinated and probably compete for insects through having a large and attractive floral display during high-flowering years. In most cases, inflorescences with a larger floral display had higher reproductive success. There was no evidence for resource limitation, while pollen limitation was evident across all sites (although this effect was relatively small). Hence, for *Aciphylla*, being a bigger inflorescence in dense, synchronous flowering populations was generally better for overall reproductive success. Hence masting may be a necessary strategy resulting from the need to have large, synchronous flowering to achieve pollinator economies of scale.

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## Appendix 1 - Floral morphology in *Aciphylla scott-thomsonii*

**Aims:** To assess whether a trend in flower number at certain locations on an inflorescence was detectable among inflorescences of all sizes, and whether seed set rate was related to this.

**Methods:** In early December prior to fruiting, while flowers were still present on *A. scott-thompsonii*, eight inflorescences were selected from the Hakataramea Pass field site. Four different sizes were chosen to represent the natural variation in inflorescence height present in the population, from very tall to relatively short. Two inflorescences of each height class, (approximately the same size), were selected from different plants. In the lab, all flowers on every fifth lateral umbel, starting from the apex downwards along the inflorescence, were counted. This was carried out in order to see whether a trend in flower number at certain locations on an inflorescence was detectable among inflorescences of all sizes. Measured at every fifth umbel was distance (cm) from the stem apex; diameter of the stem directly beneath the umbel; number of umbellules (flower clusters); and number of flowers. Regression analysis was used to test which variable was the best predictor of flower number along the stem. The two variables consistently returning the best fit were distance and diameter at every fifth umbel. This information could then be used to predict the number of flowers on a compound umbel at any given position along any inflorescence of a specified size. Later in the season when inflorescences were harvested, seed set was measured on all 58 *A. scott-thompsonii* inflorescences by counting all seeds on every fifth umbel along the stem to see if seed set rates varied with position on the inflorescence. Only grand means were presented in Chapter 2 results, but examples of how seed set rate (and flower number per umbel) varied with position along the stem are presented here. Results are only presented for one inflorescence but provide a good example of what the trends from most of the other selected inflorescences showed.

**Results:** Results show that umbels near the middle section of the flowering length of the inflorescence had the most number of flowers per umbel and higher mean seed set rates (Fig.1). Most consistently among all inflorescences sampled, stem diameter returned the higher  $r^2$  value for flower number. The trends conformed to a negative polynomial regression fit, with  $r^2$  values ranging from 0.79 to 0.96. Diameter along stem was also well

correlated with seed set rate, but generally with lower  $r^2$  values. Distance along stem was also a highly powerful predictor for both flower number and seed set rate, with all  $r^2$  values ranging between 0.85 and 0.97 for flower number, and  $r^2$  values of around 0.2 to 0.6 for seed set. Either one of these values would be useful for predicting flower number at any given point along the inflorescence. See Figure 1 for representative inflorescences showing the polynomial correlations between distance/diameter and flower number per umbel/seed set rate.

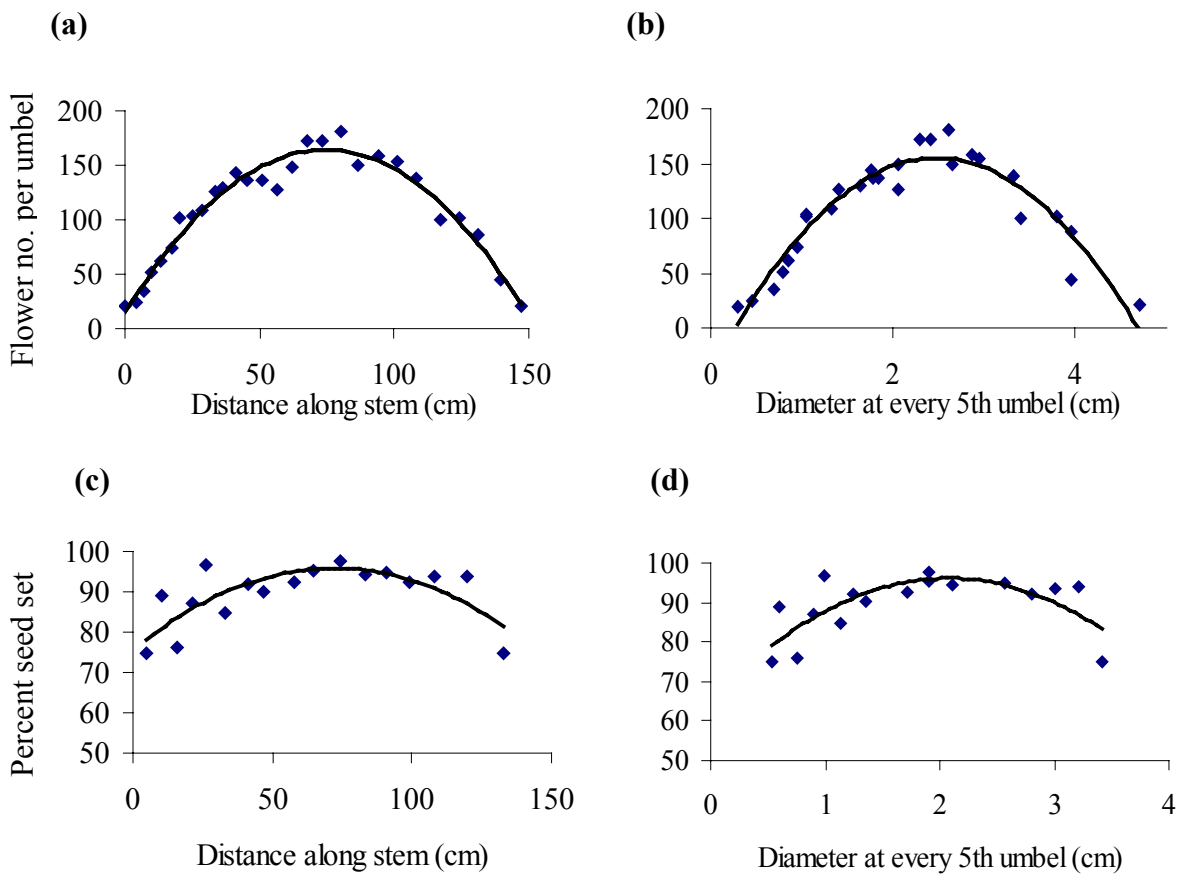


Fig.1 Polynomial regression analyses for *A. scott-thomsonii* at Hakataramea Pass, showing correlations between flower number per umbel with distance and diameter along the stem for one inflorescence (a) and (b) early in the flowering season, and between seed set rate and distance/diameter along the stem for one inflorescence (c) and (d) post harvest. Graph (a)  $r^2 = 0.96$ ,  $y = -0.03x^2 + 4.01x + 14.99$ ; graph (b)  $r^2 = 0.90$ ,  $y = -31.83x^2 + 157.71x - 40.28$ ; graph (c)  $r^2 = 0.59$ ,  $y = -0.004x^2 + 0.56x + 75.67$ ; graph (d)  $r^2 = 0.55$ ,  $y = -7.19x^2 + 29.88x + 65.13$ .

Overall, strong relationships exist between the above variables and these can be useful for estimating flower number or seed set depending on position along the inflorescence. However, to predict flower number per umbel accurately, it must be taken into account that this can vary depending on the size of the inflorescence. Fig. 2 demonstrates that inflorescences of varying flowering lengths all exhibit the same strong negative polynomial correlation, with inflorescences in the middle portion of the stem having a higher number of flowers per umbel.

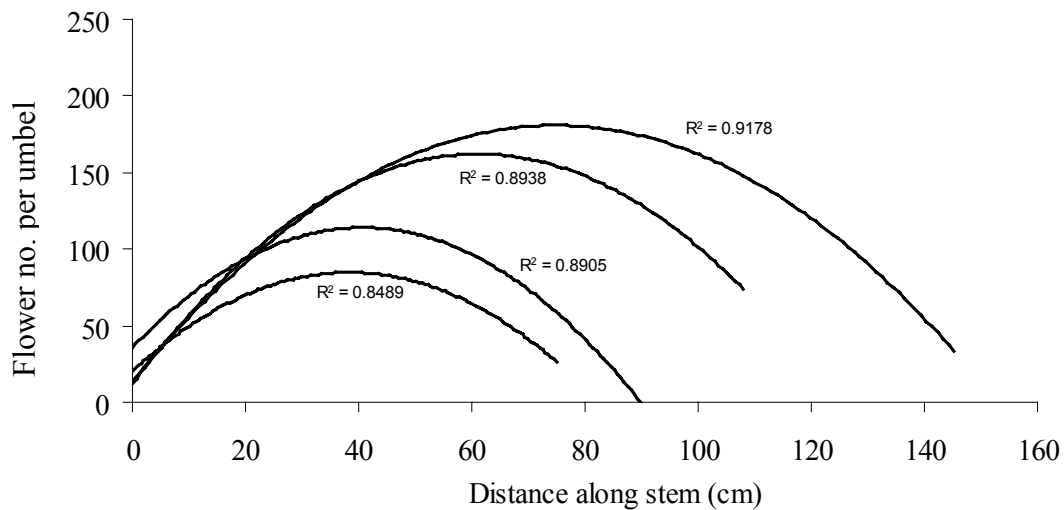


Fig. 2 Polynomial regression analyses for *A. scott-thomsonii* showing correlations between flower number per umbel with distance along stem for four *A. scott-thomsonii* inflorescences of varying sizes at Hakataramea Pass. R<sup>2</sup> values for each inflorescence are shown.

**Appendix 2** Insects from the order: Diptera (flies) found on flowers of *Aciphylla aurea*, *A. scott-thomsonii* and *A. 'lewis'* at four sites from North to South Canterbury, South Island of New Zealand (BP = Burkes Pass, HP = Hakataramea Pass, LP = Lewis Pass). Insects were caught during December 2004 and January 2005. Highlighted in bold are families that are likely to be important as pollinators of *Aciphylla*.

Family (common name)	Genus/species	Stage found	Location collected	Known to feed on	Notes
Agromyzidae (leafminer flies)	several spp.	adult	HP		Family - leaf grazers, vary small (<3mm)
<b>Bibionidae</b> (march flies)	several spp.	adult	BP, HP	nectar	
Empididae (danceflies)	at least one spp.	adult	BP, HP	nectar	family - insect predators, long proboscis
<b>Calliphoridae</b> (blowflies)	<i>Calliphora quadrimaculata</i>	adult	BP, HP	pollen/nectar	family - flower visitors
Muscidae (houseflies)	3 spp.	adult	BP, HP	nectar	family - frequent visitors to Apiaceous flowers
Phoridae (humpbacked flies)	several spp.	adult	BP, HP		family - flower visitors/breed on decaying matter
Simuliidae (sandflies/midges)	<i>Austrosimulium</i> sp.	adult	HP	nectar	
<b>Stratiomyidae</b> (soldierflies)	<i>Eulalia</i> sp.	puparium	HP		<i>Eulalia</i> adults frequent visitors to Apiaceae, good pollinators
	<i>Beris</i> sp.	adult	HP	nectar	
<b>Syrphidae</b> (hoverflies)	<i>Syrphus</i> sp.	adult	BP, HP	pollen/nectar	family - mostly smooth bodies, larvae predators of aphids/scale insects
	<i>Eristalis tenax</i>	adult	BP, HP	pollen/nectar	<i>Eristalis</i> common, important pollinators of many flowers
		larvae	BP, HP		
	<i>Helophilus</i> sp.	adult	BP, HP	pollen/nectar	<i>Helophilus</i> hairy/large
	<i>Melangyna novaezelandiae</i>	adult	BP, HP	pollen/nectar	
	<i>Melangyna</i> sp.	adult	BP, HP	pollen/nectar	
<b>Tachinidae</b> (parasitoid flies)	<i>Montanarturia dimorpha</i>	adult	HP	nectar/pollen	parasite of Lepidoptera caterpillars
	<i>Procissio cana</i> group	adult	BP, HP	nectar/pollen	parasites of larval grass grub ( <i>Costelytra zelandica</i> )
	<i>Protohystricia</i> sp.	adult	BP, HP	nectar/pollen	short proboscis, common on open/flat flowers, abundant in grasslands
		larvae	BP, HP		parasitises porina (hepialid) caterpillars
	<i>Pales</i> sp.	adult	BP, HP	nectar/pollen	
Tephritidae (fruit flies)	at least one spp.	adult	BP, HP	nectar	family unlikely to be significant pollinators
Therevidae (stiletto flies)	<i>Anabarhynchus</i> sp.	adult	BP, HP	nectar	larva in branches and rotting logs
Tipulidae (craneflies)	<i>Discobola</i> sp.	adult	LP		
	<i>Holocusia</i> sp.	adult	LP		
	<i>Zelandotipula</i> sp.	adult	LP	nectar	inhabits bogs
		larvae	LP		root feeders (generally), larva subaquatic

**Appendix 2 continued...** Insects from the orders: Lepidoptera (flies) and Coleoptera (beetles) found on flowers of *Aciphylla aurea*, *A. scott-thomsonii* and *A. 'lewis'* at four sites from North to South Canterbury, South Island of New Zealand (BP = Burkes Pass, HP = Hakataramea Pass, LP = Lewis Pass). Insects were caught during December 2004 and January 2005.

Order/Family	Genus/species	Stage found	Location coll	Food	Notes
<b>Lepidoptera:</b>					
Crambidae	<i>Orocrambus flexuosellus</i>	caterpillar adults	BP/HP BP/HP	nectar	NZ's most ubiquitous moth visit flowers, largely nocturnal but easily disturbed
Geometridae	<i>Dasyuris partheniata</i>	caterpillar	BP/HP	leaflets	nocturnal, drop to the centre of plant where they are safe
Noctuidae	<i>Agrotis ipsilon</i> (greasy cutworm)		BP		market gardens/lucerne paddocks Sth Canterbury
	<i>Graphania nullifera</i> *	caterpillar	BP/HP	leaves	deep ovoid notching of leaf petioles
<b>Coleoptera:</b>					
Anthricidae (ant-like flower beetles)	at least one sp.	adult	BP	pollen	
Cerambycidae (flower longhorn beetles)	<i>Zorion</i> sp. <i>guttigerum</i> "Blue longhorn"	adult	HP	pollen	<i>Zorion</i> most strikingly and colourful beetles in NZ fauna
Coccinellidae (ladybirds)	<i>Coccinella leonina</i> <i>C. undecimpunctata</i> <i>Adalia bipunctata</i> unidentified sp.	adult	BP/HP/LP	aphids aphids aphids aphids	possible contribution to pollination
Curculionidae	<i>Hadramphus tuberculatus</i> "Canterbury knobbed weevil" <i>Lyperobius huttoni</i> "Hutton's speargrass weevil"	adult adult	BP HP	leaves/pollen leaves/pollen	Specific to <i>Aciphylla</i> , only known site is BP Specific to <i>Aciphylla</i> , larvae subterranean and feed on roots
Helodidae	unknown	adult	HP	?	
Leiodidae (small carrion beetles)	unknown	adult	BP	?	probably feed on decaying matter, fungi, carrion
Melryidae (flower beetles)	<i>Dasytes</i> sp.	adult	HP	pollen	mainly herbivorous, found on flowering shrubs
Oedemeridae (lax beetles)	<i>Selenopalpus aciphyllae</i>	adult	BP		Live in vegetation, common on flowers or herbage
Scarabaedae (scarabaed beetles)	<i>Prodontria</i> sp. <i>Pyronota festiva</i> "manuka beetle"	adult adult	BP/HP BP		Root feeders Folivores
Staphylinidae (rove beetles)	unknown	adult	BP/HP/LP	?	live inside stalks of <i>Aciphylla</i>
Tenebrionidae (darkling beetles)	<i>Mimopeus convexus</i>	adult	BP	?	only found at a few localised sites in Mckenzie Basin

**Appendix 2 continued...** Insects from the orders: Hemiptera, Hymenoptera and Plecoptera found on flowers of *Aciphylla aurea*, *A. scott-thomsonii* and *A. 'lewis'* at four sites from North to South Canterbury, South Island of New Zealand (BP = Burkes Pass, HP = Hakataramea Pass, LP = Lewis Pass). Insects were caught during December 2004 and January 2005.

Order/Family	Genus/species	Stage found	Location coll	Food	Notes
<b>Hemiptera:</b>					
Acanthosomatidae (shield bugs)					
	<i>Oncacontius vittatus</i>	adult	LP	leaves	Live in dead flowerstalks of <i>Aciphylla</i>
Aphididae (aphids)					
	<i>Euschizaphus</i> (undescribed)		Porters Pass	sap-suckers	undescribed and rare
	<i>Cavariella aegopodii</i>		Sth Island	sap-suckers	at least five species of introduced aphids found on <i>Aciphylla</i>
	<i>Macrosiphum euphorbiae</i>			sap-suckers	
	<i>Brachycaudus helichrysi</i>			sap-suckers	
	<i>Myzus persicae</i>			sap-suckers	
<b>Hymenoptera:</b>					
Colletidae (solitary bees)	<i>Leioproctus fulvescens</i>	adult	HP	pollen	important pollinator, many hairs, short-tongued, endemic solitary bee
Cicadidae (cicadas)	<i>Kikihia</i> sp.	adult	LP		
<b>Plecoptera: (stoneflies)</b>					
Gripopterygidae	<i>Zelandoperla fenestrata</i>	adult	LP	pollen	inhabit boggy areas
	<i>Zelandobius confuses</i>	adult	LP	pollen	inhabit boggy areas

\* Only identifiable taxa have been reported in the tables above. Many other insects were found but have not yet been identified. *Aciphylla* has many specific herbivores including the common Lepidopteran *Graphania nullifera*, which were not found on the flowers of *Aciphylla* during this study (but are still reported above due to the significant damage they have on the roots in particular).

NOTES: All insects collected during this study were given to Alison Evans (Department of Conservation, DoC) under the requirements of the collection permit. *Hadramphus tuberculatus* was deposited in the Canterbury Museum and its conservation status is currently being revised to change it from “extinct” to “nationally endangered” or “nationally critical” by DoC.