Breeding systems and reproduction of indigenous shrubs in fragmented ecosystems

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Abstract

Sixteen native shrub species with various breeding systems and pollination syndromes were investigated in geographically separated populations to determine breeding systems, reproductive success, population structure, and habitat characteristics. Of the sixteen species, seven are hermaphroditic, seven dioecious, and two gynodioecious. Two of the dioecious species are cryptically dioecious, producing what appear to be perfect, hermaphroditic flowers, but that function as either male or female. One of the study species, *Raukaua anomalus*, was thought to be dioecious, but proved to be hermaphroditic. *Teucridium parvifolium*, was thought to be hermaphroditic, but some populations are gynodioecious. There was variation in self-compatibility among the four *Alseuosmia* species; two are self-compatible and two are self-incompatible. Self-incompatibility was consistent amongst individuals only in *A. quercifolia* at both study sites, whereas individuals in *A. macrophylla* ranged from highly self-incompatible to self-compatible amongst four study sites. The remainder of the hermaphroditic study species are self-compatible. Five of the species appear to have dual pollination syndromes, e.g., bird-moth, wind-insect, wind-animal.

High levels of pollen limitation were identified in three species at four of the 34 study sites. Moderate to high levels of pollen limitation were evident in 50% of the gender dimorphic populations compared with 31% of hermaphroditic populations. *Melicope simplex* populations were female-biased in 14 study plots and successful fruit set was influenced by combinations of male plant density, male flower numbers and distance to the nearest pollen. Natural fruit set in wind-pollinated species was generally higher than in animal-pollinated taxa. Populations of *Coprosma spathulata* were mostly male-biased, and male plant density and proximity influenced natural fruit set, with a correlation between low fruit set and low male density. Population recruitment was evident in 32 of the 34 sites. In *Pimelea arenaria,* recruitment failure was widespread in populations throughout the North Island despite high seed set. Gender ratios showed variation from north to south, with a lower proportion of females with increasing latitude. Many of the sixteen shrubs in this study show remarkable resilience to the effects of fragmentation of natural ecosystems, and many of the species have benefited from the creation of new edge habitat after perturbation – it may be that edges are important refugia for some native taxa, especially shrubs.

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Chapter 1

Introduction to thesis

1.1   Background

During the second half of the 20th century, the rate of species extinction reached such an unprecedented level in earth's history, that some authors have suggested the world is experiencing another major extinction event (Honnay et al. 2005). Four major human-induced processes are considered responsible for the present biodiversity crisis: over-exploitation by hunting and fishing; environmental deterioration; introduction of exotic species; and fragmentation of habitats (Honnay et al. 2005). The fragmentation of natural ecosystems is a global phenomenon and is increasingly perceived as one of the major factors influencing the persistence of species and species assemblages, and has become an important theme in conservation research (Haila 2002; Hobbs & Yates 2003). Fragmentation is described as the reduction of large expanses of natural habitat into small isolated patches that are surrounded by a matrix of transformed land usually dominated by anthropocentric activities (Saunders et al. 1991; Rathke & Jules 1993; Ward & Johnson 2005).

The size and isolation of fragments can have a major influence on population dynamics. Small remnants can only contain small populations, and species become extremely vulnerable to extinction below a certain population size, even if the quality of the habitat is optimal (Shaffer 1981). Small populations in isolated habitat remnants are particularly susceptible to increased inbreeding, decreased individual fitness, and loss of genetic variation (Rathke & Jules 1993; Murcia 1995; Aizen et al. 2002; Honnay et al. 2005).

Pollination is important as the first stage in the sexual reproduction of flowering plants, and because plants are immobile, they rely on biotic or abiotic vectors to transport pollen, and by some estimates, over 90% of angiosperms are pollinated by animals (Ashman et al. 2004; Harris & Johnson 2004). Disruption of plant-pollinator

mutualisms can occur because of the sensitivity of many flower visitors to changes in habitat quantity or quality triggered by fragmentation (Aizen et al. 2002). Although plant species can differ in their vulnerability to habitat fragmentation according to their dependence on pollination mutualism, any degradation in plant-pollinator mutualism may provoke decreases in seed number and quality, and can constitute the first step towards the demographic collapse of populations (Aizen et al. 2002). Understanding how fragmentation affects plant-animal interactions is important for developing effective tools for the management of fragmented ecosystems (Hobbs & Yates 2003).

Studies have shown that fruit and seed production is reduced in fragments due to altered pollinator visitation rates, lower pollinator abundance, or decreases in pollen transfer (e.g., Aizen & Feinsinger 1994; Bruna 2003). Pollinator specialisation and plant breeding systems are important reproductive traits in determining the degree of reproductive responsiveness of plants to habitat fragmentation (Aizen et al. 2002).

Plant breeding systems can range from those with enforced outbreeding mechanisms, such as dioecy, to those that have assured sexual reproduction via within-flower autogamous selfing (Lloyd 1992). In animal-pollinated species, the gradient of inbreeding-outbreeding will influence the degree of dependence on the pollination mutualism required for reproduction (Bond 1994). The degree of pollination specialisation can vary from extreme specialists to extreme generalists, with pollination specialists expected to be more vulnerable to mutualism failure than generalists (Bond 1994; Aizen et al. 2002). Flowering plants often produce many more flowers than fruit, but low fruit set may result from a variety of factors, including pollen limitation due to a decline in pollinators (Dalgleish 1999). Pollen limitation is described as an inadequate quantity or quality of pollen that reduces reproductive success (Ashman et al. 2004). A plant population may show no immediate effect of fragmentation if individuals are long-lived, but a study of fruit set may predict future extinction if seed set is pollen-limited and greatly reduced (Rathke & Jules 1993).

An important consequence of fragmentation is its associated increase in edge area. Edges allow potentially negative edge effects to penetrate into the fragment and affect the viability of occurring species (Young & Mitchell 1994; Honnay et al. 2005). Edge effects are the result of the interaction between two adjacent ecosystems when they are

separated by an abrupt transition (Fig. 1.1). Differences in vegetation composition and structure, and in microclimate between forest margins and forest interior are known to exist (Saunders et al. 1991; Young & Mitchell 1994; Murcia 1995). At newly created edges, changes in local microclimate, soil conditions, nutrient status, and water availability can modify the growth and reproduction of plant species, to the extent that the conditions are altered beyond their physiological tolerances, resulting in mortality and failure to regenerate (Hobbs & Yates 2003). Such fundamental change can produce a distinctly different suite of species that occupy the edge (Young & Mitchell 1994).

Fig. 1.1 An example of a kahikatea (*Dacrycarpus dacrydioides*) remnant in the Waikato region, showing a sharp boundary between the remnant and surrounding pasture.

Although not usually on the scale of human-induced fragmentation, natural disturbance is recognised as an integral component of the dynamics of many ecosystems (Hobbs & Yates 2003). Disturbance events can disrupt ecosystem, community, or population structure, but they are an important feature that facilitates regeneration of species that require perturbation of some form (Hobbs & Yates 2003). Unpredictable climatic, tectonic and volcanic disturbances are a feature of New Zealand (Meurk 1995). Massive disturbances can initiate successional trends in forest composition; however, the changes that have accompanied human colonisation may have had profound effects on regeneration (Wardle 1991).

1.2   Human-induced fragmentation in New Zealand

New Zealand is an archipelago of continental fragments and volcanic-oceanic crustal material, of Gondwanic origin, and lies between latitude 30° and 55° S (Meurk 1995). Muerk (1995) describes New Zealand's split from the Gondwana supercontinent as a "Noah's Ark carrying a small parcel of Gondwanic life as it wandered into lonely obscurity in the vastness of the Pacific Ocean". Because of the long separation time from Gondwana (80 million years ago), 82% of c. 2200 species in the vascular flora are endemic (Muerk 1995). In pre-human times about 85–90% of the total land area of New Zealand was covered with forest (McGlone 1989).

Severe impacts on the New Zealand environment and biota is linked to the arrival of the first Polynesian settlers about 1000 years ago, and by the time of widespread European settlement in the 1840s and 1850s, nearly half the original forest had been destroyed (McGlone 1989). Recent estimates of the percent loss of indigenous land cover since human settlement show a 75% loss in coastal dunes, a 75% loss in wetlands, and a 71.1% loss in indigenous forest (Leathwick et al. 2003).

Associated with the destruction of vast amounts of forest since human settlement, about 30 bird species and an unknown number of other vertebrates and invertebrates have become extinct through hunting, the introduction of predators, and habitat destruction (McGlone 1989). In the indigenous vascular plant flora, 792 described plants (34% of the total flora) are listed as threatened (deLange et al. 2004). Of the 380–400 indigenous shrub species (16% of the vascular flora) (Allan 1961; Smith-Dodsworth 1991; Wilson & Galloway 1993), 155 are currently listed in the threatened and uncommon plants inventory (deLange et al. 2004). New Zealand is considered a biodiversity 'hotspot' because of the uniqueness of its fauna and flora and the high levels of endemism. Although nearly one third of New Zealand's land mass is held in national parks, conservation parks and reserves, and managed by the Department of Conservation, major alien animal pests, e.g., possums, goats, deer, stoats, rats, and weed pests are continuing to diminish New Zealand's natural heritage (Department of Conservation 2002). The subtle impacts of fragmentation and habitat change on plant-animal interactions may be amplified into long-term effects on critical life history stages, and on the integrity of reserves and other managed landscapes (Aizen & Feinsinger 1994).

Conversely, limited fragmentation may have some benefits (see Kelly et al. 2000). For the indigenous flora of New Zealand, an understanding of the autecology of an individual species is important for identifying factors that could be influencing population persistence or contributing to population declines, and for developing sound management plans for conservation and recovery programmes.

1.3   Current knowledge about the breeding systems of indigenous shrubs

Research into flower biology began in New Zealand in the 1870s when Cheeseman described the pollination mechanism of the orchid *Pterostylis trullifolia*. Observations on native plants and their floral biology have since been reviewed by Thomson (1881), Heine (1937), Godley (1979), Lloyd (1985), Webb and Kelly (1993) and Webb et al. (1999). In a recent review of pollination systems, Newstrom and Robertson (2005) considered the breeding and sexual systems of New Zealand plants to have been relatively well studied. However, only six studies of reproduction in shrub species occur in the published literature (Newstrom & Robertson 2005).

To date, there have been no published reports of the effects of habitat fragmentation in relation to the reproductive success and persistence of indigenous shrubs in fragmented landscapes in the New Zealand.

1.4   Thesis objectives and structure

The primary objectives of this research were to investigate the breeding systems, reproductive success and population structure of native shrub species, and to assess the impacts of habitat fragmentation on population persistence. The fundamental research questions were:

> *What are the breeding systems and strategies of the study species? Is reproductive success affected by the proximity of conspecifics? Are species with certain breeding systems more likely to be affected by fragmentation effects? Are populations declining or stable?*

This thesis presents a series of chapters that represent multi-species investigations of indigenous shrubs of New Zealand. The research chapters are arranged in an hierarchical order from populations and species, to a study of pollen and ovule frequency, with chapters 3–8 in alphabetical order by species. Chapters 2–9 are presented in paper style, and as such, a degree of repetition is evident when similar issues are being addressed and/or when the same methods have been applied. Chapter 2 describes the breeding systems, reproductive success, and population structure of 16 indigenous shrub species, discusses fragmentation effects, and identifies six species for further investigation. Chapters 3–6 describe further investigations into the reproductive biology of four shrub species, *Alseuosmia macrophylla, Coprosma spathulata, Melicope simplex* and *Pimelea arenaria*. Chapter 7 reviews the breeding system of *Raukaua anomalus*. This chapter has been published in the *New Zealand Journal of Botany 43*, as MF Merrett 2005. *The breeding system of Raukaua anomalus, a small-leaved shrub from New Zealand*. Chapter 8 reviews the breeding system of *Teucridium parvifolium*. This chapter has been published in the *New Zealand Journal of Botany 45*, as MF Merrett 2005. *Gynodioecy in Teucridium parvifolium (Verbenaceae), a threatened, small-leaved shrub from New Zealand*. Chapter 9 describes the pollen-ovule ratios of shrub species.

For the purposes of this thesis, the papers are slightly amended so cross-referencing is facilitated, but differ in no substantive way from the versions submitted to the journal.

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Chapter 2

Fragmentation and plant reproduction: case studies of sixteen native shrubs

2.1   Abstract

Sixteen native shrub species representing a range of breeding systems and pollination syndromes were investigated to determine the impact of forest fragmentation on their reproductive success and population structure. Eight of the species are hermaphroditic, seven are dioecious and one is gynodioecious. Two of the dioecious species are cryptically dioecious, producing what appear to be perfect, hermaphroditic flowers, but that function as either male or female. One of the species, *Raukaua anomalus*, thought to be dioecious, proved to be hermaphroditic, and another of the species was thought to be hermaphroditic but some populations are gynodioecious. Two of the hermaphroditic *Alseuosmia* species were self-incompatible: in *A. macrophylla*, individuals and populations ranged from self-incompatible to self-compatible, whereas *A. quercifolia* were consistently self-incompatibility. The remainder of the species that produce hermaphroditic flowers were self-compatible, with SII ranging from 0.01 to 0.53. Five of the species appear to have dual pollination syndromes, e.g., bird-moth, wind-insect, wind-animal. Within the 16 species, high levels of pollen limitation (PLI > 0.75) were identified in some populations of three species, *A. macrophylla, C. spathulata,* and *C. juniperina*. Moderate pollen limitation (PLI 0.50–0.75) was present in a further seven species. Moderate to high levels of pollen limitation were evident in 50% of the gender dimorphic populations compared with 31% of hermaphroditic populations. There was no correlation between natural fruit set and the distance to the nearest pollen source in any of the study populations. Population recruitment was evident in 32 of the 34 study populations, although in varying abundance. The most likely cause of poor population recruitment appears to be lack of suitable habitat, and possibly herbivory. Most of the sixteen shrub species in this study show a remarkable resilience to the effects of fragmentation of natural ecosystems, and many of the species appear to have benefited from the creation of new edge habitat after perturbation; it is suggested that edges are important refugia for some native taxa, especially shrubs.

2.2 Introduction

Shrubs are an important component of New Zealand's natural ecosystems, comprising approximately 380–400 species (16% of the native flora). They are mostly endemic (Allan 1961; Smith-Dodsworth 1991; Wilson & Galloway 1993; Poole & Adams 1994), with 155 currently listed in the threatened and uncommon plants in New Zealand inventory (de Lange et. al. 2004). The breeding systems of several shrub species have been investigated (Primack 1979; Primack & Lloyd 1980; Godley & Smith 1981; Garnock-Jones & Molloy 1982; Powlesland 1984; Webb 1985; Lloyd & Wells 1992; Webb 1994); however, a comparative study of the reproductive success of species with various breeding systems has not previously been undertaken.

Characteristics of New Zealand flowering plants include a predominance of small, white or pale coloured flowers, simple floral structures that lack complicated adaptations for precise modes of pollination, fleshy fruits, and separate sexes (Godley 1979; Webb & Kelly 1993; Wilson & Galloway 1993; Lloyd 1985). The New Zealand flora is also characterised by a paucity of specialised pollinators and a disproportionate reliance on unspecialised insect groups that promiscuously visit a wide range of flowers, with several of the most specialised insect pollinator groups (e.g., long-tongued bees, hawkmoths, butterflies), very poorly represented (Lloyd 1985).

Flowers are among the most complex and diverse objects in the plant kingdom (Proctor et al. 1996) and their efficacy in the process of reproduction generally determines the viability of plant species. Breeding systems are defined as 'all aspects of sex expression in plants that affect the relative genetic contributions to the next generation of individuals within a species' (Wyatt 1983). Sexual expression in many plants is variable through time and/or space and thus does not fit into neat categories, resulting in confusion in terminology and a lack of standardisation, sometimes with different meanings applied at flower, plant, and population level (Sakai & Weller 1999). In a review of the terminology associated with gender and sexual dimorphism in plants, Sakai and Weller (1999) have defined the commonly used terms, in an attempt to avoid confusion. Throughout this thesis, and because of the scope of the study, I will adopt the more traditional terminology (Sakai & Weller 1999).

Breeding systems

The earliest flowering plants were almost certainly hermaphrodites, and approximately 80% of the world's flowering plants are hermaphroditic (Proctor et al. 1996). Among the hermaphroditic taxa there are a range of strategies and mechanisms to promote cross-pollination (Faegri & van der Pijl 1979; Proctor et al. 1996). Hermaphrodism is used to describe plants that produce perfect, hermaphroditic (pistillate and staminate) flowers with both male and female functions (Sakai & Weller 1999). Dioecism, when plants produce unisexual flowers on separate plants, is the most common form of gender dimorphism (Proctor et al. 1996). Gynodioecy is a form of gender dimorphism involving the production of female (pistillate) and hermaphrodite (pistillate and staminate) flowers on separate plants in the same population (Sakai & Weller 1999). Gynodioecy is regarded as an intermediate evolutionary step from hermaphrodism to dioecy, involving male-sterile individuals arising within a population of hermaphrodites (Delph & Lloyd 1996; Webb 1999). The evolutionary pathway from hermaphrodism to gynodioecy and often on to dioecy is the best documented transition to gender dimorphism, although in many genera, gynodioecy appears to be a persistent breeding system in its own right (Webb 1999).

Successful pollination is usually the main mechanism by which seeds are formed and it is a critical stage in the persistence of most plant populations (Proctor et al. 1996). The various interactions of pollination are about maintaining at least, the possibility of cross-fertilisation (Faegri & van der Pijl 1979; Proctor et al. 1996). Cross-fertilisation provides new genetic combinations, allowing populations the potential to adapt to changes in environmental conditions, and reduces the effect of potentially damaging or lethal mutations (Proctor et al. 1996). Darwin (1877) first demonstrated that out-crossed progeny were usually more vigorous than those produced by self-fertilisation.

One of the distinctive features of the New Zealand flora is the high frequency of gender dimorphism (Heine 1937; Carlquist 1966; Godley 1979; Lloyd 1985; Webb & Kelly 1993). The incidence of dioecy varies considerably in different regional floras (Sakai et al 1995). For example, dioecy occurs in 2.8% of Californian flowering plants (Sakai et al. 1995), less than 5% of the British flora, in about 20% of Mediterranean shrub flora, and in up to 25% of tropical rain forest trees (see Proctor et al. 1996). It is also common

in island floras, e.g., Hawaii where 14.7% of angiosperm species are dioecious (Bawa 1979; Sakai et al. 1995). In an analysis of the occurrence of gender dimorphism in 83 seed plant genera in the indigenous flora of New Zealand (363 genera were recognised), Webb et al. (1999) found that gender dimorphism was represented in 23% of the indigenous genera. Dioecism was the most common form of gender dimorphism, occurring in 17.9% of the New Zealand genera, and gynodioecism the second most frequent, occurring in 7.2% of the genera (Webb et al. 1999). In some cases, the presence of dioecy has not been recognised until recently e.g., *Elingamita johnsonii* (Baylis 1995), *Dysoxylum spectabile* (Braggins 1996).

In many gender dimorphic species, vestigial organs of the other sex are often present in the flower (Proctor et al. 1996; Eckhart 1999), with male flowers often larger and more showy than female flowers (Proctor et al. 1996). The smaller female flowers may be a consequence of the relationship between petal and stamen development; suppressed anther development associated with suppressed petal development (Proctor et al. 1996). In *Carpodetus serratus*, female flowers were similar in size to hermaphrodites but the stamens were shorter (Shore 1969). In the gynodioecious *Fuchsia excorticata*, *F. colensoi*, and *F. perscandens*, the female flowers were much smaller, and had small staminodes compared with the hermaphrodites (Thomson 1881; Godley 1955). The female flower is also smaller than the hermaphrodite in *Pimelea* species (Burrows 1960), and in several species of *Hebe* (Delph 1996).

Gender ratios in gender dimorphic species can vary widely between and within species, and may be equal, or biased toward males or females (Foweraker 1932; Godley 1955, 1963, 1964; Burrows 1960; Webb 1981; Connor 1984). Equal or male-dominated populations appear the most common form of gender bias in the New Zealand dioecious flora (Wild & Zotov 1930; Godley 1964; Shore 1969; Rivers 1971; Lloyd 1974; Lloyd & Webb 1977; Webb & Lloyd 1980). Godley (1964) investigated 16 native, dioecious species in natural populations and found 10 were male-biased, one was female-biased, and the remainder had more or less equal proportions of the sexes.

Reproductive strategies

The reproductive strategies of plants are diverse, and the balance between outcrossing and selfing can vary depending on life history and the ecology of the species (Faegri & van der Pijl 1979; Proctor et al. 1996). Breeding strategies can range from obligate outcrossing or dioecy to facultative selfing (Faegri & van der Pijl 1979; Proctor et al. 1996). Any incompatibility system reduces the pool of potential pollen donors and increases the probability of inadequate fertilisation (Burd 1994), but reduces the likelihood of inbreeding depression caused by selfing (Delph & Lloyd 1996).

The commonest and possibly most effective strategy that plants use to avoid self-fertilisation is self-incompatibility, where a physiological barrier makes it impossible or difficult for a flower to fertilise itself, even when the flower has been pollinated with its own pollen; a mechanism whereby the plant is able to discriminate in favour of pollen other than its own (Wyatt 1983; Seavey & Bawa 1986; Proctor et al. 1996). One of the best-studied strategies involves the prevention of a germinating pollen grain from reaching the ovule by blocking of pollen tube growth in the style (sporophytic incompatibility) (Seavey & Bawa 1986). In late-acting self-incompatibility systems, the incompatibility occurs after the pollen tube has entered the ovule, but the ovule is aborted before fertilisation (gametophytic incompatibility) (Seavey & Bawa 1986).

In a study of the reproductive biology of *Pseudowintera colorata*, Lloyd and Wells (1992) found that pollen tubes entered the micropyles after both self and cross-pollination, but self-pollinated flowers demonstrated ovular self-incompatibility. Self-incompatibility has also been demonstrated in the New Zealand shrub flora in *Hebe vernicosa* (Frankel & Hair 1937), *Pentachondra pumila* (Godley 1966), *Discaria toumatou* (Primack 1979), *Geniostoma rupestre* (Rattenbury 1980), and *Corokia cotoneaster* (Webb 1994), but the precise mechanism has not been documented for any of the species.

Heteromorphic self-incompatibility systems are distinctive because of their easily recognisable differing flower forms, usually involving different lengths of style and reciprocal positioning of anthers and stigmas in two or more morphs (heterostyly) (Ganders 1979). Heteromorphy is nearly always a characteristic of insect pollinated

flowers that contain few stamens (Ganders 1979; Proctor et al. 1996). Heterostyly is not known to occur in the New Zealand flora (Godley 1979; Webb et al. 1999).

Separation of the male and female functions in individual flowers has traditionally been viewed as a strategy to avoid self-pollination, although the role of pollinators in the evolution of these breeding system mechanisms is of current interest (Dafni 1992). A common strategy to reduce self-pollination is separation of the anthers and stigma either spatially (herkogamy) or temporally (dichogamy).

Webb and Lloyd (1986) identified various types of herkogamy, depending on floral traits such as flower shape and anther position. In dichogamy there is temporal separation in the presentation of pollen and stigmas within an individual hermaphroditic blossom, and self-pollination is prevented if pollen is shed at a time when the stigma is not receptive (Faegri & van der Pijl 1979; Lloyd & Webb 1986; Proctor et al. 1996). Two forms of dichogamy occur: protandry, where the pollen is available before the stigma is receptive, and is the commoner of the two forms of dichogamy, and protogyny, where the stigma is receptive before pollen is available (Faegri & van der Pijl 1979; Lloyd & Webb 1986; Proctor et al. 1996).

Thomson (1881) noted protandry in 37% of the 235 New Zealand hermaphrodite species he investigated, including the woody species *Cyathodes juniperina, Discaria toumatou, Elaeocarpus hookerianus*, and *Gaultheria antipoda*. Esler (1969) noted protandry in *Rhopalostylis sapida*, and Moore (1977) described strong protandry in *Ascarina lucida*. Protandry has also been reported in *Rhabdothamnus solandri* (Petri 1903), *Schefflera digitata* (Godley 1979), *Hoheria sextylosa* and *Kunzea ericoides* (Lloyd & Webb 1986), and *Vitex lucens* (Lloyd & Webb 1986; Barrell et al. 1997). Of the variety of means of disposing of the used stamens or anthers in protandrous species, probably the commonest method is abscission at the base of the filament, as occurs in many Umbellifereae (Lloyd & Webb 1986). In some species, e.g., *Hebe*, only the anthers are abscised. Other strategies include the anthers bending inwards, or outwards and downwards after pollen is presented, e.g. *Kunzea ericoides, Hoheria sextylosa* (Lloyd & Webb 1986). In *Rhabdothamnus solandri*, the anthers present initially at the roof of the gullet blossom then drop to the floor while the stigmas are presented, and in

Discaria toumatou the stigma is exposed after pollen removal, a simple but uncommon mechanism (Lloyd & Webb 1986).

In protogyny, the stigma is receptive for a time before the anthers mature, but if pollination does not occur, the stigma may remain receptive when the anthers release their pollen. Protogyny is not common among insect-pollinated plants, where protandry is more common, but is a feature of some wind-pollinated species (Proctor et al. 1996). Thomson (1881) noted that of the 235 New Zealand hermaphroditic species he investigated, only 8% were protogynous, and those were mostly herbaceous taxa. Moore (1973) describes a form of protogyny in three species and one cultivar of the *Hebe* group 'Paniculatae', where there is 'a precociously elongating style'. In *Coriaria arborea,* the styles and stigmas wither and drop before the anthers are presented Lloyd and Webb (1986).

Self-pollination is limited or prevented by both herkogamy and dichogamy; however, insect pollinators usually visit more than one flower per plant and, unless flowers open synchronously on the entire plant, or there are other self-incompatibility mechanisms, geitonogamous self-pollination can still occur (Proctor et al. 1996).

Despite the range of strategies and adaptations to avoid self-pollination, many plants are capable of self-fertilisation; it is common in many species and the norm in others (Proctor et al. 1996). As many as two-thirds of British plants are capable of self-fertilisation, although this is less common in long-lived perennials and woody species (Proctor et al. 1996), compared with only 12% of 25 forest trees in Costa Rica (Bawa 1979). In the woody flora of New Zealand, self-compatibility has been reported in *Carpodetus serratus* (Shore 1969), *Hebe amplexicaulis* (Garnock-Jones & Molloy 1982), *Hebe subalpina* (Delph & Lloyd 1991), *Pomaderris kumeraho* (Harvey & Braggins 1985), and *Vitex lucens* (Godley 1971; Barrell et al. 1997).

Habitual self-fertilisation is common in short-lived ephemeral plants that have a single flowering episode followed by death, and is a characteristic of many plants living in habitats with cold, wet weather during their flowering season. Over many generations, habitual selfing can lead to large numbers of plants becoming nearly uniform genetically (Proctor et al. 1996). Self-compatible species can increase plant fertility by

autogamous fertilisation, thus minimising dependence on pollinators for pollen transfer, and as a means of reproductive assurance when pollination is uncertain (Larson & Barrett 2000). However, although self-compatibility is a requirement for autogamy, not all self-compatible species are autogamous (Larsen & Barrett (2000). In some species, self-fertilisation serves as a risk-spreading strategy if cross-pollination does not occur. For example, styles or anthers may initially mature separately but if outcrossing does not occur, they may move into close proximity to allow for self-pollination (Proctor et al. 1996). In pollination experiments on *Phormium tenax*, Craig and Stewart (1988) demonstrated preferential outcrossing, but the ability to self-fertilise if cross-pollination did not occur. Selfing allows for rapid expansion of a population with a locally adaptive genetic combination that has proved successful, and assurance of seed set is also an advantage when there is only a single individual (Faegri & van der Pijl 1979; Proctor et al. 1996). Self-fertilisation may reduce the rate of seed set, seed weight, germination rate, and seedling establishment or survival (Proctor et al. 1996).

Apomixis occurs in plants when there is asexual reproduction without fertilisation (Proctor & Yeo 1973; Faegri & van der Pijl 1979). Examples are given of apomictic genera in which the flowers and stamens are almost vestigial, the stamens have been lost, or the anthers are empty, e.g., *Alchemilla* (Faegri & van der Pijl 1979). In some cases pollen is produced, but the grains are highly irregular and have probably lost all sexual potential. Some apomictic plants have large, open flowers that apparently function quite normally, and produce copious amounts of pollen, e.g., *Taraxicum* and *Hieracium* (Silvertown & Doust 1993). Interestingly, many apomicts apparently need pollination to start the apomictic development of seed, even if fertilisation does not appear to occur (Proctor & Yeo 1973; Faegri & van der Pijl 1979). Faegri and van der Pijl (1979) considered that apomictic populations have lost adaptability, which could restrict their ability to colonise new habitats. Silvertown and Doust (1993) considered apomicts an evolutionary dead end because they have lost their adaptive ability when environments change. Hair (1966) suggested there was strong presumptive evidence for apomictic reproduction in five triploid forms of *Pomaderris* in New Zealand. Apomixis has been reported in *Coprosma waima* and C. *robusta* (Heenan et al. 2002, 2003).

Fragmentation effects

The natural vegetation cover of every continent except Antarctica has been extensively modified by humans, leaving a legacy of natural ecosystems that have been fragmented into smaller spatially discrete units (Menges 1991; Saunders et al. 1991; Aizen & Feinsinger 1994; Gigord et al. 1999). In New Zealand, destruction and fragmentation of continuous forest tracts has occurred since human occupation, and has continued over the past 150 years since European settlement. For example, an estimated 71.1% of indigenous forest has been lost since human arrival in New Zealand (Leathwick et al. 2003). Some lowland forests in the North Island that formerly occupied extensive areas have been reduced to isolated remnants, primarily as a consequence of clearing for agriculture and urban development (Young & Mitchell 1994; Ministry for the Environment 1997). The reduction and fragmentation of plant communities is considered a threat to the maintenance of biodiversity (Saunders et al. 1991; Rathke & Jules 1993; Aizen & Feinsinger 1994; Gigord et al. 1999). Certain plant species are less likely to be found in fragments, often as a result of local extinctions, but the precise mechanisms responsible for the extinctions are usually not known, nor are the consequences of habitat fragmentation for long-term population dynamics (Bruna 2003).

Reproductive mutualisms (plants and their pollinators) are a complex web of interactions that, if broken, could cause a cascade of extinctions (Bond 1994). No mutualism is completely assured when pollinators are faced with a diversity of threats as a consequence of human activities, such as pesticide use, habitat modification, and invasions by alien plants and animals (Bond 1994). Mutualism failure is influenced not only by the degree of dependency (facultative or obligate), but by the importance of seeds in the demography of the plant (Bond 1994). An interruption or breakdown in plant-pollinator interactions could result in reduced fruit and seed set, with probable long-term consequences for population dynamics. The degree to which fragmentation may affect pollination and reproductive success is expected to vary among plant species, depending on differing breeding systems and strategies, and sensitivities to pollen transfer (Aizen & Feinsinger 1994). The success of the interaction between pollination systems and other aspects of the breeding systems is measurable ultimately in successful fruit production, dispersal, and establishment (Webb 1994). Seed

production and seedling establishment are vital for long-term population persistence; however, for most plant species it is unknown what critical threshold levels of recruitment are required for population maintenance (Bruna 2003).

Studies have shown that fruit production can be reduced in fragments as a consequence of low pollinator abundance, altered pollinator visitation rates, or decreases in pollen transfer, and that decreased recruitment in fragments is hypothesised to be a primary mechanism driving local extinctions (Rathke & Jules 1993; Aizen & Feinsinger 1994). However, certain compensatory mechanisms may ensure population persistence. For example, self-pollinating species with a diverse pollinator fauna are almost assured of seed set, and although some species with specialist pollinators may fail to set seed, they may persist by vegetative propagation (Bond 1994). Pollen limitation and subsequent reduced seed set in fragmented plant populations have been reported from a number of studies (Jennersten 1988; Ellstrand & Elam 1993; Aizen & Feinsinger 1994; Ågren 1996; Gigord et al. 1999; Hansman 2001). Pollen limitation is demonstrated empirically when female plant fertility in open-pollinated controls is lower than in flowers that have had supplemental pollination (Larson & Barrett 2000).

A variety of intrinsic and extrinsic factors are likely to contribute to pollen limitation (Burd 1994). Rathke and Jules (1993) suggested the reproductive success of plants was more likely to decline in fragments where there was pollinator specialisation and/or self-incompatibility, but that species with generalised pollinators were more likely to be reproductively successful. In a review of the effects of habitat fragmentation on the reproductive success of 46 hermaphroditic species, Aizen et al. (2002) found that a similar proportion of generalist and specialist species were negatively affected by fragmentation. Burd (1994) examined studies of 258 species, of which 62% were reported to experience significant pollen limitation. Bond (1994) provided qualitative criteria for assessing which plant attributes are most important for determining extinction risk, and determined that self-incompatible or dioecious species with pollinator and disperser specificity, obligatory disperser requirements, seed dependency for population persistence, and short-lived taxa were the most at risk from mutualism failure. Larson and Barrett (2000) reviewed the literature on 240 species and tested hypotheses concerning the association between pollen limitation and various ecological and life-history traits. Their results showed that self-compatible, autogamous,

nectariferous, and herbaceous species or those that occur in open habitats, exhibited significantly less pollen limitation than those with contrasting traits (Larson & Barrett 2000). However, they found no difference in the levels of pollen limitation between 'specialised pollinator' flowers and those with 'unspecialised' flowers (Larson & Barrett 2000). Variation in pollen limitation is influenced by a suite of factors including size and number of flowers displayed, floral longevity, ovule number, mating and pollination system, habitat type, the demography of populations, and local climatic conditions. Importantly, Larson and Barrett (2000) found that no single trait could explain the variation in pollen limitation. Chronic pollen limitation can reduce plant populations, and although reproductive or life history traits can evolve that minimise pollen limitation effects, these may not evolve at a rate fast enough to forestall extinction (Ashman et al. 2004).

Plant populations may be small, sparse, or declining for a variety of reasons, and pollen limitation-Allee effects (positive density dependence) can accelerate or exacerbate the problems in small populations when species benefit from the presence of their conspecifics (Ashman et al. 2004). Populations that rely on animal pollinators are more likely to experience Allee effects through at least three mechanisms: low-density populations are often less attractive to pollinators and therefore receive fewer pollinator visits; flowers may receive fewer conspecific pollen grains as a result of generalist pollinators visiting multiple species in foraging bouts; and there is a greater likelihood of increased selfing when pollinators visit more flowers per plant compared with larger populations (Ashman et al. 2004). Several studies have shown that selfing rates increase with decreasing plant density (Ashman et al. 2004). Small population size can also limit the number of compatible mates, especially for plants with barriers to selfing such as self-incompatibility mechanisms, dioecy, and heterostyly, and increase the relatedness among possible mates (Ashman et al. 2004).

Plant reproductive systems and strategies play a major role in the abundance and diversity of taxa in fragmented ecosystems (Lovejoy et al. 1986; Saunders et al. 1991), and since breeding systems significantly influence genetic diversity, identifying these is an appropriate initial step of investigation because seed output represents a direct, quantifiable measure that integrates numerous interacting factors, and provides an index of community integrity or 'health' (DeMauro 1993; Aizen & Feinsinger 1994).

This study presents an investigation into the breeding systems and strategies, reproductive success and population structure of sixteen native shrub species, and assesses the impacts of habitat fragmentation on population persistence. The following questions are addressed: (i) What are the breeding systems and strategies of the study species? (ii) Is reproductive success affected by the proximity of conspecifics? (iii) Are species with certain breeding systems more likely to be affected by fragmentation effects? (iv) Are populations declining or stable?

2.3   Materials and methods

Field experiments were carried out on populations of native shrub species within the Coromandel, Bay of Plenty, Waikato, King Country and Central North Island regions of the North Island. To compare any fragmentation effects, the study sites were of differing sizes, and the species comprised taxa with various breeding systems – hermaphroditic, dioecy, and gynodioecy, and pollination syndromes; entomophily, ornithophily, and anemophily – and included common and rare taxa. For each species, two geographically separated populations of differing density at sites of differing size were investigated. The exception was *Alseuosmia macrophylla* where four populations were identified because of polymorphism among populations. In total, 468 individual plants, of 16 species, in 34 populations were identified at 25 study sites (Fig. 2.1). At several of the sites, more than one species was investigated.

Study species

Alseuosmia macrophylla **A.Cunn.**

A. macrophylla (Alseuosmiaceae) is a much-branched, endemic shrub of lowland to lower montane forests from North Cape to about 38°S in the North Island, and northern South Island (Allan 1961). It flowers during spring, producing solitary or 2–5 flowered fascicles (Allan 1961). Flower colour, size, and plant growth habit is variable throughout its distribution in the North Island (unpubl. data), confirming Allan's (1961) suggestion that the species was more polymorphic than had been recognised. The fruit is a many-seeded berry that matures during autumn (Allan 1961). Four populations of *A. macrophylla* were investigated because of floristic polymorphism and variable breeding strategies in this species (unpubl. data). Study populations were located at Mamaku

Plateau, Maungatautari Mountain, Pirongia Forest Park, and Walter Scott Reserve (Fig. 2.1; Table 2.1). At each study site, *A. macrophylla* occurs under a tall, mostly closed canopy of native trees such as tawa (*Beilschmiedia tawa*) and rimu (*Dacrydium cupressinum*), but often within 50 m of forest edges and tracks. The size of the study sites ranged from 43 ha at Walter Scott Reserve, to 15 000 ha at Pirongia Forest Park (Table 2.1).
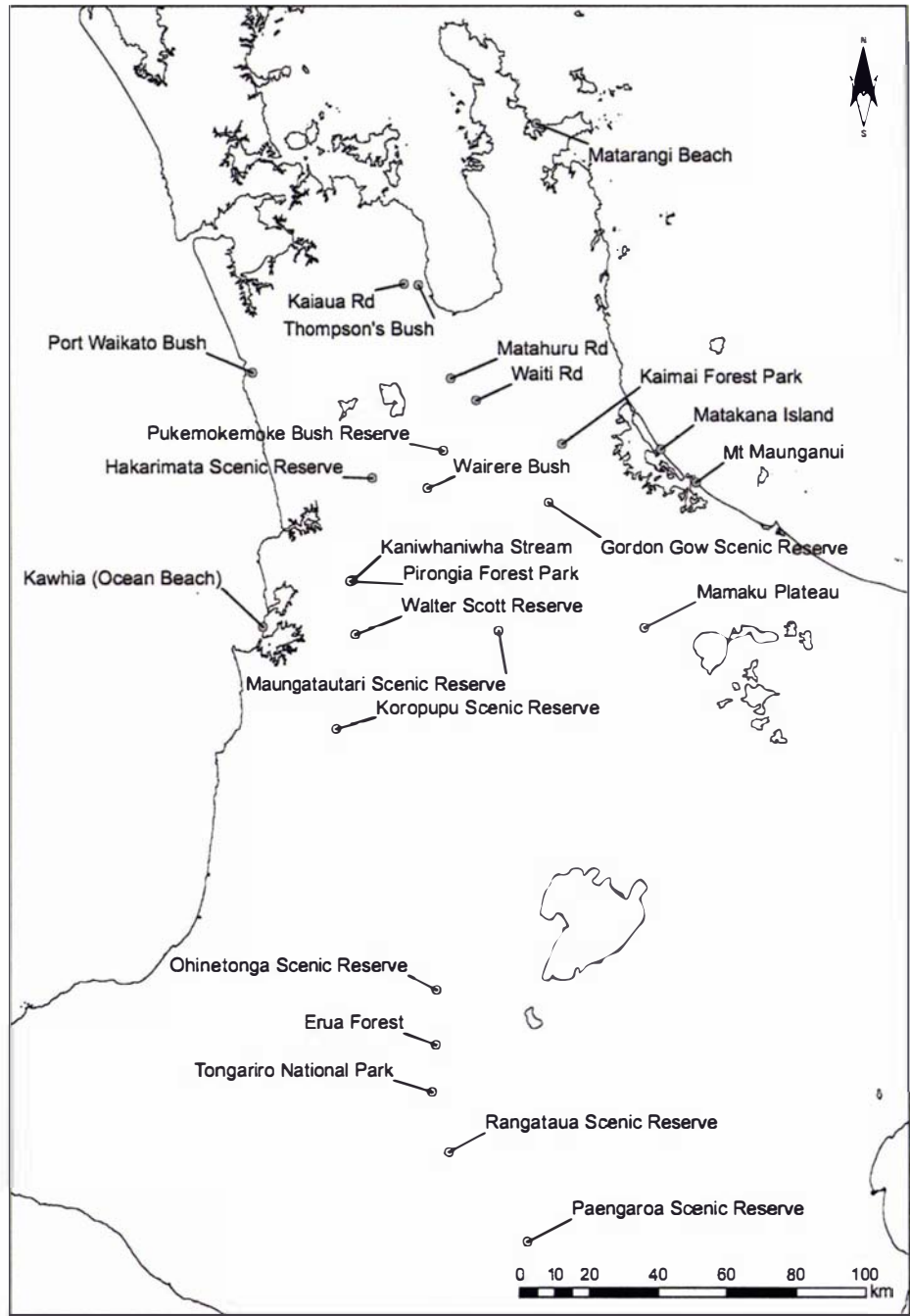
Fig. 2.1 Location of study sites of 16 native shrub species. Species and study site details are listed in Table 2.1.

Table 2.1 Study species in alphabetical order, study sites, species and site abbreviations, study site size, and study site grid references for 16 species in 34 populations. SR, Scenic Reserve, BR, Bush Reserve.

Species	Study sites	Species and study site abbreviation	Site size (ha)	NZMS 260 grid reference
Alseuosmia macrophylla	Mamaku Plateau	Alsmac MM	184	U15/775474
A. macrophylla	Maungatautari SR	Alsmac MT	2389	T15/351467
A. macrophylla	Pirongia Forest Park	Alsmac PR	15000	S15/923610
A. macrophylla	Walter Scott Reserve	Alsmac WS	43	S15/937457
A. pusilla	Erua Forest	Alspus EF	14750	S19/173269
A. pusilla	Tongariro National Park	Alspus TR	25000	S20/162131
A. quercifolia	Hakarimata SR	Alsque HK	1795	S14/988912
A. quercifolia	Pukemokemoke BR	Alsque PM	40	S14/197989
A. turneri	Ohinetonga SR	Alstur OH	148	S19/174427
A. turneri	Rangataua SR	Alstur RT	58	S20/211956
Coprosma spathulata	Hakarimata SR	Copspa HK	1795	S14/988912
C. spathulata	Pukemokemoke BR	Copspa PM	40	S14/197989
Coriaria arborea	Kaniwhaniwha Stream	Corarb KS	4	S15/931613
C. arborea	Matahuru Rd	Corarb MH	< 1	S13/218200
Cyathodes juniperina	Pukemokemoke BR	Cyajun PM	40	S14/197989
C. juniperina	Waiti Rd	Cyajun WR	< 1	S13/288136
Leucopogon fasciculatus	Hakarimata SR	Leufas HK	1795	S14/988912
L. fasciculatus	Waiti Rd	Leufas WR	< 1	S13/288136
Macropiper excelsum	Kaimai Forest Park	Macexc KF	4978	T13/537010
M. excelsum	Port Waikato Bush	Macexc PW	4.5	R13/635217
Melicope simplex	Gordon Gow SR	Melsim GG	7.4	T14/497841
M. simplex	Pukemokemoke BR	Melsim PM	40	S14/197989
Melicytus micranthus	Gordon Gow SR	Melmic GG	7.4	T14/497841
M. micranthus	Wairere Bush	Melmic WB	4.7	S14/148880
Melicytus novae-zelandiae	Matakana Island	Melnov MK	< 1	U14/823994
M. novae-zelandiae	Mt Maunganui	Melnov MN	1.5	U14/926898
Pimelea arenaria	Kawhia (Ocean Beach)	Pimare KW	4	R15/664480
P. arenaria	Matarangi Beach	Pimare MR	< 1	T10/466937
Pomaderris hamiltonii	Kaiaua Rd	Pomham KR	< 1	S12/082675
P. hamiltonii	Thompson's Bush	Pomham TB	35	S12/124472
Raukaua anomalus	Gordon Gow SR	Rauano GG	7.4	T14/497841
R. anomalus	Mamaku Plateau	Rauano MM	184	U15/775474
Teucridium parvifolium	Koropupu SR	Teupar KP	24	R16/882181
T. parvifolium	Paengaroa SR	Teupar PG	102	T21/436696

Alseuosmia pusilla Colenso

A. pusilla (Alseuosmiaceae) is a small (< 1 m tall), single-stemmed, understory, endemic shrub occurring in mature, montane native forests of the lower half of the North Island and the upper half of the South Island (Allan 1961; Gardner 1978). During November it produces sparse, hermaphroditic, pink, fragrant, mostly solitary flowers from leaf axils (Allan 1961). The multi-seeded, fleshy fruit is a berry (Allan 1961). The study sites were located within 50 m of the edge of Erua Forest (14 750 ha) and Tongariro National Park (25 000 ha), central North Island (Fig. 2.1; Table 2.1). The habitat of *A. pusilla* was under a relatively closed canopy of native trees, but where the shrub layer is relatively open.

Alseuosmia quercifolia A.Cunn.

A. quercifolia (Alseuosmiaceae) is an endemic, single-stemmed shrub that produces a small, open crown, and grows to approximately 3 m tall. It occurs most commonly in lowland native forests from the Waikato region northwards (Merrett et al. 2002). Its flowers are fragrant, nectiverous, and hermaphroditic. The fleshy, multi-seeded fruit is a berry (Allan 1961). The study populations were located in the Hakarimata Scenic Reserve (1795 ha) and Pukemokemoke Bush Reserve (40 ha) (Fig. 2.1; Table 2.1). *A. quercifolia* habitat is under a broken canopy where shade is provided, most commonly on southeast facing slopes (Merrett et al. 2002).

Alseuosmia turneri R.O.Gardner

A. turneri (Alseuosmiaceae) is an endemic, branching shrub growing to approximately 2 m tall, and occurs under mature native forests in central North Island (Gardner 1978), sometimes occurring in the same habitat as *A. pusilla*. During late spring (October–November) it produces hermaphroditic, fragrant, reddish-pink, somewhat sparse flowers with a single style and stigma and four anthers. The fleshy, multi-seeded fruit is a berry (Allan 1961). Populations were located in Ohinetonga Scenic Reserve (148 ha) and Rangataua Scenic Reserve (58 ha) (Fig. 2.1; Table 2.1). The habitat of *A. turneri* is similar to *A. macrophylla* in that it occurs most commonly under a relatively closed canopy but close to edges or near canopy gaps where light levels are higher.

Coprosma spathulata A.Cunn.

C. spathulata (Rubiaceae) is an, endemic, small-leaved dioecious shrub usually less than 1.5 m tall, and most commonly occurs on ridges and hillslopes in secondary forest under a light canopy. Its distribution is restricted to lowland forests of the northern part of the North Island, from the Waikato northwards (Allan 1961). It is anemophilous (Webb et al. 1999), and in the Waikato region it flowers in August. The fruit is a fleshy, almost-black drupe (Webb & Simpson 2001). The study populations were located in the Hakarimata Scenic Reserve (1795 ha) and Pukemokemoke Bush Reserve (40 ha) (Fig. 2.1; Table 2.1). The habitat at both sites was under a broken canopy of tall secondary forest trees such as kanuka (*Kunzea ericoides*) and tanekaha (*Phyllocladus trichomanoides*).

Coriaria arborea R.Linds.

C. arborea (Coriariaceae) is an endemic, relatively common, tall shrub/small tree that occurs most frequently in open or semi-open habitats including coastal and montane forests and shrubland in early successional communities throughout New Zealand (Allan 1961). During spring and summer it produces abundant, drooping floral racemes up to 30 cm long that comprise numerous small, hermaphroditic flowers. The fruit comprise several separate achenes enclosed by enlarged, fleshy petals (Webb & Simpson 2001). One of the populations for this study was located on a road verge at Matahuru, and the other was scattered along the margins of the Kaniwhaniwha Stream, near Mt Pirongia (Fig. 2.1; Table 2.1).

Cyathodes juniperina (J.R.Forst. et G.Forst.) Druce

C. juniperina (Epacridaceae) is an erect, spreading shrub up to 5 m tall (Allan 1961). It occurs throughout New Zealand (Allan 1961), in a disparate mix of mostly open habitats such as roadsides, in secondary and logged forest, and also in geothermally altered soils where mature, flowering plants are usually less than 50 cm tall. It flowers in spring, producing tiny, bell-shaped, white flowers, usually singly. The breeding system of the genus *Cyathodes* is described as dioecious (Godley 1957; Webb et al. 1999). The fruit is a drupe with a several-seeded endocarp (Webb & Simpson 2001). Study sites were located at Pukemokemoke Bush Reserve (40 ha), and on the road verge of Waiti Rd (Fig. 2.1; Table 2.1).

Leucopogon fasciculatus (G.Forst.) A.Rich.

L. fasciculatus (Epacridaceae) is a laxly branched spreading shrub up to 5 m tall, varying in habit according to exposure (Allan 1961). It occurs throughout New Zealand in coastal to lower montane shrubland (Allan 1961), mostly in open or semi-open habitats in secondary and logged native forest, along roadsides, and is common in geothermal areas. In spring it produces racemes of tiny, white, bell-shaped flowers, often in great numbers. The breeding system of the genus *Leucopogon* is described as dioecious (Godley 1957; Webb et al. 1999). The fruit is a drupe with a several-seeded endocarp (Webb & Simpson 2001). The study sites were located in kanuka dominated secondary forest in the Hakarimata Scenic Reserve (1795 ha) and on the road verge of Waiti Rd (Fig. 2.1; Table 2.1).

Macropiper excelsum (G.Forst.) Miq.

M. excelsum (Piperaceae) is a common, dioecious, shrub or small tree up to 6 m tall, occurring in lowland forest throughout the North Island, and to Banks Peninsula and Okarito in the South Island (Allan 1961). It flowers throughout the year, producing minute flowers on bracted spikes (Allan 1961). The breeding system of the genus *Macropiper* is described as dioecious, with entomophilous pollination (Webb et al. 1999). The fruit is a coalescence of numerous, tiny, single seeded drupes forming a compound fruit (Webb & Simpson 2001). The study sites were located at the edges of forest in the Kaimai-Mamaku Forest Park (10 000 ha), and at Port Waikato Camp bush (10 ha) (Fig. 2.1; Table 2.1).

Melicope simplex Cunn.

M. simplex (Rutaceae) is an endemic, small-leaved, divaricating shrub up to 4 m tall, occurring throughout both North and South Islands, usually on the margins of coastal and lowland forest. Webb et al. (1999) describe the breeding systems of the genus *Melicope* as dioecious and gynodioecious. In spring it produces small, greenish to white flowers in few- or many-flowered cymes (Allan 1961). The fruit is a dry, 4-celled capsule (Webb & Simpson 2001). Study sites were located at Gordon Gow Scenic Reserve (7.4 ha) and Pukemokemoke Bush Reserve (40 ha) (Fig. 2.1; Table 2.1). *M. simplex* habitat at both sites was under a broken canopy and/or on the margins of the forest.

Melicytus micranthus Hook.f.

M. micranthus (Violaceae) is an endemic, small-leaved divaricating, dioecious shrub up to 2 m tall, occurring throughout both North and South Islands in lowland forests and margins from latitude (38°S southwards (Allan 1961). In the Waikato region, *M. micranthus* occurs most commonly and abundantly under a canopy in kahikatea-dominated forest patches. During spring and summer it produces tiny, white, usually solitary flowers. The breeding system of the genus *Melicytus* is described as dioecious, with entomophilous pollination (Webb et al. 1999). The fruit is a fleshy berry (Allan 1961; Webb & Simpson 2001). The study sites were located at Gordon Gow Scenic Reserve (7.4 ha) and Wairere Bush (5 ha) (Fig. 2.1; Table 2.1). The habitat at both sites was under a tall, almost-closed kahikatea (*Dacrycarpus dacrydioides*) dominated canopy, and *M. micranthus* was the most common shrub species at both sites.

Melicytus novae-zelandiae (A.Cunn.) P.S.Green

M. novae-zelandiae (Violaceae) is an endemic, dioecious, shrub species up to 3 m tall, with a restricted, northern North Island and Three Kings distribution, mainly on coastal islands (Allan 1961). In spring it produces small, greenish flowers from the branches. The breeding system of the genus *Melicytus* is described as dioecious, with entomophilous pollination (Webb et al. 1999). The fruit is a fleshy berry (Allan 1961; Webb & Simpson 2001). Study sites were located on Matakana Island and on sand dunes at Mt Maunganui (Fig. 2.1; Table 2.1). The Matakana population occurs in localised patches, only on the seaward side of the island, in more consolidated sand of the back dunes, in a narrow strip (c. 20 m) on the margin of pine forest. The Mt Maunganui population is quite localised and also on the seaward side of the peninsula, in the back dune habitat and bounded by urban properties.

Pimelea arenaria A.Cunn.

P. arenaria (Thymelaceae) is an endemic, low-growing, much-branched, gynodioecious shrub that occurs on coastal sand dunes. Allan (1961) describes its distribution as 'throughout' but it is now known to occur in only one population in the South Island, on Farewell Spit, and over the last century its distribution in the North Island has declined as several populations have become locally extinct (Milne & Sawyer 2002). During spring and summer it produces abundant multi-flowered clusters of small, white flowers

at the tips of branches. The fruit is a single seeded, fleshy, drupe (Allan 1961). *P. arenaria* sens str. is listed in the 'gradual decline' category of the most recent lists of threatened and uncommon plants of New Zealand (de Lange et al. 2004). Study populations were located at Matarangi Beach and Ocean Beach, Kawhia (Fig. 2.1; Table 2.1). The habitat at both sites was the dune system between unconsolidated sand of the foredunes and the more consolidated back dunes. At Matarangi, wilding seedlings of the exotic, *Pinus pinaster* have established among *P. arenaria* and threaten to dominate the ecosystem by overtopping and outcompeting the native species.

Pomaderris hamiltonii **L.B.Moore**

P. hamiltonii (Rhamnaceae) is a many-branched endemic shrub/small tree up to 8 m tall, with a restricted distribution in the northern half of the North Island. It is an early successional species, most commonly occurring on roadside banks, and on the margins of secondary native forest. The fruit is a dry capsule (Allan 1961; Webb & Simpson 2001), and the seeds are enclosed in a kayak-shaped coccus. It is classified as 'sparse' in the most recent lists of threatened and uncommon plants of New Zealand (de Lange et al. 2004). Harvey and Braggins (1985) suggested the triploid *P. hamiltonii* reproduces by agamospermy. One of the study populations was on a road verge near Miranda, and the other was on the margins of a privately owned patch of native forest near Kaiaua (35 ha) (Fig. 2.1; Table 2.1).

Raukaua anomalus **(Hook.) A.D.Mitch., Frodin et Heads**

R. anomalus (Araliaceae) is a small-leaved, divaricating, endemic, bushy shrub growing to approximately 3 m tall (Allan 1961). It occurs throughout New Zealand, mainly on forest margins or within 30 m of the edge of native forests and in lowland scrub from latitude 35°S southwards (Allan 1961). It flowers late spring to early summer, and the fruit is a small, fleshy exocarp (Webb & Simpson 2001). The populations at two sites for this study were located at Gordon Gow Scenic Reserve (7.4 ha) in the Waikato region, and on the margin of native forest on Mamaku Plateau, near Rotorua (Fig. 2.1; Table 2.1). The habitat of the Gordon Gow population was within 10 m of the edge of the reserve under a broken canopy of kahikatea (*Dacrycarpus dacridioides*) and totara (*Podocarpus totara*). The Mamaku population was scattered along a small area on the eastern edge of tawa dominated forest.

Teucridium parvifolium Hook.f.

T. parvifolium (Verbenaceae) is endemic to New Zealand, and the only species in the genus (Allan 1961). It is a closely branched, soft-wooded, somewhat sprawling small-leaved shrub that grows approximately 1–2 m tall. The flowers are solitary or in few-flowered cymes, and the fruit is dry and comprises 4 nutlets surrounded by a persistent calyx (Allan 1961; Webb & Simpson 2001). It occurs in scattered populations in coastal to lowland forests in both North and South Islands (Allan 1961) and is listed in the 'Gradual Decline' category of the most recent lists of threatened and uncommon plants of New Zealand (de Lange et al. 2004). One of the study populations was located at Koropupu Scenic Reserve (24 ha) in the King Country, and the other at Paengaroa Scenic Reserve (102 ha) in central North Island (Fig. 2.1; Table 2.1). The forest habitat of *T. parvifolium* was in light wells associated with canopy gaps and at Koropupu, with limestone outcrops.

Pollination experiments

To determine breeding systems and reproductive strategies of each species, pollination treatments were carried out on tagged plants in each population, and treated as follows: 1. Controls (natural): natural pollinators allowed, stems in bud were tagged and buds counted. 2. Manual supplementary cross-pollination (crossed): during anthesis pollen from different plants was collected on a small artist's paintbrush and applied to stigmas. 3. Manual self-pollination (selfed): for hermaphroditic flowers, flower buds were bagged and during anthesis, pollen was applied directly onto stigmas with pollen collected from the same plant, and flowers rebagged. 4. Pollinators excluded (bagged): flower buds were counted then stems were enclosed in fine, mesh, fabric bags, tied with coloured twist ties, and left bagged with no further treatment. For the purposes of this study, buds were not bagged individually, precluding differentiating between passive geitonogamous and autonomous pollen transfer. The number of plants and flowers per population used in pollination experiments varied amongst species and sites, depending on species and flower numbers. Where possible, all treatments were conducted on each tagged plant, but in some cases there were too few flowers, and separate plants were used for each treatment. In all cases however, control flowers were used in conjunction with a treatment on an individual plant. Significant differences in fruit set between the

various treatments are useful in evaluating pollinator efficacy, pollen limitation, breeding strategies such as self-incompatibility, and pollinator dependency.

Fruit set was counted at various intervals after flowering, depending on fruit maturation rate. Unripe, but developed fruits were bagged (to prevent predation losses), and when ripe, fruit were collected; seeds were extracted, washed, counted, and air-dried for at least 2 weeks and then weighed.

Population density, gender and size-class structure

To measure population density and recruitment, temporary plots were established at each study site within which all individuals were counted and classified into three size/age classes: adults (flowering individuals), juveniles (non-flowering plants 20 cm or more tall), and seedlings (< 20 cm tall). To determine the effect of plant frequency, adult plant density per ha^{-1} was calculated. The distance from each tagged adult to the nearest potential out-cross pollen source was measured.

Data analysis

The percentage fruit set for individual plants and for each treatment was established by the following calculation: ((*n*. fruit/*n*. flowers) x 100), and population means generated for each species and treatment. Box plots were generated from individual plant fruit set for each treatment. The box plots depict the median (central line in each box), 25% and 75% quartiles (upper and lower limits of each box), 90th and 10th percentiles (indicated by the whiskers), and outliers, (indicated by open circles). Fruit set is a binary parameter in which the number of successes and failures are scored for each plant and treatment combination. Generalised Linear Model (GLM) analyses were used to compare fruit set between pollination treatments. Because fruit set was measured as a proportion, the GLMs used binomial error distribution and logit link function, and *F*-test significance tests, with a separate analysis for each site. For each site a simple linear regression was fitted to examine the influence of the nearest pollen source on natural fruit set. Generalised Linear Models with Poisson error distribution and logarithm link function were used to compare the number of seeds per fruit for each treatment in *Alseuosmia* species, and a two-way ANOVA was used for seed weight per fruit per treatment averaged over individual species and each treatment group. Three additional indices

were calculated to assess pollen limitation, self-compatibility (adapted from Larsen & Barrett 2000 and Riveros et al. 1998), and autogamous selfing, and are as follows:

Pollen limitation index: PLI = $1 - (I_N/I_X)$ where I_N is the percent fruit set of natural (open-pollinated controls) and I_X is the percent fruit set of crossed (supplemental cross pollen) plants, and represents the degree of pollen limitation, or inadequate pollinator activity. PLI = 0 indicates no pollen limitation in the population under study (Larsen & Barrett 2000). A PLI of > 0.75 indicate a high degree of pollen limitation (following Newstrom & Robertson 2005).

Self-incompatibility index: SII = $1 - (I_{MS}/I_{MX})$ where I_{MS} is the percent fruit set from selfed (manual self-pollination) and I_{MX} is the percent fruit set from crossed (supplemental cross pollen) flowers. SII = 0 indicates complete self-compatibility (Riveros et al. 1998). A SII of > 0.75 indicates a high degree of self-incompatibility.

Autogamous selfing index: ASI = (I_E/I_{MS}) where I_E is the percent fruit set from pollinator excluded (bagged) flowers and I_{MS} is the percent fruit set from selfed (manual self-pollination) flowers. An ASI > 0.75 indicates a high level of autogamous selfing, and an ASI of < 0.25 indicates no autogamous selfing.

2.4 Results

Hermaphrodite species

Alseuosmia macrophylla
A. macrophylla produces protandrous, fragrant, nectivarous, flowers during September–October, comprising a single style and stigma, and 4 to 6 filamentous anthers that dehisce longitudinally and are often exserted beyond the stigma (Fig. 2.2). Pollination treatments were conducted during October 2001 and ripe fruit were collected in May 2002.

Fig. 2.2 Flowers of *Alseuosmia macrophylla* from the Mamaku Plateau.

Mean natural fruit set was low at the Mamaku and Maungatautari sites, with 6.7% and 16% respectively, a result reflected by the high PLIs, particularly at Mamaku (Fig. 2.3; Table 2.2). Low fruit set in the selfed and bagged treatments at the Mamaku population suggests some form of physiological mechanism that reduces self-fertilisation. There was considerable within-population variation in the pollinator-excluded treatment at Maungatautari, influenced by two plants with 67 and 69% fruit set, and 5 plants that set no fruit. In contrast, mean natural fruit set at the Pirongia and Walter Scott sites were 74.6% and 54.7% respectively (Fig. 2.3; Table 2.2). There was a significant difference ($P < 0.01$) between natural and crossed fruit set in the Mamaku population, and combined with a high SII, a high degree of self-incompatibility was evident there (Table 2.3). The SII and ASI from Maungatautari show a moderate level of self-incompatibility and partial autogamous selfing, whereas at Pirongia and Walter Scott, natural fruit set was relatively high and with a low PLI (Table 2.2). Relatively high levels of fruit set in the self-pollination and bagged treatments at Pirongia and Walter Scott, and SII and ASI show self-compatibility and a degree of autogamy (Table 2.2).

GLM analysis showed no relationship between natural fruit set and the distance to the nearest pollen at any of the *A. macrophylla* sites (Table 2.3), suggesting factors other than pollen availability and density were implicated at sites with low natural fruit set, or that a simple measure to the nearest pollen donor does not adequately summarise the pool of potential pollen donors. The Mamaku population had the highest density of adult plants (Table 2.3), and in fact, *A. macrophylla* is the most common understory

shrub at this site, often forming dense thickets. There were no significant differences in the number of seeds per fruit between treatments at any of the sites, or seed weights between treatments (Table 2.4).

Population size-class structure shows good recruitment of seedlings and juveniles at three sites, Mamaku, Maungatautari and Pirongia, and although there were fewer seedlings at the Walter Scott site there was a higher proportion of juvenile plants (Fig. 2.4).

The Mamaku Plateau study site of *A. macrophylla* was identified for follow-up investigations into pollen limitation and pollinator effectiveness, and will be discussed in more detail in Chapter 3.

Table 2.2 Number of individual plants and numbers of treatment flowers per population, means of percent fruit set and their standard errors from four pollination treatments in eight hermaphroditic shrub species from 18 study sites in the North Island. N, natural fruit set; C, out-crossed flowers; S, selfed flowers; B, bagged flowers; PLI, pollen limitation index; SII, self-incompatibility index; ASI, autogamous selfing index. Species, study sites and their abbreviations are listed in Table 2.1.

			Pollination treatments										
			Number of flowers				Mean fruit set (%)				Indices		
Species	Site	Number of plants	N	C	S	B	N	C	S	B	PLI	SII	ASI
A. macrophylla	MM	16	511	99	81	194	6.7	50.5	8.7	0.6	0.87	0.83	0.07
A. macrophylla	MT	14	406	33	39	150	16.1	52.4	19.4	20.8	0.69	0.63	1.07
A. macrophylla	PR	9	325	33	51	90	74.6	64.3	54.5	23.9	-0.16	0.15	0.44
A. macrophylla	WS	15	323	36	46	101	55.3	56.0	61.1	43.6	0.01	-0.09	0.71
A. pusilla	EF	14	124	33	34	88	57.4	74.9	87.5	79.8	0.23	-0.17	0.91
A. pusilla	TR	30	71	27	33	35	83.2	90.0	100	87.4	0.08	-0.11	0.87
A. quercifolia	HK	39	781	212	275	383	32.9	50.6	3.1	4.4	0.35	0.94	1.42
A. quercifolia	PM	22	716	139	136	164	64.9	41.6	2.2	7.2	-0.56	0.95	3.27
A. turneri	OH	12	126	33	32	40	57.9	52.1	56.9	49.1	-0.11	-0.09	0.86
A. turneri	RT	9	76	28	31	32	58.8	92.1	94.3	30.6	0.36	-0.02	0.32
C. arborea	KS	12	834	430	205	124	89.2	94.1	67.4	77.5	0.05	0.28	1.15
C. arborea	MH	13	546	330	155	333	89.3	96.1	80.4	82.3	0.07	0.16	1.02
P. hamiltonii	KR	3	167	121	152	229	5.9	2.9	0	3.2	-1.03	1.00	-
P. hamiltonii	TB	3	309	488	461	543	8.5	2.4	3.0	3.6	-2.54	-0.25	1.20
R. anomalus	GG	14	529	110	150	288	39.6	56.9	26.7	14.0	0.30	0.53	0.52
R. anomalus	MM	10	408	130	142	184	17.2	41.8	30.3	9.4	0.59	0.28	0.31
T. parvifolium	KP	15	770	143	153	269	78.9	84.2	85.4	75.7	0.06	-0.01	0.89
T. parvifolium	PG	12	550	103	77	225	88.9	94.1	84.9	94.3	0.06	0.10	1.12

Table 2.3 Results of significance tests of pollination treatments on fruit set from binomial GLM analyses, plot sizes, adult plant density, means of distance to nearest pollen, and significance results of regression analyses on natural fruit set and pollen travel distance of eight hermaphroditic shrubs at 18 study sites in the North Island. d.f., degrees of freedom; #, insufficient data for analysis. Species, study sites and their abbreviations are listed in Table 2.1.

Species	Study site	Natural vs crossed (F, P)	Natural vs selfed (F, P)	Natural vs bagged (F, P)	Selfed vs crossed (F, P)	Plot size (m²)	Adult density (100 m²)	Mean distance to nearest pollen (m)	Natural fruit set versus pollen distance (P)	d.f.
A. macrophylla	MM	15.2, 0.004	0.07, 0.799	7.3, 0.024	21.3, 0.001	168	40.5	4.3	0.908	14
A. macrophylla	MT	19.1, 0.002	43.3, <0.001	0.9, 0.349	19.9, 0.003	150	17.3	9.3	0.601	12
A. macrophylla	PR	0.9, 0.358	3.0, 0.127	36.8, 0.001	2.1, 0.202	200	22.5	4.8	0.385	7
A. macrophylla	WS	0.5, 0.498	0.1, 0.822	2.9, 0.121	0.01, 0.938	150	24.0	2.3	0.714	13
A. pusilla	EF	0.03, 0.876	3.2, 0.092	2.6, 0.119	1.3, 0.281	20	115.0	0.6	0.622	12
A. pusilla	TR	0.8, 0.372	8.5, 0.006	0.2, 0.651	3.0, 0.116	20	195.0	0.6	0.789	28
A. quercifolia	HK	1.5, 0.236	14.5, 0.001	26.8, <0.001	44.7, <0.001	170	37.7	2.1	0.034	37
A. quercifolia	PM	2.9, 0.126	68.5, <0.001	228.3, <0.001	133.9, <0.001	250	2.8	6.7	0.637	20
A. turneri	OH	2.3, 0.194	0.2, 0.673	0.19, 0.674	2.0, 0.251	200	4.0	3.1	0.780	10
A. turneri	RT	1.6, 0.279	1.42, 0.155	3.1, 0.139	10.0, 0.051	200	7.0	2.6	0.464	7
C. arborea	KS	3.4, 0.095	3.7, 0.100	0.6, 0.521	7.3, 0.035	100	7.0	22.8	0.417	10
C. arborea	MH	2.6, 0.129	9.4, 0.021	1.1, 0.313	4.9, 0.068	150	21.3	1.9	0.353	11
P. hamiltonii	KR	#	#	#	#	200	3.0	2.5	#	1
P. hamiltonii	TB	5.4, 0.067	9.6, 0.027	2.3, 0.187	0.17, 0.690	400	2.5	2.3	0.487	1
R. anomalus	GG	3.2, 0.106	1.9, 0.186	17.9, 0.001	4.9, 0.051	300	3.3	6.5	0.452	12
R. anomalus	MM	5.6, 0.042	1.2, 0.296	2.3, 0.162	1.8, 0.211	200	6.0	1.9	0.215	8
T. parvifolium	KP	0.36, 0.559	1.1, 0.322	1.1, 0.306	0.0, 0.998	800	3.0	2.0	0.696	13
T. parvifolium	PR	0.23, 0.649	0.0, 0.929	0.14, 0.718	0.25, 0.641	200	9.5	5.8	0.395	10

Table 2.4 Mean number of seeds per fruit, and seed weight from four pollination treatments and eight hermaphrodite shrub species from 18 study sites in the North Island. N, natural; C, cross pollinated; S, self-pollinated; B, bagged. Species, study sites and their abbreviations are listed in Table 2.1.

Species	Site	Mean n. seeds per fruit				Mean seed weight (mg)			
		N	C	S	B	N	C	S	B
A. macrophylla	MM	4.2	7.8	5.7	2	3.89	3.03	4.16	4.00
A. macrophylla	MT	6.2	7.5	4.2	4.8	5.52	4.24	4.32	4.39
A. macrophylla	PR	7.3	6.5	6.9	4.2	4.58	5.38	5.30	5.47
A. macrophylla	WS	6.8	6.4	5.4	5.4	5.05	4.69	4.63	1.91
A. pusilla	EF	11.2	11.1	13.7	11.6	2.46	2.33	2.14	2.48
A. pusilla	TR	10.3	13	13.1	10	2.67	2.40	2.40	3.42
A. quercifolia	HK	6.2	6.7	3.8	11	2.65	2.47	2.76	2.61
A. quercifolia	PM	9.7	9	4.5	6.4	2.05	2.08	3.14	2.10
A. turneri	OH	4.6	5.2	4.4	3.6	6.66	6.16	6.50	5.81
A. turneri	RT	4.2	5.5	5.6	5.3	5.27	5.54	5.69	5.50
C. arborea	KS	1	1	1	1	0.45	0.52	0.27	0.38
C. arborea	MH	1	1	1	1	0.40	0.49	0.28	0.38
P. hamiltonii	KR	0.9	-	-	-	1.0	-	-	-
P. hamiltonii	TB	0.7	1.1	0.9	1.2	0.78	0.86	0.90	0.94
R. anomalus	GG	2	2	2	2	3.10	2.93	3.32	2.46
R. anomalus	MM	2	2	2	2	2.80	2.56	1.20	1.30
T. parvifolium	KP	4	4	4	4	1.00	1.08	1.04	0.96
T. parvifolium	PG	4	4	4	4	1.00	1.02	1.01	1.01

The header spans: "Pollination treatments" over both "Mean n. seeds per fruit" and "Mean seed weight (mg)".
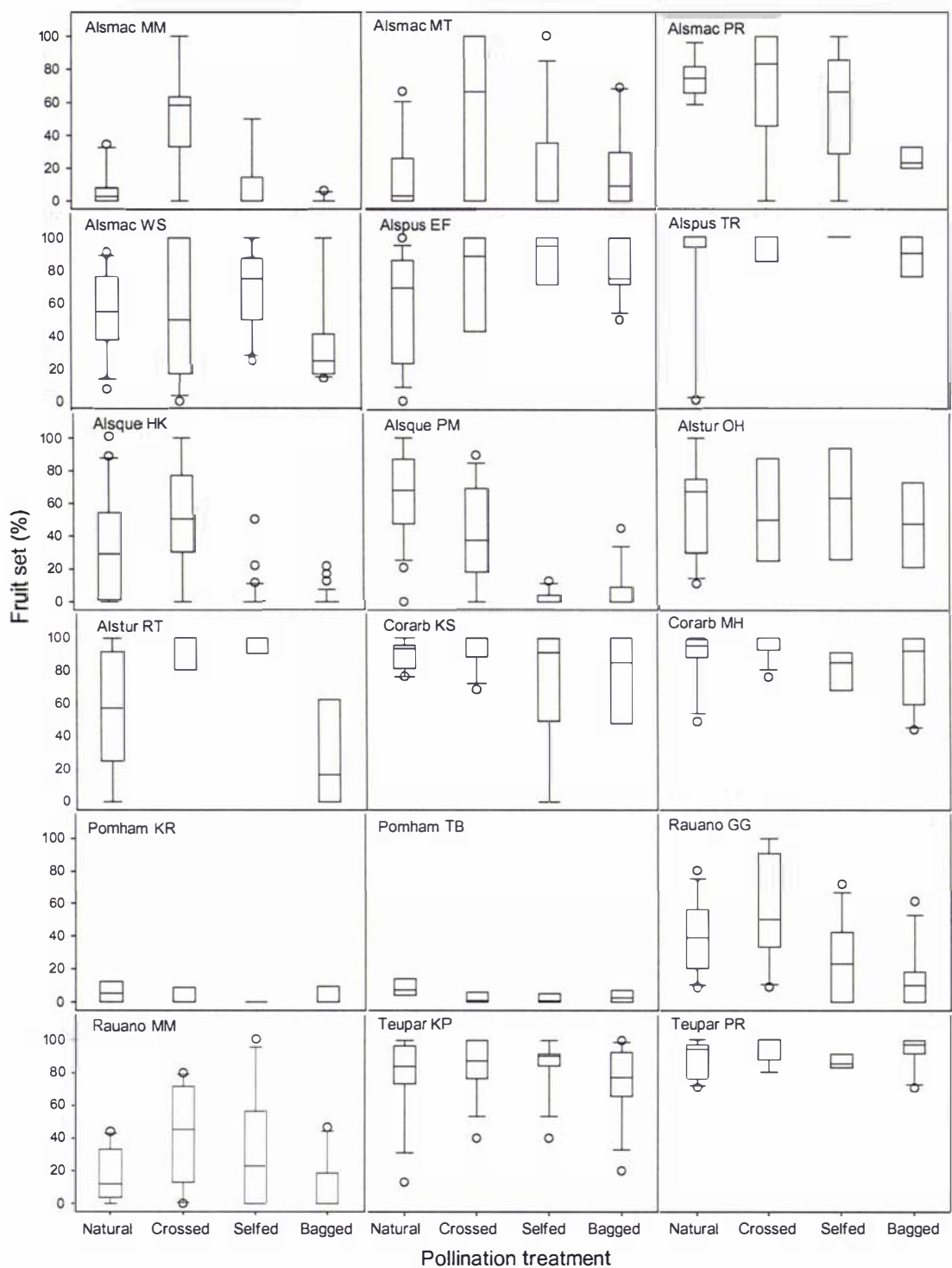
Fig. 2.3 Distribution of fruit set in eight hermaphrodite species from 18 study sites. Species, study sites and their abbreviations are listed in Table 2.1.
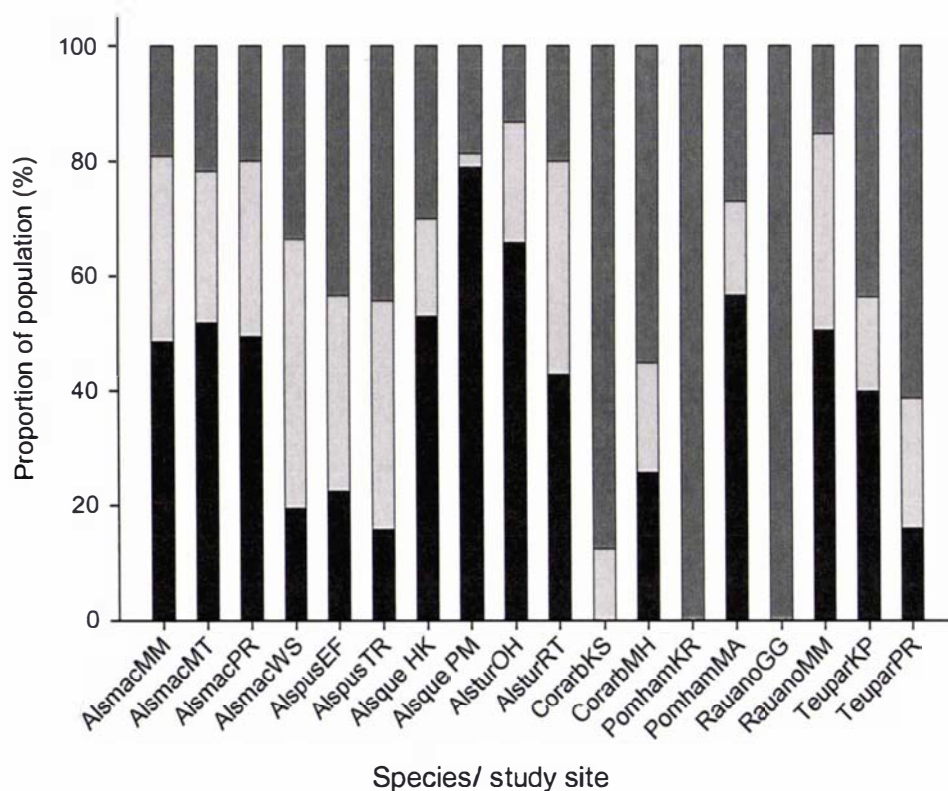
Fig. 2.4 Population size-class structure of eight hermaphroditic native shrub species at 18 study sites in the North Island. Black, seedlings; light grey, juveniles; dark grey, adults. Species, study sites and their abbreviations are listed in Table 2.1.

Alseuosmia pusilla

A. pusilla flowers are hermaphroditic, protandrous, have a single style and stigma, and the anthers are positioned more or less equal to the stigma. Pollination treatments were conducted during November 2002 and ripe fruit were collected in May 2003. The mature fruit is bright pink and highly visible (Fig. 2.5).

Mean natural fruit set in *A. pusilla* ranged from 0 to 100% at both sites, with a mean of 57.4% at Erua and 83.2% at Tongariro (Fig. 2.3; Table 2.2). There were no significant differences between natural fruit set and the crossed and bagged treatments at both sites, but there was a significant difference between natural and selfed at the TR site (Fig. 2.3, Table 2.3), a reflection of the high level of fruit set in the self-pollinated treatment (Table 2.2). High levels of fruit set in the selfed and bagged treatments, and low PLI show no pollen limitation, and low SII reflect high levels of self-compatibility in this species (Table 2.2). There was no correlation between fruit set and distance to the nearest pollen and no significant difference in the number of seeds per fruit or fruit

weight between treatments (Tables 2.3, 2.4). Adult density was high (11500 and 19500 plants per ha^{-1}) at both sites (Table 2.3), with most plants within 1 m of each other. Population size-class structures from both sites were similar, with seedlings and juveniles comprising 77% and 84% of the sampled populations, indicating population recruitment (Fig. 2.4).

Fig. 2.5 Fruit of *Alseuosmia pusilla*, Erua Forest.

Alseuosmia quercifolia

A. quercifolia flowers are produced in small clusters from leaf axils during September, and are fragrant, protandrous, and comprise a single style and stigma. (Fig. 2.6). Pollination treatments were conducted during September 2001 and ripe fruit were collected in May 2002. Mean natural fruit set at both sites ranged from 0 to 100%, with means of 33.4% and 64.9% at Hakarimata and Pukemokemoke respectively (Fig. 2.3; Table 2.2). The lower mean of natural fruit set from the Hakarimata site was influenced by 20% of the tagged plants producing no fruit. There was no significant difference between natural and cross fruit set at either site, indicating adequate levels of natural

pollination; however, a moderate PLI value (0.56) from the Pukemokemoke site shows some pollen limitation (Table 2.2). Significant differences between natural fruit set and the selfed and bagged treatments indicate self-incompatibility and autogamy, a result reflected by SII and ASI values at both sites (Table 2.2). These results suggest this species is pollinator dependent at both sites. There was no significant correlation between fruit set and distance to the nearest pollen at either site (Table 2.3). There were no significant differences in the number of seeds per fruit or seed weight between treatments, although fewer seeds were produced in the selfed treatment at both sites (Table 2.4). Seedlings were abundant at both sites, although recruitment persistence is not evident at Pukemokemoke because of low juvenile frequency (Fig. 2.4), a result similar to that recorded during an earlier survey (Merrett et al. 2002).

Fig. 2.6 Flowers of *Alseuosmia quercifolia* from Hakarimata Scenic Reserve.

Alseuosmia turneri

A. turneri produces lightly scented, pink, hermaphroditic flowers during late spring. Pollination treatments were conducted during October and November 2001, and ripe fruit were collected in May 2002. Mean natural fruit set in *A. turneri* was almost identical at both sites, with means of 57.9% and 58.8%, and ranging from 0 to 100% (Fig. 2.3; Table 2.2). There was within population variation in fruit set between pollination treatments, although there were no significant differences between treatments (Table 2.3). Relatively low PLI values indicate no pollen limitation, and very

low SII values show high levels of self-compatibility (Table 2.2). ASI values indicate a higher level of autogamy in the Ohinetonga population compared with only partial autogamous selfing at the Rangataua site (Table 2.2). There was a significant difference between natural fruit set and the distance to the nearest pollen at the Ohinetonga site (Table 2.3). There were no significant differences in the number of seeds per fruit or seed weight between treatments (Table 2.4). Although specific pollinators were not investigated, a bellbird was observed nectar feeding during the flowering season of 2001 at the Ohinetonga Scenic Reserve (pers. obs.). Population size-class structure shows a high proportion of seedlings and juveniles, indicating population recruitment (Fig. 2.4).

Coriaria arborea

C. arborea is wind-pollinated, and dichogamous, with the stigmas emerging before anther emergence and dehiscence (protogyny) (Fig. 2.7). Flower maturation is sequential, starting from the apex of the raceme, and with flowers of each gender phase present at any point in time. Temporal separation of the female and male phases could be a strategy to help avoid self-pollination; however, having flowers at each phase on a single inflorescence would appear to counteract the putative advantages of dichogamy, but is possibly an adaptation to increase the efficiency of pollen transfer. Pollination treatments were conducted during September 2002 and ripe fruit were collected in November 2002.

Fig. 2.7 Dichogamous flowers of *Coriaria arborea*, showing receptive female styles, female stage senescence and anther enlargement of the male stage.

Mean fruit set for all treatments was high at both sites, ranging from 77.5 to 96.1% (Fig. 2.3; Table 2.2). Natural fruit set was significantly higher than the self-pollination treatment from Matahuru, and significantly higher in crossed-compared with self-

pollination treatments from Kaniwhaniwha, albeit, these differences are small (Table 2.3). Low PLI and SII show *C. arborea* experienced no pollen limitation, and is highly self-compatible and autogamous (Table 2.2). Successful self-compatibility was at the raceme level rather than individual flowers, because individual flowers were not bagged separately. Each flower produced one seed per fruit, although 5 ovules were present in each of the flowers examined. There was no correlation between fruit set and the distance to the nearest pollen (Table 2.3), and no significant difference in seed weight from the various pollination treatments (Table 2.4).

Adult plants dominated the populations at both sites, and no seedlings were recorded from the Kaniwhaniwha site (Fig. 2.4). The study plants there were scattered along the margins of the Kaniwhaniwha Stream among rank pasture grasses that have effectively reduced the likelihood of seed germination.

Pomaderris hamiltonii

In spring *P. hamiltonii* produces masses of hermaphroditic, creamy-yellow flowers that comprise three styles and stigmas joined to form what appears to be a single style, and 5 anthers on hinged filaments (Fig. 2.8). The flowers appear to be protandrous, and on opening anthers are folded inwards to rest against the style below the stigmatic surface. Concomitant with flower opening and anthesis, the anthers fold outwards, away from the stigmatic surface, but as the flower matures and begins to senesce, the hinged filament folds inwards and the anthers become suspended directly above the stigma. Pollination treatments were conducted during October 2001 and ripe fruit were collected in December 2001.

Fig. 2.8 Late stage of *Pomaderris hamiltonii* flower, showing anthers positioned over the stigma.

Mean fruit set was low in all pollination treatments and at both sites, which could indicate a high rate of flower abortion, regardless of the degree of ovule fertilisation (Fig. 2.3; Table 2.2). Insufficient data precluded GLM analysis of the Kaiaua Rd results because of loss of tagged inflorescences during fruit maturation. However, there was a significant difference in natural fruit set compared with the crossed and selfed treatments from the Thompson's Bush site (Table 2.3). The low PLI values suggest no pollen limitation (Table 2.2), despite low fruit set. There was no correlation between fruit set and distance to the nearest pollen (Table 2.3), and no significant difference in the number of seeds per fruit or seed weight between the various pollination treatments (Table 2.4). The Thompson's Bush site had a high proportion of seedlings and juveniles (Fig. 2.4), although adult density was relatively low (Table 2.3). There was no population recruitment at the Kaiaua Rd site, most likely due to a groundcover of mown grass effectively preventing seed germination there.

Raukaua anomalus

R. anomalus flowers are tiny (2–3 mm), and strongly protandrous. Pollination treatments were conducted during January and February 2002 and ripe fruit (Fig. 2.9) were collected in May 2002. The breeding system was thought to be dioecious (Kirk 1899; Eagle 1975), but during the course of this study it became apparent that flowers were hermaphroditic, and a review of the breeding system and floral characteristics is presented separately in Chapter 7.

Fig. 2.9 Mature fruit of *Raukaua anomalus* showing retained styles.

There was considerable within-population variation in natural fruit set at both sites, ranging from 0 to 80%, with means of 39.6% at Gordon Gow and 17.2% at Mamaku (Fig. 2.3; Table 2.2). Fruit set from the crossed treatment was higher than natural at both sites, although analysis showed this to be significant only at the Mamaku site, and combined with a PLI of 0.59, moderate pollen limitation is evident (Tables 2.2, 2.3). Successful fruit set in hand-pollinated (26.7% and 30%) flowers and moderate SII values suggests there is a degree of self-compatibility. ASI values suggest partial autogamy although poor fruit set in the bagged treatment indicates pollinator dependence (Fig. 2.3; Table 2.2). There was no correlation between natural fruit set and distance to the nearest pollen (Table 2.3), and no significant difference in the number of seeds per fruit or seed weight from the various treatments (Table 2.4). Although natural fruit set was lower at the Mamaku site, adult density was higher, and size-class distribution shows a normal reverse J curve (Fig. 2.4). The relatively small population at the Mamaku site was clustered around the edge of native forest, whereas at Gordon Gow, the population was scattered under a canopy of kahikatea, mostly within 30 m of the edge, and there was no population recruitment (Fig. 2.4).

Teucridium parvifolium

T. parvifolium has attractive, creamy-white, zygomorphic flowers with a single, branching style, and four spherical anthers that are held almost level with the stigmas on long filaments. Pollination treatments were conducted during December and January 2002–03 and ripe fruit were collected in April and May 2003. *T. parvifolium* was thought to be hermaphroditic throughout its range. However, during hand pollinations at Paengaroa, it was noted that one of the tagged plants contained what appeared to be pistillate (female) flowers only, and subsequently an investigation of gender dimorphism in other *T. parvifolium* populations in the North Island was undertaken, and a review of the breeding system is described in Chapter 8. I have, however, retained it in the hermaphroditic group for analyses.

Fruit set in all pollination treatments was high, ranging from 75.7% to 94.3% (Fig. 2.3; Table 2.2), and there were no significant differences between the various pollination treatments at either study site (Table 2.3). These results and associated low PLI and SII values show high levels of reproductive success, self-compatibility, and autogamy

(Table 2.2). There was no significant difference between natural fruit set and pollen distance, and no difference in the number of seeds per fruit, or seed weight between treatments (Tables 2.3, 2.4). Seedlings and juveniles were present at both sites, although the distribution of size classes from Paengaroa showed adults were the dominant constituent (Fig. 2.4).

Gender dimorphic species

Coprosma spathulata

C. spathulata female flowers have 2 styles that elongate to up to 1.3 cm in length with maturity, and are covered with tiny glandular hairs for trapping pollen. The male flowers have four anthers that dehisce longitudinally, and hang from the corolla on fine filaments. Pollination treatments were conducted during August 2002 and ripe fruit were collected during April and May 2003.

Mean natural fruit set was low from the Hakarimata site, and there was a significant difference between natural and crossed fruit set at both sites (Fig. 2.10; Tables 2.5, 2.6). Pollen limitation is evident as shown by a high PLI at the Hakarimata site (Table 2.5). One plant at the Pukemokemoke site produced one fruit in the bagged treatment (Fig. 2.10; Table 2.5). This single result is unlikely to be an indication of apomixis, a strategy that has been reported in other *Coprosma* species (Heenan et al. 2002, 2003), but rather the result of some form of contamination during the bagging process. Higher levels of fruit set would be expected if *C. spathulata* was producing by apomixis, and there was no fruit set in bagged flowers at Hakarimata (Fig. 2.10). Logistic analysis shows no relationship between natural fruit set and pollen distance among sites (Table 2.6), but for the combined data from both sites, there was a significant relationship ($P = 0.05$). The number of seeds per fruit was consistently two, and there was no significant difference in seed weight between the two pollination treatments (Table 2.7). Gender ratios at both sites were female biased, particularly at Hakarimata with 86% compared with 58% at Pukemokemoke (Fig. 2.11). The proportion of seedling and juvenile plants was 77% at Hakarimata compared with 48% at the higher density Pukemokemoke site (Fig. 2.12). Further investigation into the efficacy of wind-pollination in *C. spathulata* in populations of differing densities is discussed in Chapter 4.

Table 2.5 Number of individual plants and numbers of treatment flowers per population, and means of percent fruit set from four pollination treatments on eight gender dimorphic shrub species at 16 study sites in the North Island. N, natural; C, crossed; S, selfed; B, bagged; PLI, pollen limitation index; SII, self-incompatibility index; ASI, autogamous selfing index. Species, study sites and their abbreviations are listed in Table 2.1.

			Pollination treatments										
			Numbers of flowers per treatment				Mean fruit set (%)				Indices		
Species	Site	Number of plants	N	C	S	B	N	C	S	B	PLI	SII	ASI
C. spathulata	HK	10	296	111	-	107	8.2	66.6	-	0	0.88	-	-
C. spathulata	PM	15	363	127	-	173	58.9	91.1	-	0.4	0.35	-	-
C. juniperina	PM	14	511	182	157	203	4.1	26.3	0	0	0.84	-	-
C. juniperina	WR	12	407	173	169	188	24.8	64.4	17.3	1.9	0.61	-	0.10
L. fasciculatus	HK	16	696	225	298	483	8.8	18.4	0	0.5	0.52	-	-
L. fasciculatus	WR	14	575	278	220	345	12.6	21.6	0.5	2.1	0.42	-	4.20
M. excelsum	KF	16	53	21	-	50	96.4	93.8	-	0	-0.03	-	-
M. excelsum	PW	15	57	22	-	30	100	80.0	-	0	-0.25	-	-
M. simplex	GG	16	669	229	-	325	15.8	56.2	-	0	0.72	-	-
M. simplex	PM	16	486	202	-	196	11.6	39.2	-	0	0.70	-	-
M. micranthus	GG	5	87	28	-	64	25.0	80.4	-	0	0.69	-	-
M. micranthus	WB	14	319	65	-	135	28.6	82.4	-	0	0.65	-	-
M. novae-zelandiae	MK	15	624	294	-	291	62.6	78.1	-	3.8	0.20	-	-
M. novae-zelandiae	MN	16	795	293	-	326	83.8	87.8	-	3.8	0.05	-	-
P. arenaria (F)	KW	4	140	100	-	59	55.5	50.0	-	7.3	-0.11	-	-
P. arenaria (H)	KW	13	415	116	152	245	49.8	59.7	57.3	46.8	0.17	0.04	0.82
P. arenaria (F)	MR	10	406	142	-	161	34.7	25.3	-	1.1	-0.37	-	-
P. arenaria (H)	MR	10	406	147	164	149	43.2	27.9	43.9	40.6	-0.55	-0.57	0.93

Table 2.6 Results of significance tests of pollination treatments on fruit set from binomial GLM analyses, plot sizes, adult plant density, mean distance to the nearest pollen, and significance results of regression analyses on natural fruit set and pollen travel distance of eight gender dimorphic shrubs at 16 study sites in the North Island. d.f., degrees of freedom; F, female plants; H, hermaphrodite plants. Species, study sites and their abbreviations are listed in Table 2.1.

Species	Study site	Natural vs crossed (t-ratio, P value)	Natural vs selfed (t-ratio, P value)	Natural vs bagged (t-ratio, P value)	Selfed vs crossed (t-ratio, P value)	Plot size (m^2)	Adult density (100^{-m^2})	Mean distance to nearest pollen (m)	Natural fruit set versus pollen distance (P value)	d.f.
C. spathulata	HK	228.2, <0.001	-	-	-	100	10.0	7.0	0.173	8
C. spathulata	PM	15.6, 0.001	-	-	-	215	48.8	1.1	0.905	13
C. juniperina	PM	15.8, 0.002	6.5, 0.027	8.6, 0.012	21.5, <0.001	400	3.0	8.3	0.593	12
C. juniperina	WR	2.9, 0.102	0.04, 0.848	2.9, 0.103	3.5, 0.074	240	4.2	8.2	0.282	10
L. fasciculatus	HK	3.5, 0.088	181.9, <0.001	58.0, <0.001	18.3, 0.001	74	28.4	4.6	0.395	14
L. fasciculatus	WR	1.8, 0.205	17.6, 0.002	8.9, 0.015	19.4, 0.002	128	15.6	2.0	0.558	12
M. excelsum	KF	0.02, 0.987	-	-	-	100	21.0	3.2	0.157	14
M. excelsum	PW	17.4, <0.001	-	-	-	75	49.3	1.3	0.995	13
M. simplex	GG	38.3, <0.001	-	-	-	270	5.2	4.3	0.322	14
M. simplex	PM	13.3, <0.001	-	-	-	147	18.4	14.7	0.993	14
M. micranthus	GG	16.2, 0.056	-	-	-	414	5.6	8.8	0.784	3
M. micranthus	WB	21.4, 0.001	-	-	-	280	48.2	1.5	0.851	12
M. novae-zelandiae	MK	6.5, 0.023	-	-	-	110	78.2	2.2	0.526	13
M. novae-zelandiae	MN	1.4, 0.263	-	-	-	100	80.0	2.7	0.520	14
P. arenaria (F)	KW	0.2, 0.719	-	-	-	1060	0.5	0.9	0.965	2
P. arenaria (H)	KW	0.8, 0.396	0.2, 0.658	0.02, 0.899	0.2, 0.639	-	2.3	1.8	0.242	11
P. arenaria (F)	MR	0.56, 0.465	-	-	-	300	2.9	4.5	0.267	8
P. arenaria (H)	MR	3.6, 0.091	0.01, 0.930	0.6, 0.711	2.3, 0.167	-	14.3	1.9	0.404	8

Table 2.7 Mean number of seed per fruit, number of fruit, seed weight and number of seeds for 4 pollination treatments on eight gender dimorphic shrub species at 16 study sites in the North Island. N, natural; C, out-crossed; S, selfed; B, bagged; F, female plants; H, hermaphrodite plants.

| | | Pollination treatments | | | | | | | |
| | | Mean number of seeds per fruit | | | | Mean seed weight (mg) | | | |
Species	Site	N	C	S	B	N	C	S	B
C. spathulata	HK	2	2	-	-	7.72	9.87	-	-
C. spathulata	PM	2	2	-	-	5.89	9.50	-	-
C. juniperina	PM	1	1	-	-	25.60	23.32	-	-
C. juniperina	WR	1	1	-	-	24.36	23.12	-	-
L. fasciculatus	HK	1	1	-	1	4.32	4.08	-	5.20
L. fasciculatus	WR	1	1	-	1	4.45	4.37	-	3.73
M. excelsum	KF	115	134	-	-	2.01	1.95	-	-
M. excelsum	PW	142	141	-	-	2.09	2.40	-	-
M. simplex	GG	0.62	0.47	-	-	5.29	5.36	-	-
M. simplex	PM	0.48	0.43	-	-	6.49	6.05	-	-
M. micranthus	GG	1.8	2.2	-	-	5.54	5.04		
M. micranthus	WB	2	2.2	-	-	4.91	5.20		
M. novae-zelandiae	MK	1.7	1.7	-	-	16.30	20.40	-	-
M. novae-zelandiae	MN	1.4	1.7	-	-	17.13	18.08	-	-
P. arenaria (F)	KW	1	1	-	1	7.82	8.05	-	9.53
P. arenaria (H)	KW	1	1	1	1	8.70	8.88	9.02	8.87
P. arenaria (F)	MR	1	1	-	1	2.97	2.99	-	1.95
P. arenaria (H)	MR	1	1	1	1	3.09	2.99	3.39	3.31
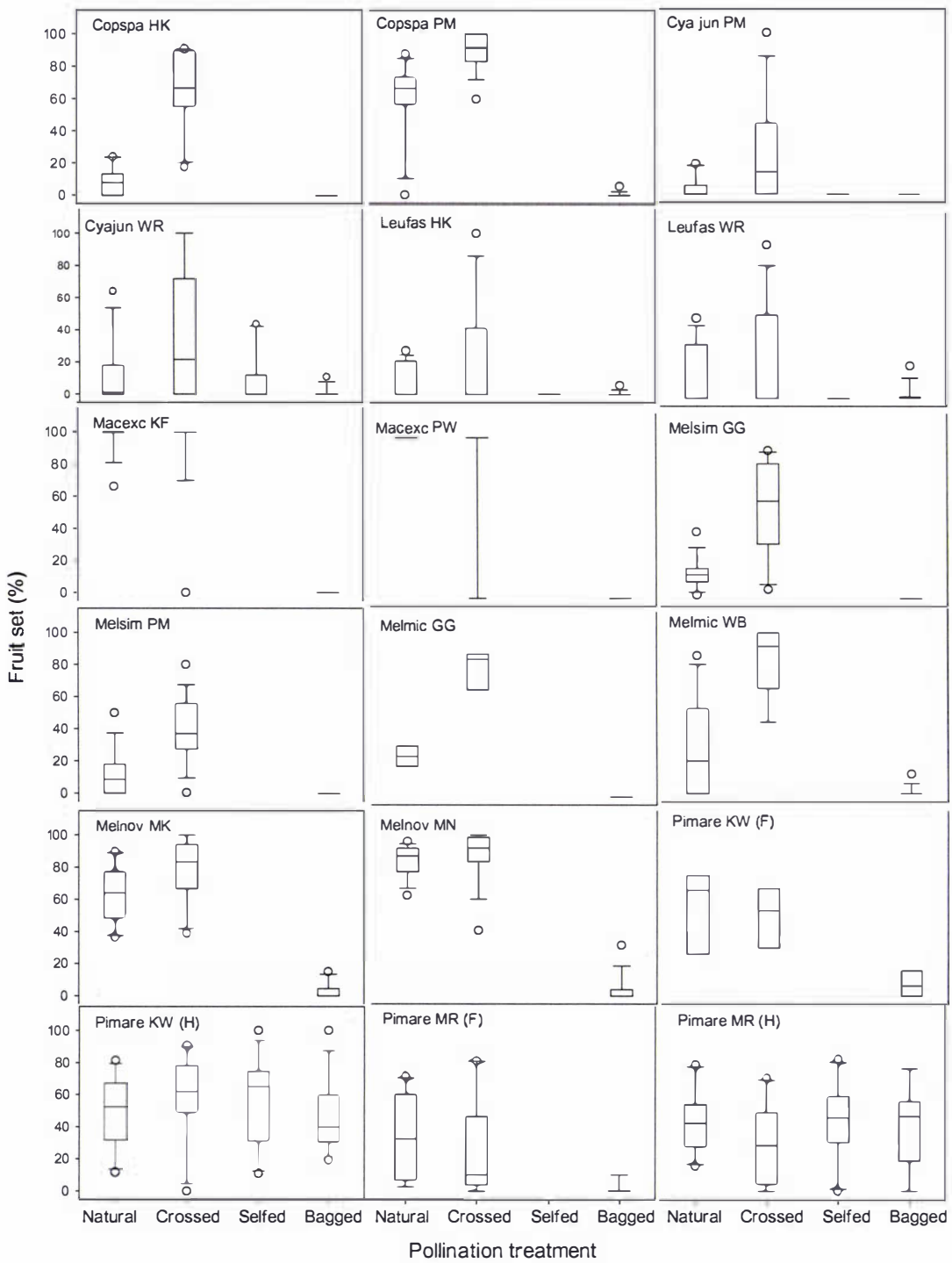
Fig. 2.10 Distribution of fruit set in eight gender dimorphic species from 16 study sites. F, female plants; H, hermaphrodite plants. Species, study sites and their abbreviations are listed in Table 2.1.
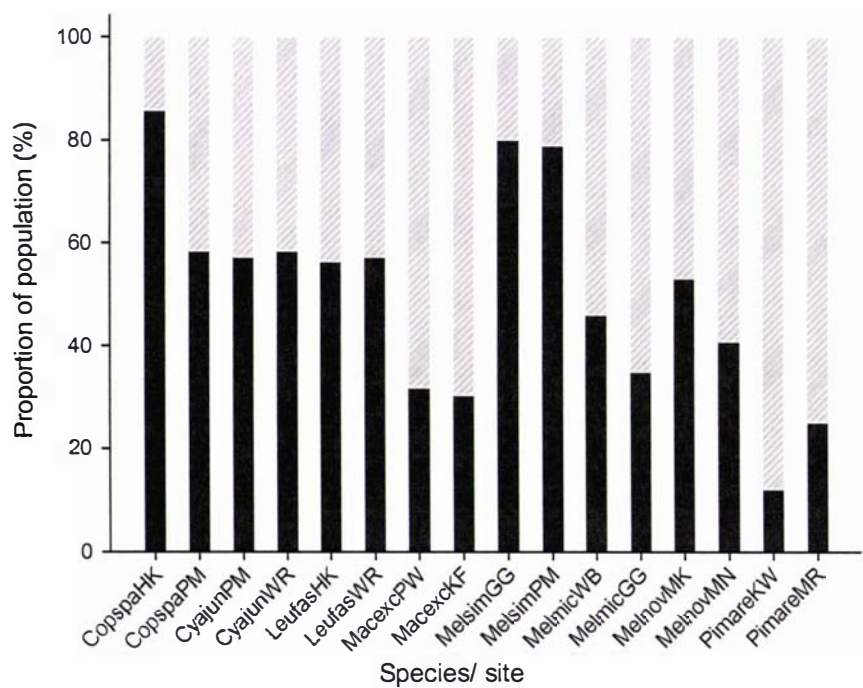
Fig. 2.11 Distribution of gender ratios in gender dimorphic shrub species. Black, females; grey, males or hermaphrodites. Species, study sites and their abbreviations are listed in Table 2.1.
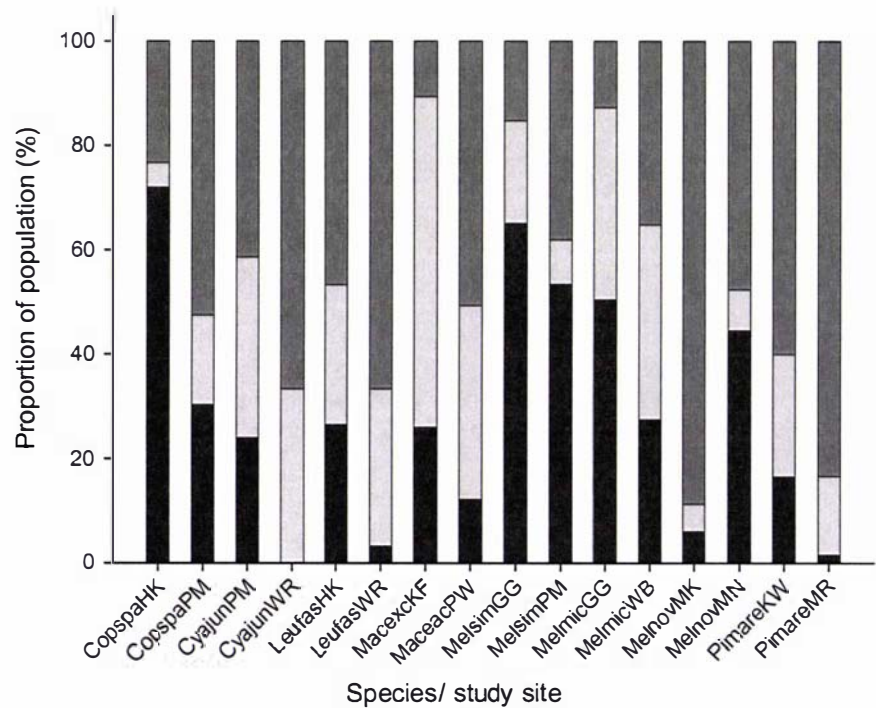
Fig. 2.12 Population size-class structure of eight gender dimorphic, native shrub species at 16 study sites in the North Island. Black, seedlings; light grey, juveniles; dark grey, adults. Species, study sites and their abbreviations are listed in Table 2.1.

Cyathodes juniperina

Flowering was sparse on some *C. juniperina* plants, particularly where their habitat was under a tall kanuka canopy at the Pukemokemoke site. The small, white, bell-shaped, insect-pollinated flowers occur singly or in pairs, and superficially appear to be hermaphroditic (Fig. 2.13), although the breeding system is described as dioecious by Webb et al. (1999). Godley (1957) described cryptic dioecy in *Cyathodes* in the South Island, and during the fruit set counting stage of this study, I noted that some of the tagged plants did not produce fruit in any of the pollination treatments. A combination of this and a lack of old fruit on these plants made it evident that this species was also cryptically dioecious in the study populations. Fruit set in bagged and selfed treatments also suggests some plants produce hermaphroditic flowers (Fig. 2.10; Table 2.5). There were no visible differences in the flower morphology of the different genders. Of the tagged, treatment plants 43% and 42% (Fig. 2.11) were putative males at Pukemokemoke and Waiti Rd respectively, and because of this the results of pollination treatments on the putative male plants were excluded from analyses. The fruits persist on the plant for up to 3 years, changing from white to brown with age. Pollination treatments were conducted during September 2002 and ripe fruit were collected in April and May 2003.

Fig. 2.13 Example of a typical flower (cut to show detail) of *Cyathodes juniperina* showing pistil, and dehisced anthers with pollen. The gender of the flower was indeterminate.
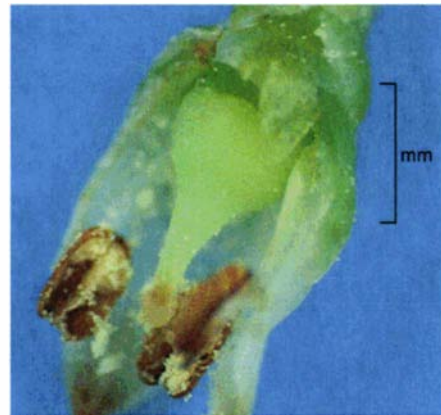
Mean natural fruit set was low, particularly at Pukemokemoke (6.9%), and there was a significant difference between fruit set from natural and crossed pollination treatments at both sites (Fig. 2.10; Tables 2.5, 2.6). Fruit set in the selfed and bagged treatments in plants at the WR site indicates some flowers are hermaphroditic, and populations may comprise plants that produce male, female, and hermaphroditic flowers. A high PLI

value (0.84) from Pukemokemoke indicates pollen limitation (Table 2.5). There was no relationship between fruit set and the distance to the nearest pollen (Table 2.6). The seeds of this species are enclosed within a single, very hard, endocarp (Webb & Simpson 2001), and were not removed for separate weighing; however, there was no significant difference in the weight of the endocarp between the various pollination treatments (Table 2.7). Population size-class structures show adult plants are the dominant size-class, although at relatively low density at both sites (Fig. 2.12; Table 2.6). No seedlings were present at the Waiti Rd site (Fig. 2.12).

Leucopogon fasciculatus

L. fasciculatus produces racemes of tiny, white, bell-shaped, insect-pollinated flowers (Fig. 2.14). Pollination treatments were conducted during September 2002 and ripe fruit were collected in May 2003. As noted with *Cyathodes juniperina*, the flowers of *L. fasciculatus* superficially appear hermaphroditic. However, the results of pollination experiments indicate this species is also cryptically dioecious, with the additional interesting feature that some plants may produce hermaphroditic flowers; fruit set in the bagged treatment in two plants at each site suggests either some individuals produce functionally hermaphroditic flowers that reproduce autogamously, or there was some form of contamination in the bag, but this seems unlikely. No visible differences in the morphological structure of flowers from each study plant were discernible. The results from putative males were excluded from fruit set analyses.

Fig. 2.14 Cut flower of *Leucopogon fasciculatus* showing pistil, and dehisced anthers with pollen.

Mean natural fruit set was 20% and 29% whereas the mean fruit set from cross-pollination experiments was 42% and 50% (Fig. 2.10; Table 2.5). Although the results of binomial GLMs show no significant difference between these two treatments, the PLI shows moderate levels of pollen limitation at both sites (Table 2.5). Of the tagged, treatment plants 57% and 56% from Hakarimata and Waiti Rd respectively, were putative males (Fig. 2.11). There was no relationship between fruit set and the distance to the nearest pollen (Table 2.6).

The seed of this species are enclosed within a single endocarp (Webb & Simpson 2001) and were not removed for separate weighing. There was no significant difference in the weight of the endocarp between the various pollination treatments (Table 2.7).

Population size-class structures show adults dominating at both sites (Fig. 2.12), and few seedlings were present at the Waiti Rd site (a roadside population), probably a result of limited available habitat and roadside maintenance. *L. fasciculatus* is an early successional species that requires open, disturbed ground for seed germination and establishment, but over time, faster growing, taller species such as kanuka (*Kunzea ericoides*) eventually overtop it, leading to plant senescence, as was apparent at the Hakarimata site.

Macropiper excelsum

M. excelsum produces numerous flowering spikes from late winter through to autumn. Each flowering spike comprises numerous, sessile stigmas on females or bilobed anthers on males (Fig. 2.15). The anthers on male spikes mature sequentially from the apex of the spike to the base. Webb et al. (1999) describe this species as entomophilous, however, male spikes release masses of dry pollen that is readily dispersed by wind, indicating wind-dispersed. The female inflorescences have tiny drops of what is probably nectar scattered amongst the flowers (Fig. 2.15), perhaps suggesting a dual-pollination syndrome: wind and insect. The stigmas on female spikes darken and senesce once pollinated, and there is one ovule per stigma. An interesting feature of the female spikes was their longevity when bagged: the stigmas remained receptive for several weeks. Pollination treatments were conducted during September 2002 and ripe fruit were collected in January 2003.
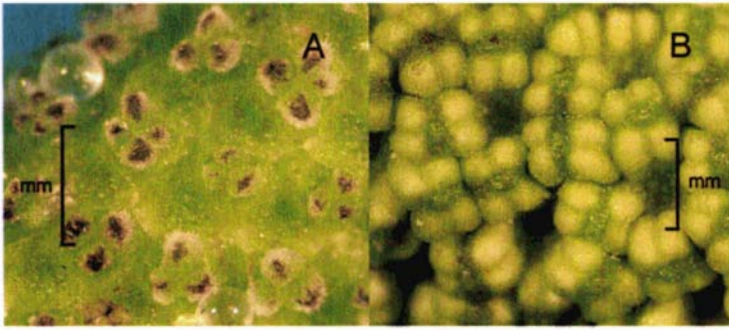
Fig. 2.15 Close-up of part of the dioecious flowering spikes of *Macropiper excelsum* showing female stigmas (A) and undehisced anthers (B).

Mean natural fruit set was very high at both study sites, reflected by low PLI values, and although there was a significant difference between the natural and cross-pollinated treatments at Port Waikato, this was not a reflection of poor fruit set in the naturally (Tables 2.5, 2.6). There was no significant difference in the mean number of seeds or seed weight between treatments (Table 2.7).

Gender ratios show males plants dominated by more than double at both sites (Fig. 2.11). Population size-class structure differed between the two study sites, with seedlings and juveniles comprising 89% at the Kaimai site, whereas at Port Waikato, they comprised 49% (Fig. 2.12).

Melicope simplex

M. simplex produces small, white flowers, often in great numbers, particularly on plants that receive higher light levels such as on the edge of native forests or in canopy gaps. The anthers of male flowers are held on relatively long filaments that elongate with maturation. Anthesis is two-staged, with two sets of four anthers maturing sequentially (Fig. 2.16). Female flowers have vestigial stamens, and a single pistil (Fig. 2.16). The ovaries consistently contain eight ovules but usually produce a maximum of four seeds, although capsules of five seeds were occasionally noted. Pollination treatments were conducted during October 2002 and ripe fruit were collected in January 2003. The capsule splits when mature, and the black, glossy seeds emerge and hang from a very fine filament, and are presumably dispersed by wind.
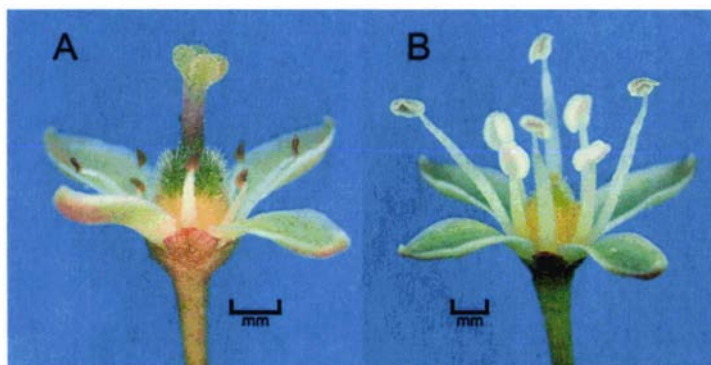
Fig. 2.16 Flowers of the dioecious shrub *Melicope simplex*. The female flower (A) shows vestigial stamens and the male flower (B) shows the reduced, non-functional pistil and two sets of four anthers at different stages of maturation.

Mean natural fruit set was less than 20% from both study sites, and there was a significant difference between the natural and crossed fruit set (Fig. 2.10; Tables 2.5, 2.6). The PLI values (0.72 & 0.70) show moderately high pollen limitation at both sites (Table 2.5). There was no relationship between natural fruit set and pollen distance (Table 2.6), and no significant difference in the number of seeds per fruit, or seed weight between the natural and cross-pollination treatments (Table 2.7).

Gender distribution was female-biased at both sites, and comprised 80% of the population (Fig. 2.11). Population size-class structure shows seedlings and juveniles comprised 85% and 62% at Gordon Gow and Pukemokemoke respectively (Fig. 2.12). Adult density was higher at Pukemokemoke than at Gordon Gow (Table 2.6).

Further investigations into the reproductive success in populations of this species was conducted, and are discussed in Chapter 5.

Melicytus micranthus

M. micranthus produces tiny (2–3 mm) white flowers (Fig. 2.17), over several months during spring, summer, and autumn. The extended flowering season observed during this study reflects the same behaviour found in a South Island study, where flowering was described as asynchronous, i.e. producing a few flowers at a time over an extended period (Powlesland et al. 1985). Male flowers were slightly larger than females, and in

this study were produced in greater numbers. The male flowers have five, sessile, 4-lobed anthers that open sequentially, dehiscing longitudinally to release the pollen (Fig. 2.17). In female flowers the number of ovules per ovary ranged from two to four. Pollination treatments were conducted during November 2001 and ripe fruit were collected in May 2002.
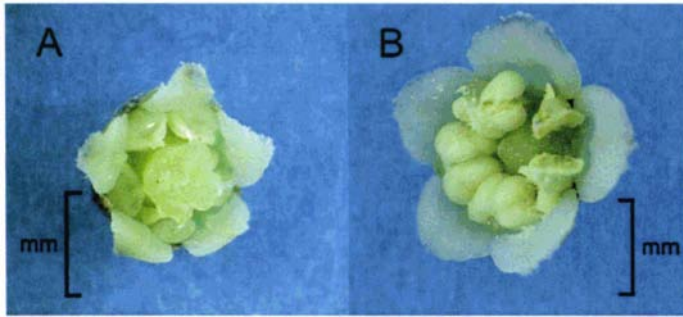
Fig. 2.17 Flowers of the dioecious shrub *Melicytus micranthus* from Wairere Bush. The female flower (A), and the male flower (B) showing sequential anthesis.

There was a significant difference between mean natural fruit set and the crossed treatment at both sites, and the moderately high PLI values show some pollen limitation at both sites (Fig. 2.10; Tables 2.5, 2.6). There was no relationship between natural fruit set and pollen distance (Table 2.6), and no significant difference in the number of seeds per fruit, or seed weight between the natural and cross-pollination treatments (Table 2.7). Adult density was low at Gordon Gow compared with Wairere (Table 2.6), and the gender distribution was male-biased at both sites, comprising 65% of the sampled population at Gordon Gow and 54% at Wairere (Fig. 2.11). Seedlings and juveniles comprised 87% and 65% of the population at Gordon Gow and Wairere respectively, indicating adequate population recruitment (Fig. 2.12).

Melicytus novae-zelandiae

M. novae-zelandiae produces large numbers of small, bell-shaped flowers, which were more abundant on male plants than on female plants. For example, a large male plant growing in an open site in full sun is likely to have its branches entirely covered with flowers, whereas flowers on female plants were more scattered and less abundant. *M. novae-zelandiae* male flower structure is similar to male *M. lanceolatus* flowers as described by Powlesland (1984), with predominant apical appendages that surround, extend beyond, and completely enclose the anthers (Fig. 2.18). In a study of the

reproductive biology of three species of *Melicytus*, Powlesland (1984) determined they were all insect-pollinated. In *M. novae-zelandiae*, however, I would suggest a combination of wind and insect. For example, during pollination treatments for this study, in addition to observing insect visitation (mainly blowflies) to flowers, I found that lightly tapping the branches on a male plants produced clouds of pollen that were readily dispersed by the ubiquitous coastal wind. Wind pollination is also supported by a high number of pollen grains per flower, and a pollen-ovule ratio indicative of wind-pollinated species when compared with other entomophilous species in this study (see Chapter 9). Pollination treatments were conducted during September 2002 and ripe fruit were collected in May 2003.
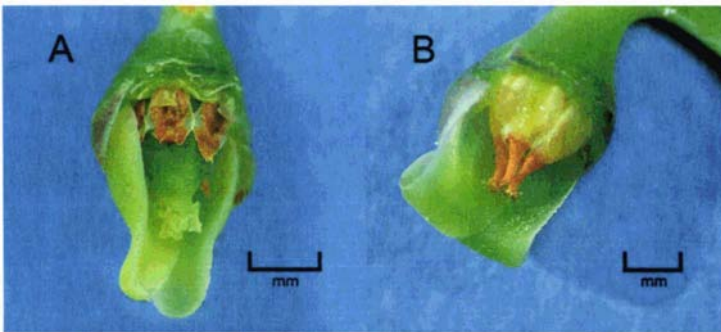
Fig. 2.18 Cut flowers of the dioecious shrub *Melicytus novae-zelandiae*. The female flower (A) shows a tri-lobed, wavy-margined stigma and reduced apical appendages, and the male flower (B) shows the orange-coloured apical appendages that completely enclose the anthers.

Mean natural fruit set was relatively high at both sites, particularly at Mount Maunganui, and although the cross-pollinated flowers produced slightly more fruit, this was significant only at Matakana (Fig. 2.10; Tables 2.5, 2.6). Very low PLI values show no pollen limitation (Table 2.5), and there was no relationship between natural fruit set and pollen distance (Table 2.6). A small number of fruit were produced in the bagged treatments (Fig. 2.10; Table 2.5), but I consider this to be a consequence of wind-dispersed pollen infiltrating the bagging material, rather than apomixis. There was no significant difference in the number of seeds per fruit, seed weight at either site (Table 2.7).

The Matakana Island population was male-biased, comprising 59% of the sample, whereas at Mount Maunganui, there was a slight female-bias, comprising 53% (Fig. 2.11). Adult plants dominated at both sites, comprising 89% at Matakana Island and 48% at Mt Maunganui (Fig. 2.12).

Pimelea arenaria

P. arenaria produces small, white flowers in terminal clusters (Fig. 2.19), and hermaphrodite flowers are larger than females. Pollination treatments were conducted during December 2002 and ripe fruit were collected in January and February 2003. An additional study of the gender distribution, fertilisation success, and size-class structure of *P. arenaria* at several populations in the North Island was undertaken, and will be discussed in Chapter 6.

Fig. 2.19 Clusters of female (A) and hermaphroditic (B) flowers of the gynodioecious coastal shrub *Pimelea arenaria* from Matarangi.
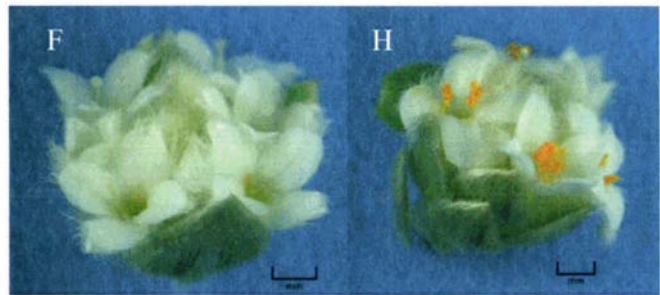
Mean natural fruit set in the female plants at Kawhia was higher than in the hermaphrodites (Fig. 2.10; Table 2.5). Conversely, higher fruit set occurred in hermaphroditic plants at Matarangi (Fig. 2.10; Table 2.5). There was no significant difference between natural fruit set and the cross-pollination treatments in either gender at either site (Table 2.6). The small number of fruit set in female bagged treatments at both sites was possibly the result of accidentally bagging hermaphroditic flowers, as plants of both gender are often intertwined. I also noted that some plants at Kawhia produced both female and hermaphroditic flowers, albeit in separate clusters. Very low SII values (0.04, 0.10) for hermaphroditic plants at both sites indicate high levels of self-compatibility, and high ASI values (> 0.75) show autogamous fertilisation (Table 2.5). There was no relationship between natural fruit set and pollen distance in either gender at either site (Table 2.6). There was no significant difference in seed weight

between treatments at either site. However, the seeds from Kawhia appeared larger, and the mean seed weight was more than double than those from Matarangi (Table 2.7).

Although population density of adult plants of both genders was low (47 females and 292 hermaphrodites/ha^{-1}) at Kawhia (Table 2.6), the overall population was much larger, but the plants were scattered along a 2–3-km stretch of the sand dunes. At Matarangi, plants were in closer proximity to each other (233 females and 1433 hermaphrodites/ha^{-1}) and covered a smaller total area. The gender ratio was hermaphroditic-biased at both sites, comprising 88% at Kawhia and 75% at Matarangi (Fig. 2.11). Size-class distribution showed adults dominating at both sites, with seedlings comprising 16.7% at Kawhia, and only 1.7% of the sampled population at Matarangi (Fig. 2.12).

2.5   Summary

The breeding systems amongst the 16 study species included hermaphrodism, dioecy and gynodioecy, and animal and wind-pollinated taxa (Table 2.8). The breeding strategy was variable only amongst the four *Alseuosmia macrophylla* populations, ranging from self-incompatible to self-compatible (Table 2.8). Pollen limitation was variable among populations and within species (Table 2.8). Autogamous selfing was present in eight of the nine species that produce hermaphroditic flowers (Table 2.8).

Table 2.8 Breeding system, pollination vector, and reproductive strategy, pollen limitation indices (PLI) range, and autogamy in 16 indigenous shrub species. H, hermaphroditic; D, dioecious; G, gynodioecious; A, animal-pollinated; W, wind-pollinated (anemophily); SI, self-incompatible; SC, self-compatible; AS, autogamous self-fertilisation; PA, partial autogamy.

Species	Breeding system	Pollination vector	Breeding strategy	PLI (range)	Autogamy (range)
Alseuosmia macrophylla	H	A	SI–SC	-0.16–0.87	AS–PA
A. pusilla	H	A	SC	0.08–0.23	AS
A. quercifolia	H	A	SI	-0.56–0.35	-
A. turneri	H	A	SC	-0.11–0.36	AS –PA
Coprosma spathulata	D	W/A	-	0.35–0.88	-
Coriaria arborea	H	W	SC	0.05--0.07	AS
Cyathodes juniperina	D	A	-	0.61--0.84	-
Leucopogon fasciculatus	D	A	-	0.42–0.52	-
Macropiper excelsum	D	W/A	-	-0.03– -0.25	-
Melicope simplex	D	A	-	0.70–0.72	-
Melicytus micranthus	D	A	-	0.65--0.69	-
Melicytus novae-zelandiae	D	W/A	-	0.05--0.20	-
Pimelea arenaria (F)	G	A	SI	-0.11– -0.37	-
P. arenaria (H)	G	A	SC	-0.17– -0.55	AS
Pomaderris hamiltonii	H	-	SC	-2.54– -1.03	AS
Raukaua anomalus	H	A	SC	0.30--0.59	PA
Teucridium parvifolium	H	A	SC	0.06	AS

2.6   Discussion

Plant species are characterised by complex integrated traits that influence a myriad of interactions, and different causal mechanisms among species can influence population growth and persistence (Aizen et al. 2002). Population persistence and viability are dependent on an uninterrupted sequence of major events, from flowering, cohort establishment, and survival to reproductive maturity (Fig. 2.20), and if an extended breakdown in one or more of these events becomes a frequent occurrence, population decline and eventually, local extinction could be expected. Rathke and Jules (1993) suggested long-lived species may not show immediate effects of fragmentation, but that future extinctions are predicted in cases of persistent pollen-limitation. In this study of native shrubs, breeding strategies, reproductive success, population size-class structures, density and habitat were investigated, and were used to assess the likely consequences for these species of ecosystem fragmentation.
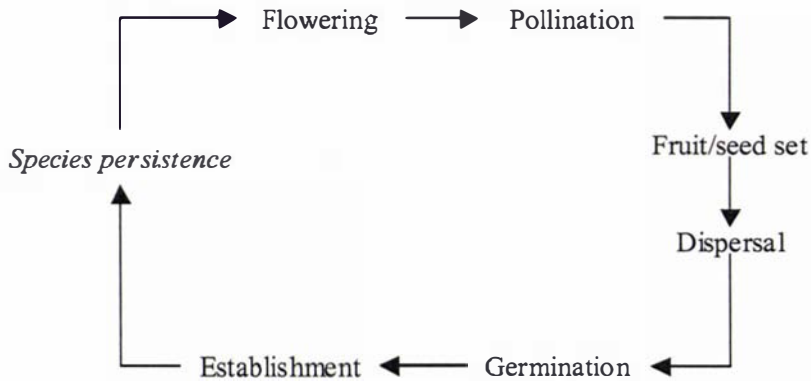
Fig. 2.20 Path diagram of the sequence of events required for plant population persistence.

Breeding systems and strategies

Of the sixteen species of this study, eight have proved to be hermaphroditic, seven dioecious, and one gynodioecious. One species, *Raukaua anomalous*, was previously thought to be dioecious, but during the course of this study was identified as hermaphroditic (see Chapter 7 for more details). One of the hermaphroditic species, *Teucridium parvifolium*, has been found to have gynodioecism in two populations in the North Island (see Chapter 8). Six of the hermaphroditic species are animal-pollinated, and one is wind-pollinated. *Pomaderris hamiltonii* was included in the animal-pollinated group for analyses because insect pollination was not eliminated as a potential pollination mechanism. The three dioecious wind-pollinated species show indications of a dual (wind and animal)-pollination syndrome; in the past, the dispersal of *Coprosma spathulata* pollen may also have been animal assisted by movement amongst the undergrowth by foraging native birds. *Macropiper excelsus* and *Melicytus novae-zelandiae*, previously reported as entomophilous, both display traits that suggest wind-pollination, and cryptic dioecy has been verified in the North Island in populations of *Cyathodes juniperina* and *Leucopogon fasciculatus*. Cryptic dioecy is where perfect, hermaphroditic flowers produce male and female organs, but function as either male or female (Mayer & Charlesworth 1991). Cryptically dioecious species are known from several unrelated families and has been reported in several genera including *Pimenta,*

Rosa, Schiedea, Solanum, and *Wikstroemia* (Mayer & Charlesworth 1991), and Humeau et al. (1999) reported cryptic dioecy in *Dombeya* species on La Réunion. In the New Zealand flora, cryptic dioecy has also been reported in the genus *Pennantia* (Icacinaceae) (Godley 1979; Webb 1996) and in *Dysoxylum spectabile* (Meliaceae) (Braggins et al. 1999).

In total, three species, one hermaphrodite (*Alseuosmia macrophylla*) and two gender dimorphic (*Coprosma spathulata, Cyathodes juniperina*), at three study sites showed high levels of pollen limitation (PLI > 0.75). The Mamaku *A. macrophylla* population also showed a high degree of self-incompatibility. Both *A. quercifolia* populations were highly self-incompatible, with one population showing moderate levels of pollen limitation. Self-incompatibility is a breeding strategy where a physiological barrier makes it impossible or difficult for a flower to fertilise itself. In most self-incompatible plants, pollen tube growth is inhibited on the stigma or in the style (Seavey & Bawa 1986). Seavey and Bawa (1986) also described late-acting self-incompatibility systems whereby fertilisation is prevented by inhibitory systems that operate in the ovary. Investigation of these mechanisms was beyond the scope of this study, but the self-incompatible *Alseuosmia* species have the potential for further investigation of the specific mechanisms preventing self-pollination. Self-incompatible taxa are more likely to show signs of pollen limitation if they have specialised pollinators and if pollinator abundance has declined (Wyatt 1983; Seavey & Bawa 1986; Proctor et al. 1996), with the degree of pollinator service an important predictor (Bond 1994). However, many New Zealand shrub species are relatively long-lived, i.e. c. 100 years, and it is possible their reproductive output would naturally vary from year to year, but because of their longevity, the consequences of occasional or frequent poor seed set would not necessarily have a significant impact in the long term. Some of the self-compatible populations are autonomous selfers; these taxa are reproductively assured and therefore relatively immune from pollination failure. Compensatory mechanisms such as self pollination, generalist pollination fauna, reduced dependence on seeds, and vegetative reproduction may ensure persistence in habitats where pollinator services are unreliable (Bond 1994).

Despite pollen limitation and self-incompatibility in the *Alseuosmia* species, population size-class structures at those sites showed a typical reverse J distribution, indicating

population recruitment despite low fruit set. *A. macrophylla* is known to be bird pollinated, and moth pollination has been identified (see Chapter 3), and poor fruit set may be the result of a decline in native pollinators, which in turn may be a consequence of predation by introduced mammals (Elliott 1996; Wilson et al. 1998; Wilson et al. 2003; Innes et al. 2004; Kelly et al. 2005). The cause of relatively low (<20%) natural fruit set in the Mamaku *Raukaua anomalus* population is not clear. This species can produce many hundreds of flowers during one season, and although natural fruit set may be low, the number of seeds produced on each plant could be relatively high. The population size-class structure at this site shows adequate seed has been produced for recruitment.

In the gender dimorphic taxa, high levels of pollen limitation (> 0.75) were evident in two species, the wind-pollinated *Coprosma spathulata,* and the cryptically dioecious *Cyathodes juniperina*. The second population of *C. juniperina* also showed moderate levels of pollen limitation. Moderate pollen limitation (PLI 0.50–0.75) was evident in a further three species: *Leucopogon fasciculatus* in one population*, Melicope simplex* in two populations*,* and *Melicytus micranthus* in two populations.

There were few (14%), male plants in the *Coprosma spathulata* (HK) population; a factor most likely influencing the low natural fruit set there. Seed germination was evident at this site, with seedlings comprising 72% of the population. However, seedlings tended to be clustered beneath female plants, and because juveniles comprised only 5% of the population, seedlings were apparently not surviving. This could possibly be due to unsuitable habitat characteristics or herbivory.

Moderately low fruit set in populations of *Cyathodes juniperina* and *Leucopogon fasciculatus* may be biased by their cryptic dioecy due to failure to consistently identify plant gender correctly. Cryptic dioecy is often difficult to detect because the functionally unisexual morphs appear to have perfect, hermaphroditic flowers (Mayer & Charlesworth 1991). The persistent, non-functional organs may have been retained because of the role they play in the reproductive process, such as pollinator attraction (Mayer & Charlesworth 1991). For *C. juniperina* and *L. fasciculatus*, assessing the degree of pollen limitation more accurately would require identifying the gender of each plant before conducting pollination treatments. Habitat conditions are likely to be a

factor in low fruit set and seed germination in both species. For example, low flower numbers in the forest environment of one population of *C. juniperina* was evident, compared with the roadside site. Reduced floral abundance may negatively affect the floral attractiveness of plants or rewards to potential pollinators (Proctor et al. 1996), and poor flowering may be attributable to reduced light levels associated with maturing trees and increased canopy closure.

Low natural fruit set in both populations of *Melicope simplex* may be exacerbated by female-biased (80%) populations. However, floral abundance in *M. simplex* is similar to *R. anomalus*, in which many hundreds, and possibly thousands of flowers can be produced each season, counteracting the proportionally low fruit set. Seedling and juvenile plant numbers were quite high at both sites, but like *C. spathulata*, they were more common beneath female adult plants, and in areas with higher light and reduced groundcover.

Burd (1994) suggested pollen limitation, the difference between natural pollination and supplementary hand pollination, is a common, and natural, phenomenon, and is supported by analysis of the published results of 258 species. He suggests highly self-incompatible species would gain greater benefit, relative to natural pollination, from the artificial application of excess outcross pollen compared with self-compatible species, implying that the results may be biased. Haig and Westoby (1988) proposed that at an evolutionary equilibrium female fecundity would be limited by both pollen and resources, and argue that if pollen limited seed set, selection would favour increased allocation to pollinator attraction. Burd (1994) suggested that pollen limitation is driven by the investment of resources into pollinator attraction (floral advertisement and rewards), which would require more flowers, or ovules per flower, than would be fertilised. An excess of flowers may indirectly result in the production of fruits of superior quality (Burd 1998). Fig. 2.21 (following the method of Burd 1994) illustrates the distribution of pollen limitation in the 16 species of this study, with each plotted symbol representing the outcome for one population over one flowering season. The majority of cases (76.5%) show fruit set was enhanced by hand outcrossing (Fig. 2.21), which appears to support Burd's (1994) observations.
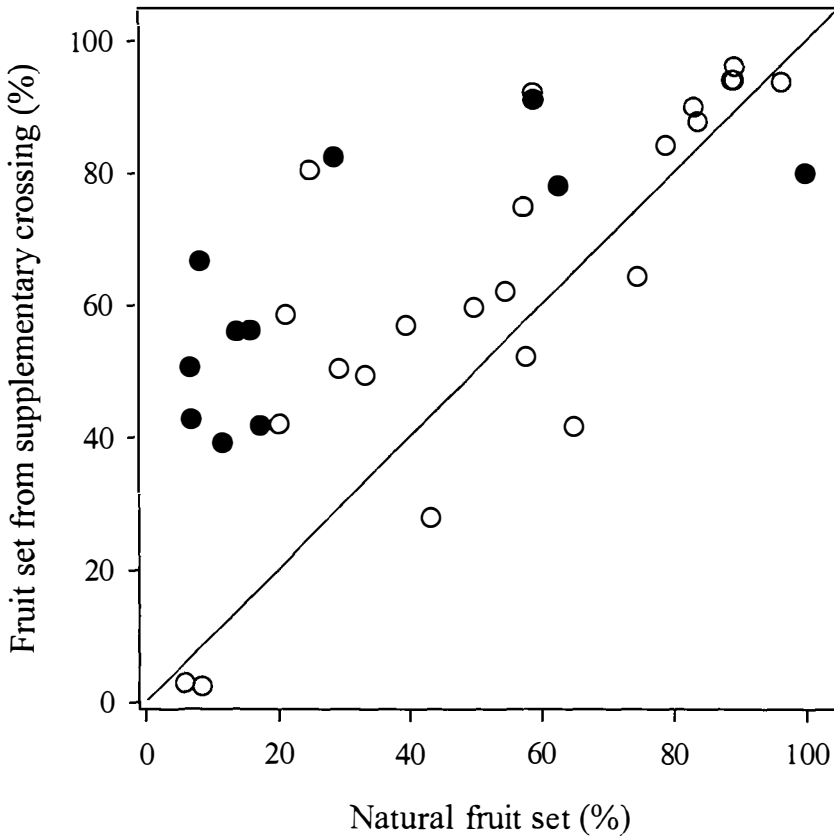
Fig. 2.21 Comparison of percent fruit set in natural pollination and supplementary outcrossing (Y-axis) and natural (X-axis) fruit set for 16 species from 34 study sites. Each plotted symbol represents one species per study site combination. The x-y line is given for reference. Points above the line indicate higher fruit set in outcrossed pollination treatments compared with natural pollination, and points below, the opposite. Filled symbols indicate species at sites with a significant difference between natural and crossed fruit set (see Tables 2.3, 2.6). Of the taxa with symbols below the x-y line, the means of natural fruit set in all populations except one (*Pomaderris hamiltonii*), were higher than 40%.

Bawa and Webb (1984) investigated flower, fruit and seed abortion in seven tree species, and found that low seed set was not generally the result of insufficient pollinators or compatible pollen. They suggest that because plants produce flowers to meet the vagaries of pollination, eventual fruit and seed set may closely match available resources, which may function as a mechanism for regulating the quality and quantity of a seed crop (Bawa & Webb 1984). Bawa and Webb (1984) also raised the possibility that flowers on the same branch in the immediate vicinity of hand-pollinations could produce fewer fruit than those close to natural pollinations because of reallocation of resources, and subsequently influence the degree of putative pollen limitation. Ashman

et al. (2004) suggested examination of pollen limitation at the population level should involve the entire plant life cycle, from germination rate, and seedling survival, to adult survival because of the potential effects of pollen supplementation on resource allocation. Because of the difficulties with such a proposal in long-lived shrub species, Ashman et al. (2004) suggested a modelling approach that explores the sensitivity of population growth rate to seed production under various life history and environmental disturbance scenarios would provide considerable insight into population-level effects of pollen limitation. Ashman et al. (2004) did not believe the current evidence allows a satisfactory assessment of the causes or consequences of pollen limitation, and that for plant species of conservation concern, knowledge of the critical threshold density at which plants will become extinct as a result of decreased pollinator activity is essential. Eight of the study species that produce hermaphrodite flowers can reproduce autonomously, and are thereby reproductively assured to some extent.

Too few studies have been conducted into the breeding systems and strategies of New Zealand's native shrubs to determine the frequency of self-incompatibility in the shrub flora. For example, Newstrom and Robertson (2005) list only seven published accounts where experimental tests of breeding systems in animal-pollinated species have been conducted on shrubs. Self-incompatibility has been identified in *Hebe vernicosa* (Frankel & Hair 1937), *Pentachondra pumila* (Godley 1966), *Discaria toumatou* (Primack 1979), *Geniostoma rupestre* (Rattenbury 1980), *Pseudowintera colorata* (Lloyd & Wells 1992), and *Corokia cotoneaster* (Webb 1994). Self-compatibility has been demonstrated in seven of the eight hermaphrodite species of this study (although one species is variable), which appears contradictory to the results of Newstrom and Robertson (2005), that 80% of trees and shrubs are self-incompatible. But, as stated by Newstrom and Robertson (2005), the degree of self-incompatibility is still poorly known, particularly in the shrub flora.

Fragmentation effects

The study sites in this investigation were of various vegetation types, included a range of fragment sizes, and comprised forest and non-forest habitats (e.g., sand dunes, stream margins, and roadside banks). The study shrub species that occur in native forest remnants have presumably persisted for at least several decades; their habitats are

mostly in public reserves, and have experienced minimal anthropocentric disturbance (such as logging, fire, stock grazing) over the past 50 or more years.

In fragmented ecosystems where population size has been reduced, seed set could be reduced to the extent that species persistence is threatened. In this study, long-term population persistence was evaluated by measuring *in situ* size-class structures. The results show species at seven of 34 sites had poor or no population recruitment: *C. arborea* KW, *P. hamiltonii* KR, and *R. anomalus* GG in the hermaphrodite group and *Cyathodes juniperina* WR, *Leucopogon fasciculatus* WR, *Melicytus novae-zelandiae* MK, and *Pimelea arenaria* MR in the gender dimorphic group. Two of these, *C. juniperina* and *L. fasciculatus*, also had moderate pollen limitation (PLI 25–75). In each of the seven species, the results suggest lack of seedlings could be related to characteristics of the habitat that preclude seed germination. For example, the ground beneath and around adult plants of *C. arborea* KW was completely covered by dense pasture grasses and other groundcover species, and mown grasses formed a dense groundcover under the roadside *P. hamiltonii* KR population; both conditions effectively preventing seed germination. Low light levels under an almost-closed canopy are the probable causes of germination and recruitment failure in the *R. anomalus* GG, population. The habitat of the *Cyathodes juniperina* and *Leucopogon fasciculatus* WR populations was the verge and banks of a rural road, also home to weedy exotic plant species; the verges are regularly maintained by mowing, an activity that damages mature plants. A dense groundcover of grasses and other exotic species reduces available habitat and commonly prevents seed germination of native taxa. The cause of poor recruitment in the *Melicytus novae-zelandiae* MK population is not clear, although the close proximity of tall pine trees (*Pinus pinaster, P. radiata*) may affect light levels and habitat availability. Poor recruitment is widespread in *Pimelea arenaria* populations, despite adequate seed set, and the specific cause has not been identified. Further research is required to determine whether herbivory of fruit and/or seedlings is a contributing factor.

The presence of seedlings but absence of juvenile plants in the *A. quercifolia* PM site, was most likely the result of seedling herbivory by introduced mammals such as hares and rabbits (Merrett et al. 2002), or possibly rats (Daniel 1973; Innes 1979; Best 1969; Moors 1985; Miller & Miller 1995; Wilson et al. 2003). Field observation suggests recruitment failure in *C. spathulata* at the HK site may be due to a lack of suitable

habitat, primarily because reduced light levels associated with increasing canopy closure do not favour seedling establishment, or as a result of seedling removal by herbivory.

Much has been written about the negative impacts of fragmentation and edge effects, and that biological diversity is threatened because of the magnitude of habitat alteration, degradation and destruction as a result of human activity (Laurance & Yensen 1991; Saunders et al. 1991; Primack 1993; Murcia 1995; Davies-Colley et al. 2000; Gehlhausen et al. 2000). However, natural disturbances are a normal feature of native ecosystems, and although rarely on the scale of destruction that is associated with human colonisation, the impacts are none the less similar. The oceanic climate of the New Zealand archipelago is described as unpredictable by Meurk (1995), with a wide range of episodic disturbance events that have influenced plant communities and ecosystem dynamics, including volcanic eruptions (Clarkson 1990; Smale & Smale 2003), earthquakes (Cullen et al. 2003), fire (McGlone 1989; Meurk 1995), windstorms and cyclones (McGlone & Webb 1981; Shaw 1983; Jane 1986; Wardle 1991; Wells et al. 1998), landslides (Payton 1988; Wells et al. 1998); flooding (McGlone 1989; Cullen et al. 2003), and drought (Jane & Green 1983; Wardle 1991; Wells et al. 1998).

Openings formed by natural disturbances and canopy collapse favour regeneration, particularly of early successional species. At all the sites, the study populations occurred on, or within 30–50 m of the edge, or were associated with gaps in the canopy. Fragmentation, logging, road construction and track formation in native forests, especially over the past 100 to 150 years, have provided disturbed soil and higher light conditions, and these have been colonised by early successional native species, including many shrub taxa. Young and Mitchell (1994) investigated forest edges to identify the conservation implications of preserving interior forest conditions and found that the edge environment of lowland broadleaf forests favoured a number of light-tolerant native species. However, it would seem that although forest interior native trees have been negatively affected by fragmentation, a number of species have been favoured, and it may be that edges are important refugia for some native taxa, especially shrubs. Kelly et al. (2000) found that flower predation of the mistletoe *Peraxilla tetrapetala* was significantly lower in plants on isolated trees compared with those on

trees in continuous forest. Montgomery et al. (2003) reported higher fruit set on edges, possibly because of increased resources e.g., higher light, and/or pollinator behaviour. A characteristic of New Zealand plants is the predominance of small, white or pale coloured flowers, simple floral structures, separate sexes, and fleshy fruits (Godley 1979; Bawa 1980; Lloyd 1985; Lloyd & Webb 1986; Webb & Kelly 1993; Wilson & Galloway 1993), and there is a disproportionate reliance on unspecialised insect groups that promiscuously visit a wide range of flowers (Lloyd 1985). Several of the species in this study, although unproven, appear to have dual-pollination syndromes, a feature that could suggest a form of 'bet-hedging' to ensure fertilisation in the event of pollination failure of one of the pollination vectors. In this context, generalist pollinators, and multiple pollination vectors, as well as self-compatibility in hermaphroditic species, have enabled small or isolated plant populations to persist, contribute to the seed bank, and thus take advantage of newly created habitat after a disturbance event such as fragmentation.

2.7   Acknowledgements

2.8   References

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Chapter 3

Floral biology, and bird and moth pollination in *Alseuosmia macrophylla*, an endemic shrub from New Zealand

3.1 Abstract

Alseuosmia macrophylla populations on the Mamaku Plateau flower for 6 weeks, but with peak flowering approximately for 14 days. The flowers display an unusual form of herkogamy, with differing stigma/anther positions. There was a significant negative relationship between herkogamy and natural fruit set, the greater the distance between anthers and stigma, the lower the fruit set. Nectar production ceased after removal of its standing crop. The mean nectar concentration was slightly less (11.5%) than nectars typical of other bird-pollinated flowers (typically 15–25%). Flower damage from nectar robbing was common, and although nectar was entirely removed during robbing, this factor apparently did not negatively affect fruit set. On one occassion, white-eyes were observed robbing flowers. *A. macrophylla* is mostly self-incompatible at this study site, and has a dual bird/moth pollination syndrome. Fruit set in nocturnal (moth)-exposed flowers (14.2%) was higher than in diurnal (bird)-exposed flowers (10.7%) although not significantly so, suggesting that moths may be as effective as pollinators as bellbirds. Widespread pollen limitation was evident with < 30% natural fruit set recorded in all six study plots compared with > 50% fruit set following supplemental cross-pollination. One potential threat to the long-term persistence of *A. macrophylla* in the Mamaku Plateau would be the loss of pollinators, despite the 'bet-hedging' dual-pollination syndrome. Because of floral polymorphism and variation in breeding strategy in *A. macrophylla* within its range, the results of this study do not necessarily apply to other populations.

3.2 Introduction

Fragmentation and modification of New Zealand's native forests as a consequence of clearance, fire, logging, and the introduction of exotic mammals over the past 150 years has resulted in changes in plant community structure and function, particularly the

decline in native pollinators. Reduced pollinator abundance is particularly important for plant species with specialised pollination systems, and these may become vulnerable as fragmentation continues (Bond 1994). An understanding of the breeding systems and strategies of plant species is an important step in assessing their capacity to persist in fragmented ecosystems (Bond 1994). Reproductive mutualisms (plants and their pollinators) are a complex web of interactions that, if broken, could cause a cascade of extinctions (Bond 1994). No mutualism is completely assured when pollinators are faced with a diversity of threats as a consequence of human activities (Bond 1994). Mutualism failure is influenced not only by the degree of dependency (facultative or obligate), but by the importance of seeds in the demography of the plant (Bond 1994). Reduced fruit and seed set as a consequence of an interruption or breakdown in plant-pollinator interactions could negatively impact on population dynamics. The degree to which fragmentation may affect pollination and reproductive success is expected to vary among plant species, depending on differing breeding systems and strategies, and sensitivities to pollen transfer (Aizen & Feinsinger 1994). Pollen limitation of plant reproduction occurs when a shortage of acceptable pollen limits seed set, and may occur on the scale of individual flowers, entire plants, or whole populations (Montgomery et al. 2001; Ashman et al. 2004). Examples of pollen limited fruit set have been reported worldwide (Burd 1994; Ashman et al. 2004).

Darwin (1877) recognised that floral characteristics could provide a guide to a plant's breeding system and that various floral features could promote outcrossing. Floral characteristics can also be an indicator of pollination vectors. For example, Faegri and van der Pijl (1979) describe generalised characteristics that identify blossoms into various pollinator groups, e.g., phalaenophily (moth pollinated), ornithophily (bird pollinated). The concept of pollination syndromes has been used to describe these plant-pollinator interactions, but Faegri and van der Pijl (1979) stressed they were generalisations only. For example, ornithophilous (bird-pollinated) flowers are typically characterised by several common features such as red or orange tubular flowers, abundant dilute nectar, high pollen-ovule ratios, numerous anthers, and lack of floral scent (Faegri & van der Pijl 1979), but in New Zealand at least, birds visit many flowers without these characters (Castro & Robertson 1997; Robertson et al. 2005).

The flowers of many plant species receive visits from a wide variety of animal groups spanning diverse orders of vertebrates or invertebrates (Hingston & McQuillan 2000; Robertson et al. 2005). The characteristics of flowers will be influenced by those pollinators that visit most frequently and effectively, but not necessarily exclusively (Stebbins 1970). The effectiveness of different flower visitors as pollinators is important for understanding plant-pollinator relationships, because although a plant may receive a variety of visitor types, only some may be successful pollinators (Robertson et al. 2005). In biotic pollination, an effective attractant is required to initiate a visitor reaction, and the 'food urge' promotes the majority of blossom visits (Faegri & van der Pijl 1979). The energy demands of most pollinators are met from nectar, with larger pollinators requiring greater and more concentrated quantities of nectar (Faegri & van der Pijl 1979).

A common expectation is that large-bodied animals, such as birds, are capable of travelling considerable distances and are not affected by plant isolation, as could be expected in fragmented habitats, whereas small bodied pollinators such as flies and small bees are assumed to be less capable of moving between isolated plants (Ghazoul & Uma Shaanker 2004). Floral clustering is energy saving for a pollinator, but there is a risk of geitonogamous self-fertilisation if self-incompatibility mechanisms are not in place. A number of mechanisms can reduce potential self-fertilisation in hermaphroditic flowers, including physiological barriers in homomorphic flowers, heteromorphy (heterostyly), and herkogamy (Ganders 1979; Faegri & van der Pijl 1979; Wyatt 1983; Seavey & Bawa 1986; Webb & Lloyd 1986). Heteromorphism is nearly always associated with self-incompatibility systems and usually involves heterostylous flowers (Ganders 1979), but it is not known to occur in the New Zealand flora (Godley 1979; Webb et al. 1999). Herkogamy is the spatial separation of pollen and stigmas in hermaphroditic flowers and is usually interpreted as a mechanism that restricts self-pollination (Faegri & van der Pijl 1979; Webb & Lloyd 1986).

The aim of this study was to develop an understanding of the reproductive biology of *Alseuosmia macrophylla,* and is a continuation of the initial study as described in Chapter 2. The following questions are addressed: (i) What are the floral characteristics of *A. macrophylla*? (ii) What is the breeding system of *A. macrophylla*? (iii) Is

reproductive success affected by the proximity of conspecifics? (iv) What are the pollination vectors of *A. macrophylla*?

3.3   Materials and methods

Study species

Alseuosmia macrophylla (Alseuosmiaceae) is an endemic, understory shrub of native forests in New Zealand. It is the most widely distributed of the five currently recognised *Alseuosmia* species, occurring in both North and South Islands (Allan 1961; Gardner 1978; Merrett & Clarkson 2000). Its floral anatomy includes a long tunnel-form corolla, red corolla colour, epipetalous stamens, and inferior ovary (Gardner 1976). The relatively large flowers in the study area are scented, produce nectar, and are up to 3 cm in length (Merrett & Clarkson 2000), with a single style and stigma and four to six filamentous anthers that dehisce longitudinally. There is, however, considerable variation in floral morphology and colour throughout its range in the North Island (unpubl. data). *A. macrophylla* is the most common understory shrub in the Mamaku Plateau study area, often forming relatively dense thickets, particularly within 50 m of the forest margin. It has a sprawling, multi-stemmed, liane-like habit, with stems reaching up to 5 m in length. The stems are relatively flexible and are able to produce adventitious roots when they come in contact with the ground surface; an event that occurs when debris, such as a tree branches, fall from canopy or sub-canopy species onto living *A. macrophylla* stems. This often causes the stems to bend downwards, make contact with the ground surface, produce adventitious roots, and eventually vegetatively reproduce another plant. As a result, close-neighbour clusters of what appears to be individual plants, may be genetically identical. Nectar feeding on *A. macrophylla* by stitchbird (*Notiomystis cincta*) has been reported from Little Barrier Island (Gravatt 1969; Angehr 1986). Nectar feeding by bellbirds (*Anthornis melanura*) on flowers of two *Alseuosmia* species, *A. macrophylla* and *A. turneri*, has been observed on several occasions (Merrett pers obs.; Merrett et al. 2002). Moth pollination of *Alseuosmia* was proposed by Merrett et al. (2002), based on floral characteristics, and the capture of a moth feeding on *A. quercifolia*.

Study area

The Mamaku Plateau study area (Fig. 3.1) is part of the Kaimai Mamaku Forest Park, a large tract of native forest that forms a boundary between the Waikato region and the Bay of Plenty. The Park comprises approximately 37 000 ha with two distinct areas, the Kaimai Range and the Mamaku Plateau. The forested area on the Mamaku Plateau is currently described as tawa (*Beilschmiedia tawa*) forest, but was formerly rimu-tawa before systematic logging and fires (Nicholls 1974). It reaches an altitude of approximately 700 m a.s.l., and has an annual rainfall ranging from 2250 to 2600 mm. The study sites were located in tawa-dominated forest, within 50 m of the forest margin along alongside the main road (SH5) between Tirau and Rotorua (Fig. 3.1).
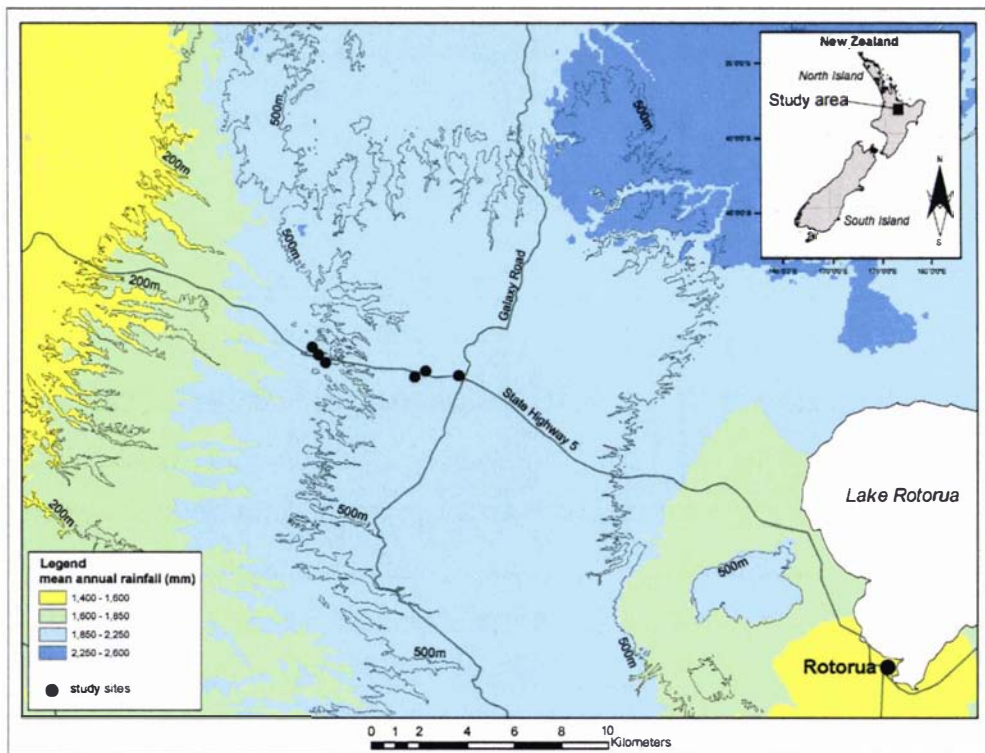
Fig. 3.1 Location of six *Alseuosmia macrophylla* plots, rainfall gradients, and contour lines of the Mamaku Plateau study area.

Phenology

Flowering periodicity was measured by monitoring 1213 flowers on tagged branches of 33 plants. Initially flower buds were counted at mature bud stage, and followed by

counting of open flowers at 7-day intervals after the start of anthesis, and until all flowers had senesced.

Nectar was sampled from 148 flowers from 17 plants during the 2003 flowering season to determine its volume and concentration. Flower buds were initially bagged until anthesis, the standing crop (SC) of nectar was collected, and then flowers were either rebagged or left exposed to pollinators (natural). Nectar was collected from the same flowers for both treatments 24 and 48 hours after the initial standing crop sampling. Volume was measured with calibrated $25\mu l$ capillary tubes, and percent concentration (in sucrose equivalents) was determined with a Bellingham & Stanley hand refractometer. Total sugar quantity of nectar sample per flower in grams was calculated by multiplying sugar concentration by nectar volume, as described by Dafni (1992).

In 2003, herkogamy was investigated by measuring *in situ* the distance from the stigma to the anthers in 903 flowers from 70 tagged plants with digital callipers.

Observation made during 2002 had shown some *A. macrophylla* flowers were damaged by nectar robbing, so during the flowering season of 2003, 840 flowers on 48 tagged plants were scored as robbed or not robbed and the proportion of robbed flowers per plant was compared with natural fruit set to assess whether nectar robbing affected fertilisation. In many cases, different flowers were used to measure herkogamy and nectar robbing.

Breeding system and reproduction

Investigations were undertaken during the 2002 and 2003 flowering seasons to assess the breeding system and reproductive success of *Alseuosmia macrophylla* (for 2002 see Chapter 2). To compare between two flowering seasons, fruit set was measured from four pollination treatments in 2003, using the same methods as described in section in Chapter 2 and reiterated as follows. 1. Controls (natural): natural pollinators allowed, stems in bud were tagged and buds counted. 2. Manual supplementary cross-pollination (crossed): during anthesis pollen from different plants was collected on a small artist's paintbrush and applied to stigmas. 3. Manual self-pollination (selfed): for hermaphroditic flowers, flower buds were bagged, during anthesis pollen collected from

the same plant was applied directly onto stigmas, and flowers rebagged. 4. Pollinators excluded (bagged): flower buds were counted then stems were enclosed in fine, mesh, fabric bags, tied with coloured twist ties, and left bagged with no further treatment. The number of treatment flowers per plant and treatment plants per plot varied amongst the six plots. The number of flowers available for pollination treatments in 2003 was lower than desirable in some plots as a result of frost and freezing winds that killed many flower buds during the period between the initial preparatory pre-anthesis tagging and bagging, and the actual pollination treatments. To measure whether natural fruit set was influenced by pollen travel distance, investigations were undertaken in separate sites of differing density on the Mamaku Plateau in 2003 (Fig. 3.1). Six 25 m x 25 m (625 m^2) plots were established within populations, and within each plot, the position of all adult (flowering) *A. macrophylla* plants was established by measuring their distance from tapes placed along plot X and Y axes (Fig. 3.2). Plants within these plots were used for the pollination treatments as described above. Two indices were calculated to assess pollen limitation and self-incompatibility and are described in Chapter 2 and reiterated as follows. Pollen limitation index: $PLI = 1 - (I_N/I_X)$ where I_N is the percent fruit set of natural (open-pollinated controls) and I_X is the percent fruit set of crossed (supplemental cross pollen) plants, and represents the degree of pollen limitation, or inadequate pollinator activity. $PLI = 0$ indicates no pollen limitation in the population under study (Larsen & Barrett 2000). PLI of > 0.75 indicates a high degree of pollen limitation (following Newstrom & Robertson 2005).

Self-incompatibility index: $SII = 1 - (I_{MS}/I_{MX})$ where I_{MS} is the percent fruit set from selfed (manual self-pollination) and I_{MX} is the percent fruit set from crossed (supplemental cross pollen) flowers. $SII = 0$ indicates complete self-compatibility (Riveros et al. 1998). SII of > 0.75 indicates a high degree of self-incompatibility.

Pollination vectors

To investigate pollinators, during the 2003 flowering season, clusters of newly opened flowers with nectar were monitored, using time-lapse video equipment with an infrared camera, at various times from dawn to dark to record pollinator activity (Table 3.1). Nocturnal and diurnal flower exposure experiments were undertaken during the 2004 flowering season to determine the efficacy of bird versus moth pollination. For the

nocturnal pollination experiments, branches were bagged while in bud and after anthesis, flowers were unbagged immediately before dusk, then rebagged at dawn. Groups of flower were subjected to four separate nocturnal moth visitation exposure periods (1, 2, 3, and 4 nights). At the completion of each of the four exposure periods, flowers were rebagged, and left for 6 weeks until all flowers had senesced. Fruit set was counted in January 2005. For diurnal pollination, the same procedures were applied, but the flowers were exposed for diurnal pollination; unbagged at dawn and then rebagged at dusk. To compare diurnally and nocturnally exposed flowers and natural fruit set, flower buds and the subsequent fruit set were counted on tagged branches on all treatment plants.

Table 3.1 Date, start time and length of video footage of flowers of *Alseuosmia macrophylla* at the Mamaku Plateau.

Date (2003)	Start time	Recording duration (hrs)
23 Oct.	17.35	3
24 Oct.	17.10	3
27 Oct.	17.40	3
3 Nov.	18.45	6
4 Nov.	5.45	6
4 Nov.	17.15	6
5 Nov.	5.45	12
5 Nov.	17.40	3
6 Nov.	11.40	6

Data analysis

The means of nectar volume, percent concentration, and total sugar concentration (g/L) were calculated. Box plots were generated from the sugar concentration (g/L) of nectar from the standing crop, 24 hour and 48 hour samples from individual flowers from 2003. A two-sample unpaired t-test was used to compare natural and bagged standing crop of nectar volume and concentration. Box plots were also generated from individual plant fruit set from four pollination treatments for 2002 and 2003. The box plots depict the median (central line in each box), 25% and 75% quartiles (upper and lower limits of each box), 90th and 10th percentiles (indicated by the whiskers), and outliers, (indicated

by open circles). To compare fruit set between the pollination treatments and years, percent fruit set was determined by calculating the proportion of fruit with the number of buds for each treatment on each plant and analysed using a generalised linear model (GLM) with binomial error distribution and logit link function, and F-test significance tests. Percent fruit set, and their means and standard errors were calculated for individual plants and for each plot. To evaluate factors influencing reproductive success at the plot level, GLM analyses, as described above, were used to identify relationships between plot averages for natural fruit set and distance to outcrossed pollen, herkogamy, and nectar robbing, and between natural fruit set and fruit set from caged flowers, and nocturnally- and diurnally-exposed flowers.

3.4   Results

Phenology

Flowering periodicity in 2004 was 6 weeks, with peak flowering occurring over a 14-day period, 3 weeks from the start of anthesis (Fig. 3.3). Nectar commonly filled half of the corolla tube, and mean nectar quantities in sucrose equivalents from the standing crop from natural flowers was 3.24 mg, with a range of 0.21 mg to 11.3 mg per flower (Fig. 3.4). In the bagged treatment, the mean nectar concentration was similar to the natural flowers at 3.52 mg, and ranged from 0.19 to 10.4 mg. There was a reduction in concentration at each of the succeeding (24 hrs and 48 hrs) collection times after standing crop sampling (Fig. 3.4). There was no significant difference in nectar concentration between the natural or bagged treatments (Fig. 3.4; Table 3.2).

Stigma/anther position is variable amongst same age flowers, suggesting variable herkogamy, i.e. variation in the distance between stigmas and anthers, ranging from exserted stigmas, stigma and anthers equal, and inserted stigmas. Among flowers from all the plots ($n = 359$), stigmas exserted beyond the anthers was the most frequent position (63.2%) (Fig. 3.5, 3.6; Table 3.3), stigmas and anthers were equal to each other in 32.9% (Fig. 3.6, 3.7), and inserted below the anthers in 3.9% of the flowers (Fig. 3.6; Table 3.3). Within plots, flowers with exserted stigmas was the most common position in five of the six plots; the exception was plot 2 where the most common position was equal (Table 3.3). It was noted that in some flowers buds, the stigma was precocious, i.e. exserted before anthesis (Fig. 3.8). Among plots, the mean exserted distance

between stigma and anthers ranged from 1.6 mm in plots 2 and 5, to 2.5 mm in plots 4 and 6, and for inserted stigmas, the mean distance ranged from 1.1 mm in plot 6 to 3.6 mm in plot 4 (Table 3.3). Logistic analysis (of absolute values) shows a significant relationship between fruit set and herkogamy (Table 3.4).

Nectar-robbed flowers were present in all plots, ranging from 15.2% in plot 5 to 81.3% in Plot 1 (Table 3.5). Amongst all flowers measured, logistic analysis showed no significant effect of robbing on natural fruit set (Table 3.4). Nectar robbing by a flock of white-eyes (*Zosterops lateralis*) was observed on one occasion; the birds perched on the stems and lifted the flower upwards with their beak near the base of the corolla, piercing and tearing the corolla for more than half its length to feed on the nectar.
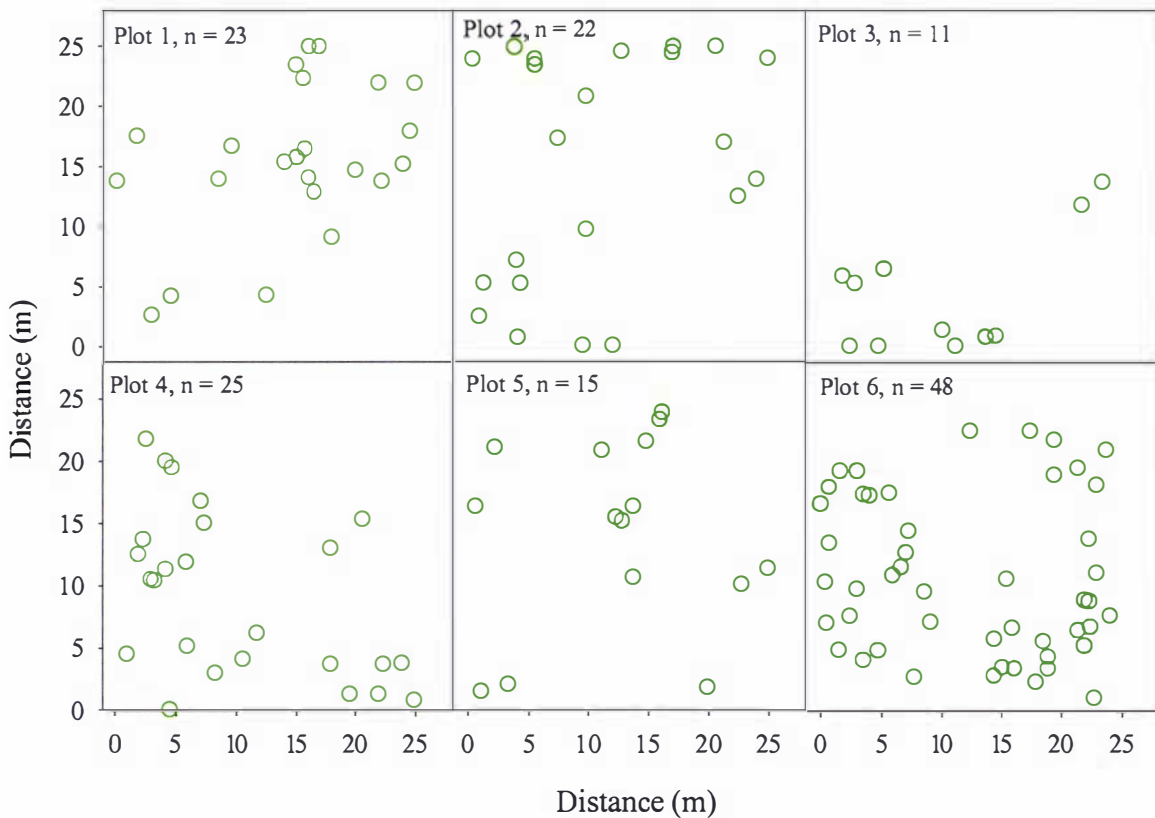
Fig. 3.2 Distribution and density of *Alseuosmia macrophylla* individuals in six 625 m² study plots in the Mamaku Plateau area.

Fig. 3.3 Flowering periodicity of 1213 flowers on 33 *Alseuosmia macrophylla* plants at the Mamaku Plateau, 2004.
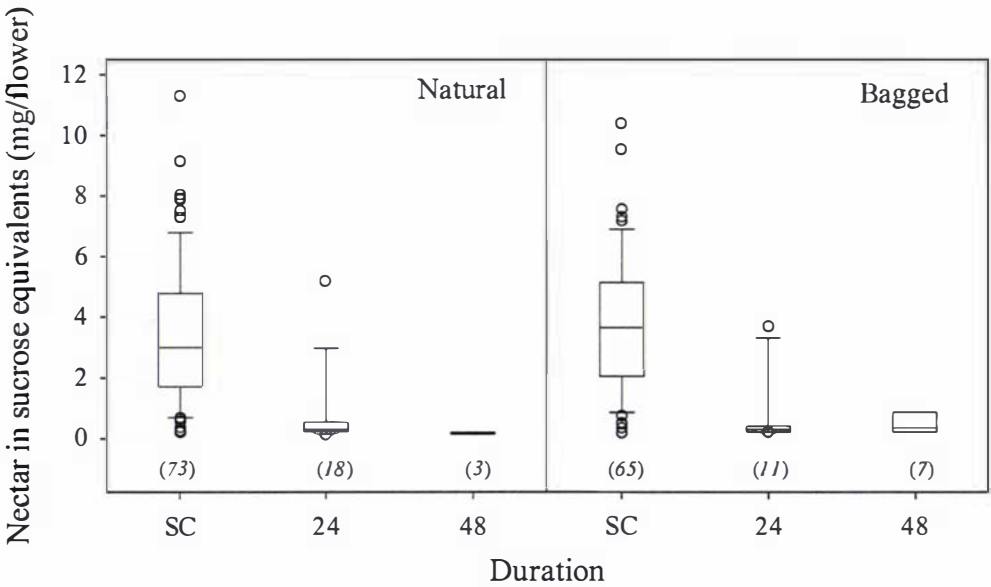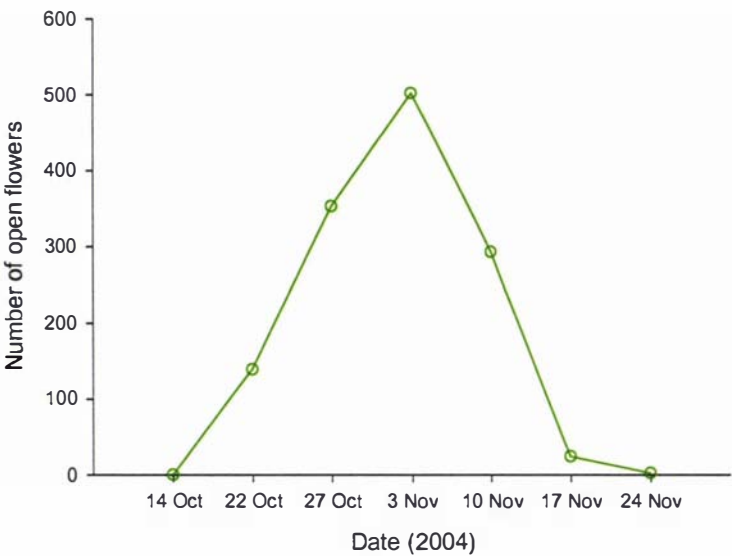
Fig. 3.4 Distribution of nectar in sucrose equivalents in g/L from *Alseuosmia macrophylla* flowers, 2003, at the Mamaku Plateau population for natural and bagged treatments for three sampling periods: SC, standing crop, after 24 hours, and after 48 hours from the same flowers, to test for nectar replenishment. The numbers of flowers per sampling period are in parentheses.

Fig. 3.5 *Alseuosmia macrophylla* flowers from the Mamaku Plateau, and an example of morphs with an exserted stigma.
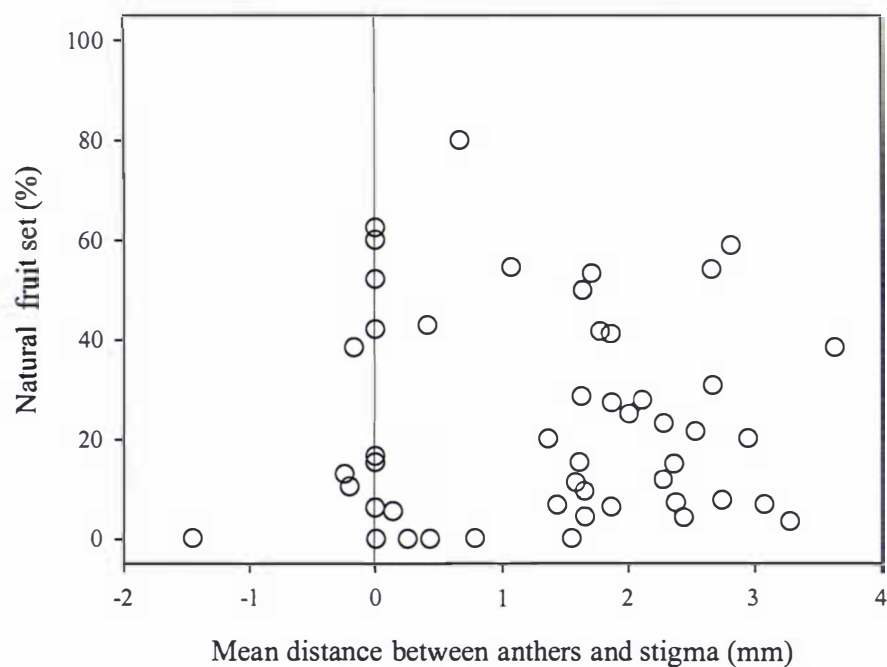
Fig. 3.6 Relationship between plant means of variable herkogamy (stigma/anther separation) and natural fruit set in 1040 flowers from 49 *Alseuosmia macrophylla* plants from the Mamaku Plateau, 2003. Each symbol represents an individual plant. Flowers above 0 have stigmas exserted beyond the anthers, those at 0 have stigma and anthers at the same level, and those below zero have the stigma positioned below the anthers.

Fig. 3.7 *Alseuosmia macrophylla* flowers showing stigma and anthers at more or less equal position.

Fig. 3.8 *Alseuosmia macrophylla* flower bud from the Mamaku Plateau showing a precocious stigma.

Table 3.2 Nectar characteristics: means of nectar volume, percent sucrose concentration, and sucrose equivalents in mg per flower of the nectar standing crop from naturally exposed and bagged *Alseuosmia macrophylla* flowers from the Mamaku Plateau, and results of two-sample unpaired *t*-tests between natural and bagged samples.

| | Treatment | | Natural vs | |
Nectar	Natural	Bagged	bagged (*t, P*)	d.f.
Volume (µl)	27.0±17.3	28.9±15.4	0.69, 0.493	136
Sucrose equivalents (%)	11.6±1.7	11.9±1.8	1.10, 0.273	136
Sucrose quantity per flower (mg)	3.4±2.3	3.7±2.2	-	

Table 3.3 The number of flowers, the proportion of flowers per plot with exserted, equal and inserted stigmas, and the minimum (Min,)mean and maximum (max) stigma-anther distance in *Alseuosmia macrophylla* flowers from six study plots in the Mamaku Plateau, 2003.

| | | | Proportion of flowers (%) with stigma position in relation to anthers | | | Stigma-anther distance(mm) | |
Plot number	Number of plants	Number of flowers	Exserted	Equal	Inserted	Exserted Min (mean) max	Inserted Min (mean) max
1	15	80	64.9	24.7	10.4	0.9 (2.3) 4.6	0.6 (1.8) 3.5
2	5	56	21.7	73.9	4.3	0.8 (1.5) 2.9	1.4 (1.4) 1.4
3	4	46	50.0	45.5	4.5	0.9 (1.8) 3.1	1.2 (1.2) 1.2
4	12	200	60.6	38.0	1.4	1.2 (2.5) 4.1	3.6 (3.6) 3.6
5	6	17	66.7	22.2	11.1	1.1 (1.6) 2.4	1.5 (1.5) 1.5
6	28	504	71.3	27.4	1.3	0.9 (2.5) 5.6	1.0 (1.1) 1.2
Totals	70	903	63.2	3.9	32.9	-	-

Table 3.4 GLM of effect of predictor variables (herkogamy, nectar robbed flowers, and distance to nearest pollen) on the plant means of fruit set for *Alseuosmia macrophylla* flowers, Mamaku Plateau, 2003. d.f. for *t* = 35.

Variable	Estimate of coefficient	SE	*t*-ratio	*P* value
Constant	-0.511	0.253	-2.02	0.043
Herkogamy	-0.2438	0.0790	-3.09	0.002
Robbing	-0.00927	0.005	-1.80	0.072
Pollen distance	0.0848	0.0734	1.16	0.248

Table 3.5 Percent frequency of nectar robbing of 840 flower on 70 plants in six *Alseuosmia macrophylla* study plots in the Mamaku Plateau, 2003.

Plot number	Number of plants	Number of flowers	Robbed flowers (%)	Mean fruit set (%)
1	15	80	81.3	5.6
2	5	56	41.1	18.7
3	4	46	15.2	9.6
4	12	200	32.5	27.9
5	6	20	15.0	1.2
6	28	438	35.6	26.9

Fruit set

The results from pollination treatments show that the mean natural fruit set was 6.7% in 2002, 18.3% in 2003, and 4.9% in 2004 (Table 3.6). There was a significant difference in natural fruit set between 2002 and 2003, and between 2003 and 2004, but not between 2002 and 2004 (Table 3.6).

In 2003, mean natural fruit set among the six study plots ranged from 1.2 % to 27.9% (Fig. 3.9; Table 3.7). Significantly higher fruit set from crossed compared with natural pollination in both 2002 and 2003 indicates pollen limitation. The PLI shows all plots (except plot 5 because of insufficient data), were pollen limited, with plots 1, 2 and 3 having indices greater than 0.80 (Table 3.7). The predominantly low fruit set from the self-pollination and bagged treatments combined with high SII shows *A. macrophylla* to be mostly self-incompatible and suggests that in most plants, in conjunction with herkogamy, there is probably some form of physiological mechanism to prevent self-fertilisation.

Adult *A. macrophylla* density ranged from 200 to 800 plants per ha^{-1} (Table 3.8). The mean distance from an individual plant to the nearest pollen source ranged from 1.7 m in plot 5 to 2.7 m in plots 1 and 2 (Table 3.8). For the 2002 flowering season, logistic analysis shows no relationship between natural fruit set and the distance to the nearest pollen (Table 3.4), and in conjunction with the increased fruit set from cross-pollinated

flowers compared with natural pollination, factors other than pollen distance and availability apparently contribute to low natural fruit set.

Table 3.6 Mean fruit set for four pollination treatments over 3 years and significance tests of pollination treatments from the 2002–2003 *Alseuosmia macrophylla* treatments from the Mamaku Plateau. Significance of differences in pollination treatments from binomial GLMs, and between years from two sample unpaired *t*-tests.

	Mean fruit set (%)			2002 vs 2003 (*P* value)	2002 vs 2004 (*P* value)	2003 vs 2004 (*P* value)
Treatment	2002	2003	2004			
Natural	6.7	18.3	4.9	<0.001	0.602	<0.001
Crossed	50.5	69.7	-	0.065	-	-
Selfed	8.7	5.5	-	0.560	-	-
Bagged	0.6	1.7	-		-	-
Natural vs crossed (*P* value)	0.004	<0.001	-	0.337	-	-
Natural vs selfed (*P* value)	0.799	0.013	-		-	-
Natural vs bagged (*P* value)	0.024	<0.001	-		-	-
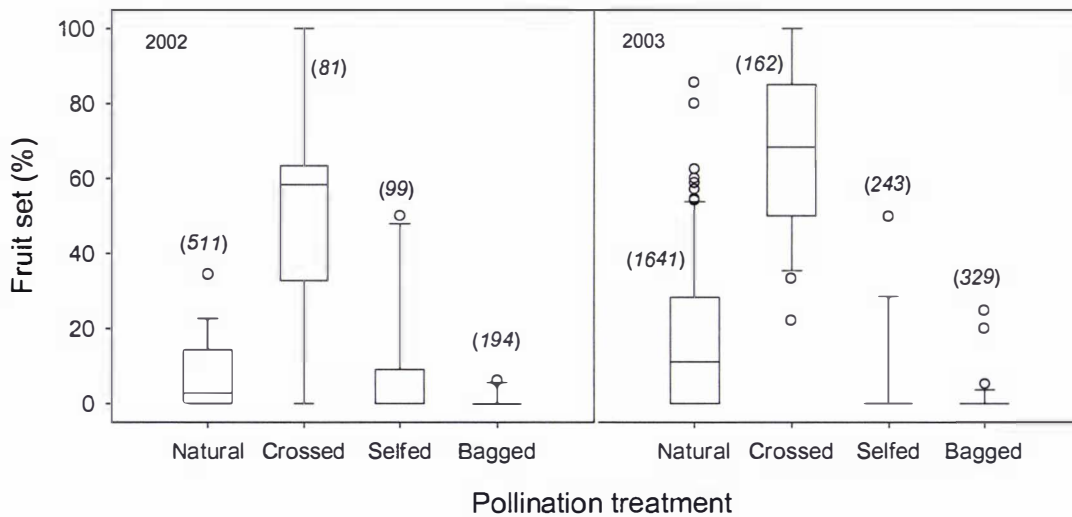
Fig. 3.9 Distribution of *Alseuosmia macrophylla* fruit set from four pollination treatments at the Mamaku Plateau, 2002 and 2003. The number of flowers for each treatment are in parentheses.

Table 3.7 Mean fruit set for four pollination treatments, pollen limitation index (PLI), and self-compatibility index (SII) from six *Alseuosmia macrophylla* study plots in the Mamaku Plateau, 2003. § = insufficient data.

Plot number	Mean fruit set (%)				Indices	
	Natural	Crossed	Selfed	Bagged	PLI	SII
1	5.6	53.3	0	4.0	0.86	1
2	18.7	95.0	16.7	3.6	0.86	0.72
3	9.6	51.1	8.3	4.0	0.83	0.95
4	27.9	67.1	0	0.6	0.52	1
5	1.2	§	§	0	§	§
6	26.9	75.3	5.7	0	0.72	0.95

Table 3.8 Number of plants per plot, mean distance to the nearest pollen source (male plant), and approximate population density from six *Alseuosmia macrophylla* study plots in the Mamaku Plateau, 2003.

Plot number	Number of plants/plot	Mean distance to nearest pollen (m)	Approximate density (ha^{-1})
1	23	2.4	400
2	22	2.7	300
3	11	1.8	200
4	25	2.2	400
5	15	2.9	200
6	48	1.8	800

Pollination vectors

Video footage was captured of both bellbird and moth visitation to *A. macrophylla* flowers. No other insects were seen visiting flowers. The two recorded episodes of moth visits were on separate days during dusk, at 7.40 pm on 23 October 2003, and 8.15 pm on 5 November 2003. In one of the moth video sequences, two moths were nectar-feeding on the same plant at the same time. A further two video tapes showed moths in the distance, but they did not visit the flowers in the camera view. Bellbird nectar feeding was captured on three separate occasions: 6 am and 6.40 am on 5 November 2003, and at 11.45 am on 6 November 2003. Personal observation and video footage of bellbird nectar-feeding demonstrates quick flower visitation behaviour, taking only a few seconds for each flower, with the bird moving along branches, and from branch to branch to feed from many flowers on any one plant. In contrast, moth visitation

appeared to take 20 to 30 seconds to nectar feed from one flower, and involved visiting a few (2 to 4) close-proximity flowers before flying away. The relatively large moth (c. 2.5 cm long) has not been able to be identified from the video footage due to lack of clarity of its features.

Logistic analysis of the results of diurnal and nocturnal exposure experiments and caged and natural fruit set, to compare pollinator effectiveness, shows a significant difference between natural fruit set and the three-exposure nocturnal flowers ($P = 0.002$), and a negative difference between natural fruit set and the caged flowers ($P = 0.006$) (Table 3.9). The highest mean fruit set in diurnally-exposed flowers, at 10.7%, was achieved after four exposure periods, whereas nocturnal-exposed flowers had the highest mean fruit set (14.2%) after three exposures (Table 3.9). Inexplicably, there was more than double the mean fruit set from these two treatments than the mean natural fruit set (5.9%). Caged flowers that excluded birds produced a mean fruit set only 2.5% (Table 3.9), suggesting other pollinators were deterred by the exclosure netting.

Table 3.9 Pollination treatment, number of flowers per treatment, mean fruit set and results of significance tests from binomial GLM of *Alseuosmia macrophylla* flowers in natural, caged, and bird and moth exposure treatments, 2004.

Treatment	Number of exposures	Number of flowers	Mean fruit set (%)	Natural fruit set vs treatment (F, P)
Natural	-	1249	4.9	-
Caged	-	473	2.5	-2.73, 0.006
Diurnal	1	39	0	-0.60, 0.547
"	2	43	0	-0.70, 0.483
"	3	53	8.6	-0.91, 0.362
"	4	42	10.7	-0.68, 0.498
Nocturnal	1	40	3.5	0.11, 0.916
"	2	58	1.0	-0.56, 0.573
"	3	65	14.2	3.11, 0.002
"	4	45	1.2	-0.75, 0.455

3.5   Discussion

Alseuosmia macrophylla flowers have characteristics that identify them as both ornithophilous (bird) and phalaenophophilous (moth) pollinated (Faegri & van der Pijl 1979). The most important bird-pollinator features are the red colouring and abundant nectar, whereas their fragrance, both diurnally and nocturnally, is a moth-pollinator feature (Faegri & van der Pijl 1979). Nectar is primarily a sugar solution and the main floral reward for many pollinators, and the standing crop of nectar is a measure of resource availability at a single point in time (Kearns & Inouye 1993). The sugar concentration of nectar varies among species, and may change during the course of a day because of evaporation, production rate, and moisture uptake from the air, with bird, bat and butterfly flowers tending to produce nectars in the 15–25% range (Kearns & Inouye 1993; Proctor et al. 1996). The standing crop of *A. macrophylla* nectar was in concentrations slightly less (11.5%) than typical bird-visited flowers (15–25%), and also weaker than *Phormium tenax* nectar (mean of 17.2% over 4 years), as recorded by Craig and Stewart (1988). One of the most interesting results from this study has been confirmation of a bird and moth dual-pollination, a syndrome that has been infrequently reported in the native flora. For example, Clout and Hay (1989) suggested the flowers of *Sophora* (kowhai species) are visited by birds, bees, butterflies and moths, and Newstrom and Robertson (2005) reported that winter-flowering *Dysoxylum spectabile* (kohekohe) flowers are shared by birds and moths. Mixed bird-moth pollination syndromes have also been reported from various other parts of the world, including Australia (Crome & Irvine 1986; Dalgleish 1999), Bolivia (Kessler & Krömer 2000), Macaronesia (Olesen 1985), North America (Pleasants & Waser 1985; Lange et al. 2000), and Tasmania (Hingston & McQuillan 2000). Having floral characters that attract both bird and moth pollinators suggests a form of 'bet-hedging' to ensure fertilisation in the event of pollination failure of one of the pollinator syndromes.

As an hermaphroditic plant species which is predominantly self-incompatible, reproductive success in *A. macrophylla* in the Mamaku Plateau population is dependent on pollinator visitation for pollen transfer. There are several possible reasons, individually or collectively, for low fruit set at this area including, floral characteristics; pollinator behaviour, abundance or density; nectar robbing; and possibly, weather conditions.

Floral characteristics

A. macrophylla flowers have two sets of characteristics that help reduce self-fertilisation: (1) homomorphic partial self-incompatibility was indicated by the lack of fruit set in the self-pollination and bagging treatments, and is a strategy where a physiological barrier makes it impossible or difficult for a flower to fertilise itself; the commonest and possibly most effective strategy (Wyatt 1983; Seavey & Bawa 1986). It is not possible to estimate the degree of self-incompatibility in the New Zealand woody flora, as only a small number of breeding system studies have been reported (Newstrom & Robertson 2005). However, self-incompatibility has been demonstrated in *Cordyline kaspar* (Beever 1981), *C. pumilio* (Beever 1983), *C. australis* (Beever & Parkes 1996), *Corokia cotoneaster* (Webb 1994), *Discaria toumatou* (Webb 1985), *Geniostoma rupestre* (Rattenbury 1980), *Hebe vernicosa* (Frankel & Hair 1937), *Pentachondra pumila* (Godley 1966), and *Pseudowintera colorata* (Godley & Smith 1981; Lloyd & Wells 1992). In *P. colorata*, the self-incompatibility reaction occurs in the ovules (Lloyd & Wells 1992), but for most species the precise mechanism is unknown.

(2) *A. macrophylla* flowers display an unusual form of herkogamy (spatial separation of sexual organs), where flowers have various style lengths and stamen positions, but with each form not confined to an individual plant. The herkogamous flowers fit into the 'ordered herkogamy' class, as described by Webb and Lloyd (1986), which is common in bell, gullet, and tube blossoms. In 'ordered herkogamy', the orientation of the pollinator is controlled by the tubular shape of the blossom and by the nectar being at the bottom of the corolla, resulting in the pollinator having only a single contact with both pollen and stigma (Webb & Lloyd 1986). It is possible that the anther contact area on the pollinators is not effective at depositing pollen on the stigma of *A. macrophylla*.

Variability in self-incompatibility amongst some populations of *A. macrophylla* suggests an apparent development of self-compatibility and autonomous selfing (see Chapter 2). Further investigations of other populations of this species would increase our knowledge of the range of this variation.

Although the overall flowering duration was 6 weeks, peak flowering (at both individual plant and population level) was for the middle 14 days of this period. This,

combined with individual flower longevity of about 7 days, indicates a relatively short time frame when the flowers are available to pollinators.

Pollinator behaviour

Only one bellbird was seen, or heard, at any one time over the three flowering seasons of this study, leading to the assumption that bellbird density was low. Eight indigenous bird pollinators of the New Zealand flora were listed by Godley (1979), including the self-introduced silvereye (*Zosterops lateralis*). New Zealand's mainland forests, however, have lost a large number of bird species and/or they have suffered a severe reduction in abundance (Holdaway 1989; Robertson et al. 1999; Anderson & Craig 2003; Kelly et al. 2005). The three main flower-visiting bird species remaining on mainland New Zealand are tui (*Prosthemadera novaeseelandia*), bellbird (*Anthornis melanura*), and silvereye (also known as white-eye) (*Zosterops lateralis*) (Newstrom & Robertson 2005). Tui are often present in areas where *Alseuosmia* species occur, including the Mamaku Plateau, but I have never seen them perched on plants in the low shrub layer, or nectar feeding on *Alseuosmia* flowers. Gravatt (1969) recorded bellbird and stitchbird (*Notiomystis cincta* (this species is now extinct on the mainland)) in the 'lower understory' on Little Barrier Island, but not tui. Rasch and Craig (1988) reported that only the stitchbird fed on *Alseuosmia* flowers on Little Barrier Island.

One bellbird could potentially visit hundreds of flowers over a 14-hour diurnal period (during late October, early November), compared with a potentially longer feeding time and fewer flower visits by moths. However, because of their apparent emergence only during dusk, moths appear to have a small window of opportunity for nectar feeding (approximately 1 hour), and it could be assumed birds would be considerably more effective as pollinators than moths. Birds could not only potentially pollinate large numbers of flowers over a much longer period of time compared with moths, but they would be able to ingest larger quantities of nectar because of their larger body size. However, if bird population numbers were very low, but moth populations very high, then moths could be more effective as pollinators. Neither moth nor bellbird population numbers were measured in this study.

The highest mean fruit set from flowers exposed to moth pollination was 14.2% compared with 10.7% from bird-exposed flowers, although the difference was not

significant. The result suggests moths may be more effective than bellbirds as pollinators of this species at this site. However, this result may also be an indication of a relatively large population of moths, or low bellbird population. Testing pollinator effectiveness from a single visit to flowers and subsequent fruit set would help determine whether moth or bird pollination was the most efficacious. Anderson (2003) compared the relative effectiveness of bird and insect visitors as pollinators and, because of higher fruit set in bird-exposed flowers, argued that the importance of native birds as pollinators had been underestimated. Robertson et al. (1999) also emphasised the importance of maintaining native bird populations as pollinators and dispersers, not only for mistletoe species, but for many other native plants of New Zealand. However, despite the presence of at least one bellbird at the study site, a possible explanation for low natural fruit set is that the nectar-gathering technique of a bellbird is somehow ineffective at transferring pollen in *A. macrophylla*. For example, the corolla mouth of this morph is relatively narrow and may deter a bellbird, but the nectar fills approximately half of the corolla tube, which could mean that a bellbird is not required to insert its beak into the corolla to access the nectar, and thus avoids collecting pollen on its facial feathers. In an illustration of the tongue of a bellbird, McCann (1964) shows it to be over 2 cm in length, and presumably the beak is a similar size. When its tongue/beak length is compared with *A. macrophylla* corolla length of approximately 3 cm (Merrett & Clarkson 2000) it is possible that nectar can be accessed by a bellbird from far enough away to avoid contact with pollen grains. The slightly smaller stitchbird may be more effective as a pollinator because of the presence of bristles on the chin and lower forehead (Higgins et al. 2001). Although a greater proportion of the measured study flowers had anthers positioned above the stigma, the mean distance was only 2.3 mm, and may not be great enough to come in contact with any facial feathers of a bellbird. In this context, it would seem plausible that flowers of native species with well-exserted anthers, e.g., tree fuchsia (*Fuchsia excorticata*), mistletoes (*Peraxilla* spp.), pohutukawa, (*Metrosideros excelsum*), in combination with smaller quantities of nectar positioned deeper in the corolla or inflorescence, could be more suited to effective bellbird pollination compared with species with the floral features of *A. macrophylla*.

Further studies of self-incompatible *A. macrophylla* populations with similar flower morphologies, where there are higher densities of bellbirds, and also where stitchbirds

are present, e.g., Little Barrier Island, could help explain whether pollinator population size is influencing fruit set, and ultimately, contributing to population persistence. The efficacy of bird pollination could also be tested with single-visit studies.

Nectar robbing

Nectar production in *A. macrophylla* was not continuous during the life of the flower; very little additional nectar was produced after nectar removal, and flowers commonly senesced within the following 24 hours, regardless of the age of the flower. The average quantity of sugar in nectar from natural *A. macrophylla* flowers at 11.6% was slightly lower than reported for bird, bat and butterfly-pollinated flowers, i.e. 15–25% range (Proctor et al. 1996), and also lower than those reported for the bird pollinated *Peraxilla* species (Ladley et al. 1997). Sucrose equivalents of nectar from eight native plant species visited by 'honeyeaters' on Kapiti Island ranged from 0.13 to 7.54 mg per flower (Castro & Robertson 1997) and thus the 3.4 mg per flower in *A. macrophylla* fits comfortably within this range. The two primary components to nectar robbery are physical damage to the flower, and extraction of nectar by the robbing animal. Nectar robbing by flocks of white-eyes has potential, albeit in a subtle way, to affect fruit set because although the style did not appear damaged, the 'reward' for a pollinator visit has been removed, and it may be that the flower becomes unattractive in subtle ways once the nectar has gone. A bellbird, for example, is likely to visually detect the absence of nectar, and a moth is unlikely to find a nectarless flower worth visiting. Temeles and Pan (2002) found that robbed male-phase flowers exported less pollen than unrobbed flowers for two apparent reasons: rejection by legitimate animal visitors, and shorter duration by visitors owing to lower nectar rewards. However, Navarro (2001) reported a positive correlation between nectar robbing and fruit set in a hummingbird-pollinated ericaceous species, and Richardson (2004) found that nectar robbers sometimes conferred benefits. Both the Temeles and Pan (2002) and Richardson (2004) studies investigated the effects of bumblebee robbers, which evidently only remove part of the nectar volume. In this study, the flowers that had been robbed had no residual nectar, inevitably inferring greater impact for legitimate visitors.

Weather conditions

An additional possible cause of poor fruit set in *A. macrophylla* in some years is adverse weather conditions during peak flowering. The study sites on the Mamaku Plateau are at altitudes ranging from 400 to 550 m a.s.l., and the area is prone to cold, wet conditions throughout the year. Rain is frequent, with mean annual rainfall ranging from 1850 to 2250 mm (see Fig. 3.1), and if it persisted for an extended period over the peak flowering season, fruit set would inevitably be affected. Persistent rain could negatively affect pollinator activity, pollen viability, pollen transfer, flower attractiveness to a pollinator, flower longevity, and nectar volume and concentration. Rain during peak flowering was identified as a cause of low fruit set in one population of *A. quercifolia* (Merrett & Clarkson 2000).

This investigation of the floral biology of *Alseuosmia macrophylla* is an example of how a species-specific study can contribute to our knowledge and understanding of plant reproduction, and the importance of effective pollinator activity in self-incompatible species. One of the potential threats to the long-term persistence of *A. macrophylla* in the Mamaku Plateau would be the loss of pollinators, despite its 'bet-hedging' co-pollination syndrome. Native birds and moths are both vulnerable to predation by introduced mammals such as possums, rats, and mustelids, as well as from their natural predators. *A. macrophylla* has persisted in many native ecosystems, despite fragmentation and logging, and is probably relatively long-lived, perhaps up to 150 years or longer. In the short term, relatively low annual fruit set in longer-lived species will not negatively impact on population persistence but in the longer term, e.g. > 100 years, population persistence will only be assured if pollinator abundance remains adequate.

Like all *Alseuosmia* species, *A. macrophylla* is highly palatable, particularly to goats and deer, and is a good example of an indicator species for measuring exotic herbivore abundance. It is an important contributor to the native forest ecosystems by providing nectar and a fleshy fruit, and presumably helps support two types of pollinators. However, as a cautionary note, although *A. macrophylla* is common in many native forests in the North Island and in the north-eastern area of the South Island, it is

extremely polymorphic, and the results of this study do not necessarily apply to other populations (see Chapter 2).

3.6 Acknowledgements

3.7 References

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Chapter 4

The efficacy of wind pollination in *Coprosma spathulata*, a dioecious shrub

4.1   Abstract

Coprosma spathulata is a small-leaved, endemic, dioecious, wind-pollinated, understorey shrub, mostly less than 2 m tall, with a restricted distribution in lowland native forests from the Waikato region northwards. It is relatively habitat-specific, occurring in discreet sub-populations, mainly on ridges in secondary native forest, usually under a broken canopy. Flowers are produced over a 6-week period during late winter and early spring, and are typical of wind-pollinated species: female flowers have two exserted, papillae-covered stigmas that elongate with maturation, and male flowers have 4 well-exserted anthers attached to long filaments that dehisce longitudinally. Wind pollination appears to be generally effective in this species, despite its forest habitat. Population gender ratios were mainly male biased, although variable among the plots. Reproductive success was high (76.9–96.8%) in six of the eight study plots, influenced by the close proximity of male plants, and numbers of male flowers. Poor fruit set in two of the plots was primarily due to insufficient pollen abundance at one site, and associated with a dense shrub layer at the other site. Population recruitment was evident in all the study plots.

4.2   Introduction

Anemophily (wind pollination) has been reported in 18% of angiosperm families (Ackerman 2000), and is the dominant pollination syndrome in some environments. It is particularly common in higher latitudes and elevations, especially in temperate areas (Culley et al. 2002), and in open-structured, and floristically less diverse vegetation, e.g., savanna, and northern temperate deciduous forests (Whitehead 1969). For example, approximately 30% of the North American, and 40–60% eastern United States forest trees are anemophilous (Whitehead 1969; Regal 1982), and almost all conifers (Cupressaceae, Pinaceae, and Podocarpaceae), grasses (Poaceae), sedges (Cyperaceae),

and rushes (Juncaceae) are wind-pollinated (Proctor et al. 1996). Wind pollination is also generally considered a significant reproductive mode in early successional systems, and in remote island floras, e.g., Hawaii (Carlquist 1966; Regal 1982; Whitehead 1969, 1983), Juan Fernández Islands (Anderson et al. 2001; Bernadello et al. 2001). In contrast, Seres and Ramírez (1995) recorded only 8.1% anemophily in a tropical cloud forest in Venezuela, and Koutnik (1987) estimated it in only 12% of species in the South Africa Cape flora. In New Zealand, 29% of the indigenous genera are anemophilous, although a large proportion of these are monocotyledons or gymnospermae (Webb et al. 1999).

Wind-pollination is a relatively passive process controlled primarily by microclimate factors, and its effectiveness is benefited by certain environmental characteristics including an open vegetation structure, close spacing of conspecific plants, production of large numbers of pollen grains, the timing of pollen release, wind velocities within an acceptable range, and relatively low humidity and rainfall during flowering (Whitehead 1983). Pollen dispersal by wind is a function of the terminal velocity of the pollen grain, controlled largely by its size, and wind velocity (Whitehead 1969). Niklas (1985a) suggested that wind-pollinated plants released pollen when environmental cues such as wind speed and humidity levels, favoured pollen transport. Wind gradients are usually very steep both within and above vegetation canopies, with an increase in turbulent flow accompanied by a decrease in vegetation density (Burd & Allen 1988). As a result, taller plants should have their pollen dispersed over a wider area leading to increased impacts with receptive stigmas. Because of their structural and floristic diversity, lack of deciduousness, and frequency of rainfall Whitehead (1969) considered tropical rain forests to be unsuitable for effective wind pollination, particularly as wind velocities are very low within the forest. However, Bawa and Crisp (1980) reported wind pollination in a small, understory, dioecious tree species from a lowland, tropical, rain forest in Costa Rica, and although they suggested the syndrome was not as rare in these ecosystems as had previously been thought, there is a lack of published accounts of the syndrome in forest ecosystems.

In forests, wind velocities are relatively low, and trees and shrubs of other species can pose physical barriers to pollen transport (Whitehead 1969). Pollen capture is influenced by the size, shape, and orientation of the collecting object, and there is

evidence that foliage and reproductive structures such as bracts and sepals may increase the probability of pollen capture by deflecting air currents towards the stigma (Niklas 1985a, b, 1987; Niklas & Buchmann 1985; Honig et al. 1992). Wind-pollinated floral structures are usually designed to maximise pollen entrainment and capture and include unisexual flowers; large, well-exposed anthers often hanging on long filaments or in catkins; stigmas large, exposed, and often finely divided and feathery; perianth small, absent or insignificant; attractants absent; and pollen grains produced in great quantities (Faegri & van der Pijl 1979; Proctor et al. 1996). The pollen grains of anemophilous species are usually small compared with entomophilous species, and pollen viability declines more rapidly than that of most animal-pollinated taxa (Bassani et al. 1994).

Gender dimorphism and strongly marked synchronous dichogamy are common in wind-pollinated taxa (Darwin 1877; Charlesworth 1993; Proctor et al. 1996). Dioecism (plants with unisexual flowers) is the most common form of gender dimorphism in the indigenous flora of New Zealand, occurring in 17.9% of the genera (Webb et al. 1999). Gender ratios in dioecious species can often vary widely between and within species, and may be equal, or biased toward males or females. Godley (1964) investigated 16 native, dioecious species in natural populations and found 11 were male-biased, three species, *Plagianthus betulinus, P. divaricatus* and *Coprosma grandifolia*, had more or less equal proportions of the sexes, and two *Aciphylla* species, *A. aurea* and *A. scott-thomsonii*, were slightly (51.6–64.3%) female-biased. Delph (1999) reviewed 45 dioecious species and found female-biased population in 15% of the taxa. Sex ratios in natural populations have important consequences for population dynamics that may be either stabilised or destabilised, depending on the impact of density-dependence (Bessa-Gomes et al. 2004).

An Allee effect, as originally proposed, is defined as a phenomenon whereby reproductive opportunities at low population densities are reduced because individuals are mate-limited (see Hackney & McGraw 2001; Dennis 2002). An Allee effect occurs when individuals benefit from the presence of conspecifics (Courchamp et al. 1999; Stephens & Sutherland 1999), which is a requirement for reproductive success in dioecious species. In small plant populations, important pollination interactions may be weakened; resulting in lowered seed set, which could increase extinction risk (Stephen & Sutherland 1999; Hackney & McGraw 2001). Empirical evidence of an Allee effect

caused by pollen limitation is mostly confined to animal-pollinated plant species, although wind pollinated species can also be limited by a lack of pollen (Knapp et al. 2001; Sork et al. 2002; Koenig & Ashley 2003; Davis et al. 2004). Koenig and Ashley (2003) suggested pollen limitation in wind-pollinated species could be the driving force behind synchronous masting, a characteristic of anemophilous trees. Masting is described as a well-defined, synchronous pattern of annual variability in seed production (Kelly 1994; Herrera et al 1998; Kelly et al. 2001). In a survey of 296 data sets, the seed output in wind-pollinated species were significantly more variable than animal-pollinated species (Herrera et al. 1998; Kelly & Sork 2002).

Coprosma spathulata is a small, endemic, dioecious, wind-pollinated shrub of indigenous forests of New Zealand (Allan 1961). This study investigates the reproductive success of *C. spathulata*, and assesses the factors that influence fruit set in an anemophilous species in a forest environment, and was initiated because poor fruit set was recorded in one population of this species during an earlier investigation (see Chapter 2). For this investigation, the following questions are addressed: (i) How effective is wind pollination in an understory shrub species? (ii) Are gender ratios consistent among geographically separated populations? (iii) Is reproductive success affected by the ratio and proximity of male plants? (vi) Are populations stable or declining?

4.3   Materials and methods

Study species

Coprosma J.R.& G.Forst. (Rubiaceae) is a Pacific Ocean-centred genus of about a hundred species (Gardner 2002), and one of New Zealand's largest genera, comprising some 53 species and subspecies, which are widely represented throughout the country (Allan 1961; Poole & Adams 1994; Parsons et al. 1995). They occur in virtually every ecosystem from coastal to montane habitats, and 22 species occur in forests, shrubland or scrub (Allan 1961; Poole & Adams 1994). The New Zealand species are all anemophilous (Webb et al. 1999). Wild and Zotov (1930) described hermaphroditic or monoecious flowers in several *Coprosma* species. Allan (1961) described the genus as prevailingly dioecious, and Webb et al. (1999) referred to instances of cosexuality in some species or populations but specific examples were not listed. However, Lloyd and

Horning (1979) found no indication of mixed-sex flowers on *Coprosma pumila* from Macquarie Island.

Coprosma spathulata is a small-leaved, endemic, dioecious, understorey shrub, mostly less than 2 m tall, with a restricted distribution in lowland native forests in northern North Island, from the Waikato region (37°S) northwards (Allan 1961). It flowers during late winter and early spring (August–September). The fruit is an almost-black, two-seeded drupe (Webb & Simpson 2001).

Study sites

An initial investigation to determine the breeding system and reproductive success of *C. spathulata* was undertaken during the flowering seasons of 2002 at two study sites in the Waikato region (Hakarimata Scenic Reserve and Pukemokemoke Bush Reserve) (see Chapter 2). The following three pollination treatments were carried out: 1. Controls (natural): natural pollinators allowed, stems in bud were tagged and buds counted. 2. Manual supplementary cross-pollination (crossed): during anthesis pollen from different plants was collected on a small artist's paintbrush and applied to stigmas. 3. Pollinators excluded (bagged): flower buds were counted then stems were enclosed in fine, mesh, fabric bags, tied with coloured twist ties, and left bagged with no further treatment.

Further investigations were undertaken during the 2003 flowering season to assess the influence of male plant frequency on reproductive success. Eight 900-m^2 plots were established in populations of *C. spathulata* of differing density in the Waikato region (Fig. 4.1). Three of the 2003 study plots (PM1, PM2 & PM3) were located at different altitudes (60, 100 & 150 m a.s.l.) in Pukemokemoke Bush Reserve, a 40-ha hill-country, forested remnant that rises to 166 m a.s.l. Four of the plots (HK1, HK2, HK3, & HK4) were located at differing points and at various altitudes (60 to 100 m a.s.l.), along the Hakarimata Range, an 1800-ha Reserve of native forest that extends 19 km, and which rises steeply from the surrounding undulating pastureland. It is characterised by steep ridge and gully systems, rising to 374 m a.s.l. at its highest point (Department of Lands & Survey 1984). Much of the forest on the Hakarimata Range has suffered past modification as a result of logging, fires, and clearance for pasture, particularly on the lower slopes, and is in various stages of recovery. Plot KR was located 100 m a.s.l. on

the Karakariki Range, a southern extension of the Hakarimata Range, and with similar geography, altitude and vegetation. Two of the eight study plots HK1 and PM1, were established in the same place as the 2002 study.

Within each plot, the position of all individuals was recorded by tape measures placed along the X and Y axes, with a third tape used to measure the distance from the axis tapes. For each individual, plant gender was recorded, female plants were identified with numbered tags, buds were counted on tagged branches, and the number of open flowers was counted on all male plants. Fruit set was counted in December 2003, when the fruit were well developed, but not yet ripe. Two mature stigmas were collected from each tagged female plant in each plot, set in slides with fuchsin gel, and the number of pollen grains on each stigma were counted using a compound microscope. To measure flowering periodicity, buds and flowers were counted in PM1 at 3- to 4-day intervals; for male plants, the number of buds on tagged branches were counted, for females, open flowers were counted. Additional female flower buds were enclosed in cellophane bags to exclude pollen, and at each visit, the number of open flowers were counted. The number of seedlings within each plot was counted, and the following habitat and vegetation characteristics were recorded for each plot: estimated percent cover of canopy, shrub and groundcover layers; the DBH of all trees with a diameter greater than 3 cm; and combined tree basal area (m^2/ha^{-1}).

Data analysis

To compare fruit set between pollination treatments for the 2002 data, the number of fruit for the number of buds on each plant from each site were analysed using a Generalised Linear Model (GLM) with binomial error distribution and logit link function, and F-test significance tests, as described in Chapter 2, with a separate analysis for each site. Pollen limitation in the 2002 treatments was calculated using a pollen limitation index: PLI = $1 - (P_N/P_X)$, where P_N is the percent fruit set of naturally pollinated, control flowers and P_X is the percent fruit set by plants that received supplemental cross pollen. PLI = 0 indicates no pollen limitation (Larsen & Barrett 2000). A PLI > 0.75 indicates a high degree of pollen limitation (following Newstrom & Robertson 2005). For the 2003 data, the percent natural fruit set for individual plants, and plant means were calculated for each site, and box plots were generated from

individual plant fruit set for each plot. The box plots depict the median (central line in each box), 25% and 75% quartiles (upper and lower limits of each box), 90th and 10th percentiles (indicated by the whiskers), and outliers, (indicated by open circles). To determine factors influencing reproductive success, a scatter plot matrix was initially used to evaluate relationships between mean percent fruit set, male plant ratios, male flower frequency, and vegetation variables for each plot. Two-sample unpaired *t*-tests were used to analyse differences in natural fruit set in plots HK1 and PM1 between each year, and between fruit set and numbers of male flowers with a separate analysis for each plot. The distance to the nearest pollen source (male plants), and the frequency of pollen grains on stigmas using individual plants were analysed using GLM analyses. GLM with binomial error distribution and logit link function, and *F*-test significance tests, were also used to identify relationships between fruit set and male flower frequency, and percent canopy, shrub and ground cover.
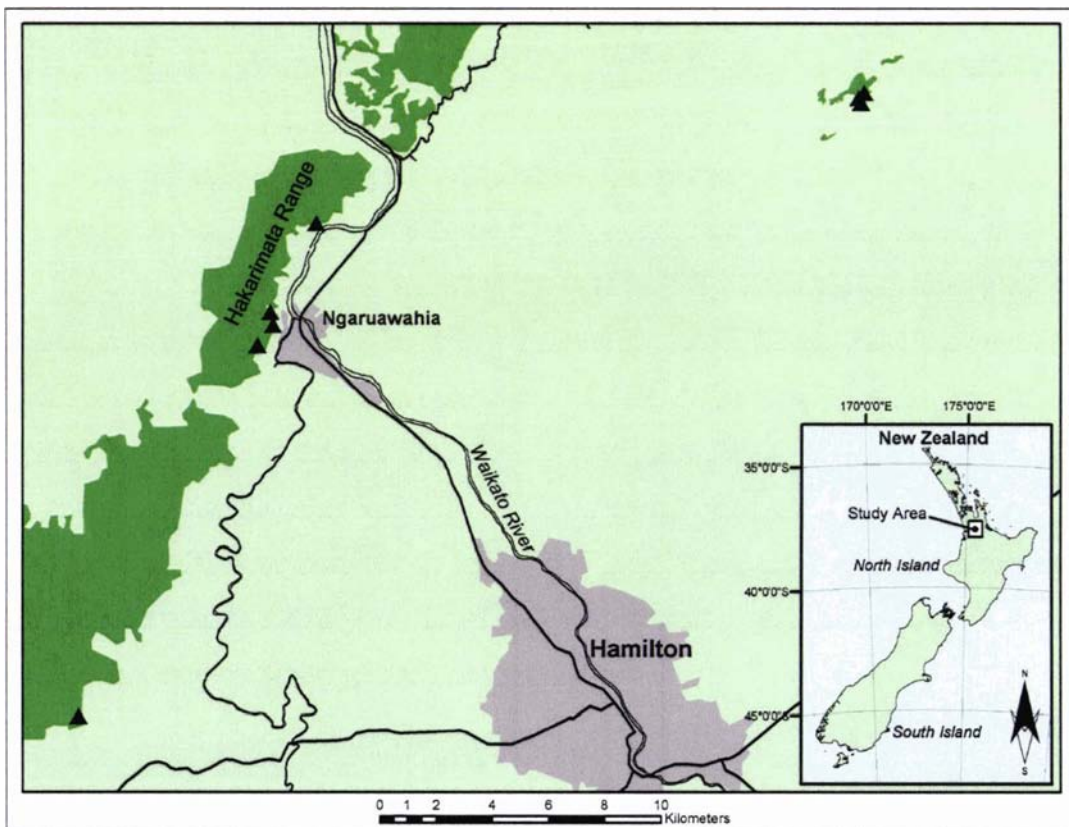
Fig. 4.1 Location of eight *Coprosma spathulata* study plots in the Waikato region. HK, Hakarimata; KR, Karakariki; PM, Pukemokemoke.

4.4   Results

Field searches for suitable study sites showed *C. spathulata* to be relatively habitat-specific, with its presence evidently restricted by topography and vegetation characteristics, with discrete sub-populations present, mainly on ridges, in secondary native forest (approximately 50–100 years old), usually under a broken canopy.

Floral characteristics

C. spathulata female flowers have two exserted stigmas that elongate up to 1.5 cm in length with maturity, and are covered with tiny papillae for trapping pollen. The number of ovules per ovary was consistently two. The male flowers have four well-exserted anthers that dehisce longitudinally, and hang from the corolla on long filaments. The anthers dehisce more or less simultaneously, within a few hours of emergence from the bud. Flowering duration was spread over a 6-week period in both male and female plants, with peak flowering in female plants occurring approximately 10 days from commencement (Fig. 4.2). In male plants, however, there was an initial, almost synchronous flowering, followed by a steady reduction in the number of open male flowers (Fig. 4.2). Bagged female stigmas remained receptive for longer than unbagged stigmas, and many (68%), were still in a receptive state when the experiment was terminated after 6 weeks, at the end of natural flowering (Fig. 4.2). No insects were observed visiting flowers.

Population density

Amongst the eight plots of 2003, there was considerable variation in *C. spathulata* density, ranging from 13 to 259 individuals per 900 m^2 (Fig. 4.3). Gender distribution was also variable amongst the plots, but populations were male-biased in seven of the eight plots, ranging from 28.6% ($n = 6$) in plot HK1, to 72.2% ($n = 187$) in plot PM1 (Fig. 4.3; Table 4.1).

Fruit set

In 2003, mean fruit set amongst the plots ranged from 15.8±5.1% in plot KR to 96.8±1.5% in plot HK4 (Fig. 4.4; Table 4.1). One plant at the Pukemokemoke site produced one fruit in the bagged treatment (see Chapter 2) but this single result is

unlikely to indicate apomixis, a strategy that has been reported in other *Coprosma* species (Heenan et al. 2002, 2003), but was probably the result of some form of contamination. Higher levels of fruit set in bags would be expected if *C. spathulata* was producing by apomixis, and there was no fruit set in bagged flowers at Hakarimata. In 2002, the mean natural fruit set was 8.2% at HK1 and 58.9% at PM1 (Table 4.3).

In 2003, the number of male flowers recorded in each of the eight plots ranged from a low of 66 in plot KR to 3379 in plot PM1 (Table 4.1). The mean distance from flowering female plants to the nearest pollen source (male plant) ranged from 0.82 m in plot PM1, to 7.66 m in plot HK1 (Table 4.2). There was a significant, positive relationship between fruit set and the number of male flowers in six of the study plots; the exceptions were plots HK1 and KR (Table 4.1).

The was a significant difference in natural fruit set between 2002 and 2003 at both sites, and between natural and crossed fruit set at both sites in 2002 (Table 4.3). In 2002, a high PLI (0.88) at HK1 site shows a high level of pollen limitation (Table 4.3). For the 2003 plot data, regression analysis shows a significant relationship between fruit set and distance to the nearest pollen source in plots HK1 and PM2, indicating decreased fruit set with increased male plant distance (Table 4.2). However, although the distance to male plants was similar in Plots PM2 and KR, there was no relationship between fruit set and pollen distance, but the number of male flowers in Plot KR was very low, suggesting this was a factor contributing to low fruit set (Tables 4.1, 4.2). This plot had very few plants of either sex, and as a result, pollen density would be expected to be low. The threshold pollen travel distance appears to be approximately 7 m before fruit set is reduced to less than 20% (Fig. 4.5).

Pollen grains on stigmas

The mean number of *C. spathulata* pollen grains per stigma ranged from 4.8 grains in plot KR to 69.1 grains in plot HK3 (Table 4.2). There was a significant, positive relationship between the number of pollen grains per stigma and fruit set from five of the eight plots; the exceptions were plots HK2, HK3, and KR (Table 4.2).

Population structure and vegetation characteristics

The number of seedlings per plot ranged from 16 to 187 (Table 4.4), but there was no significant relationship between seedling frequency and the numbers female or male plants, fruit set, or vegetation cover classes.

Canopy cover was similar amongst plots, ranging from 80% to 95% (Table 4.4). The shrub layer cover was variable, and ranged from 20% to 95%. The ground cover ranged from 20% to 45% (Table 4.4). There was a significant relationship ($P < 0.001$) between fruit set and the canopy, shrub and layer ground cover (Table 4.5). The vegetation composition was relatively similar among the plots, with *Kunzea ericoides* (kanuka) and *Phyllocladus trichomanoides* (tanekaha) the two most common canopy species, occurring in all plots (Table 4.6), and indicative of mid-stage, secondary, native forest. The number of tree species in the plots ranged from 5 in plot HK2 to 15 species in plot PM2, and tree basal area ranged from 32.9 m^2/ha^{-1} in plot HK4 to 54.5 m^2/ha^{-1} in plot PM3 (Tables 4.4, 4.6).
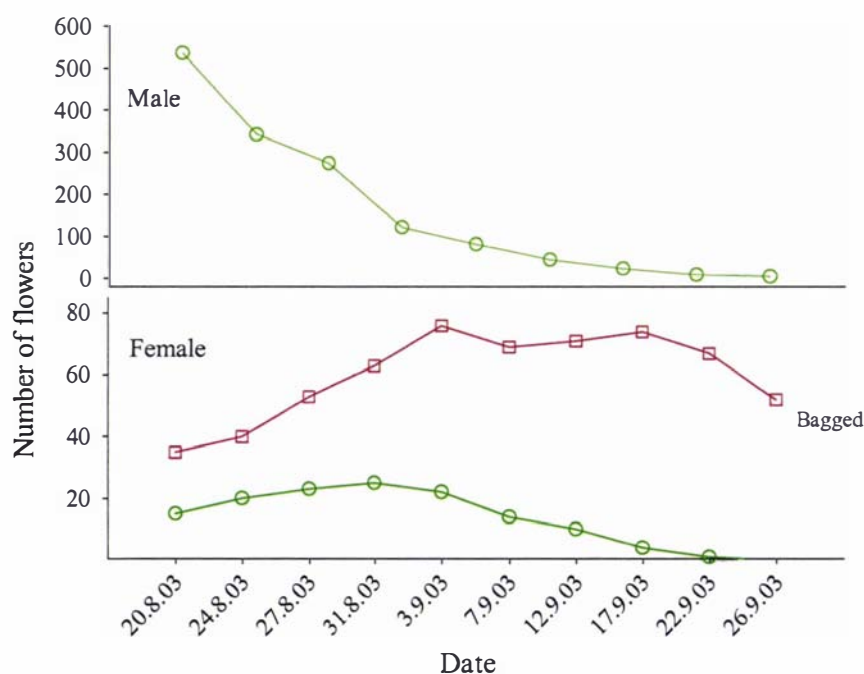
Fig. 4.2 Flowering duration for male, female, and bagged female *Coprosma spathulata* flowers, plot PM1, 2003. ○, natural; □, bagged.
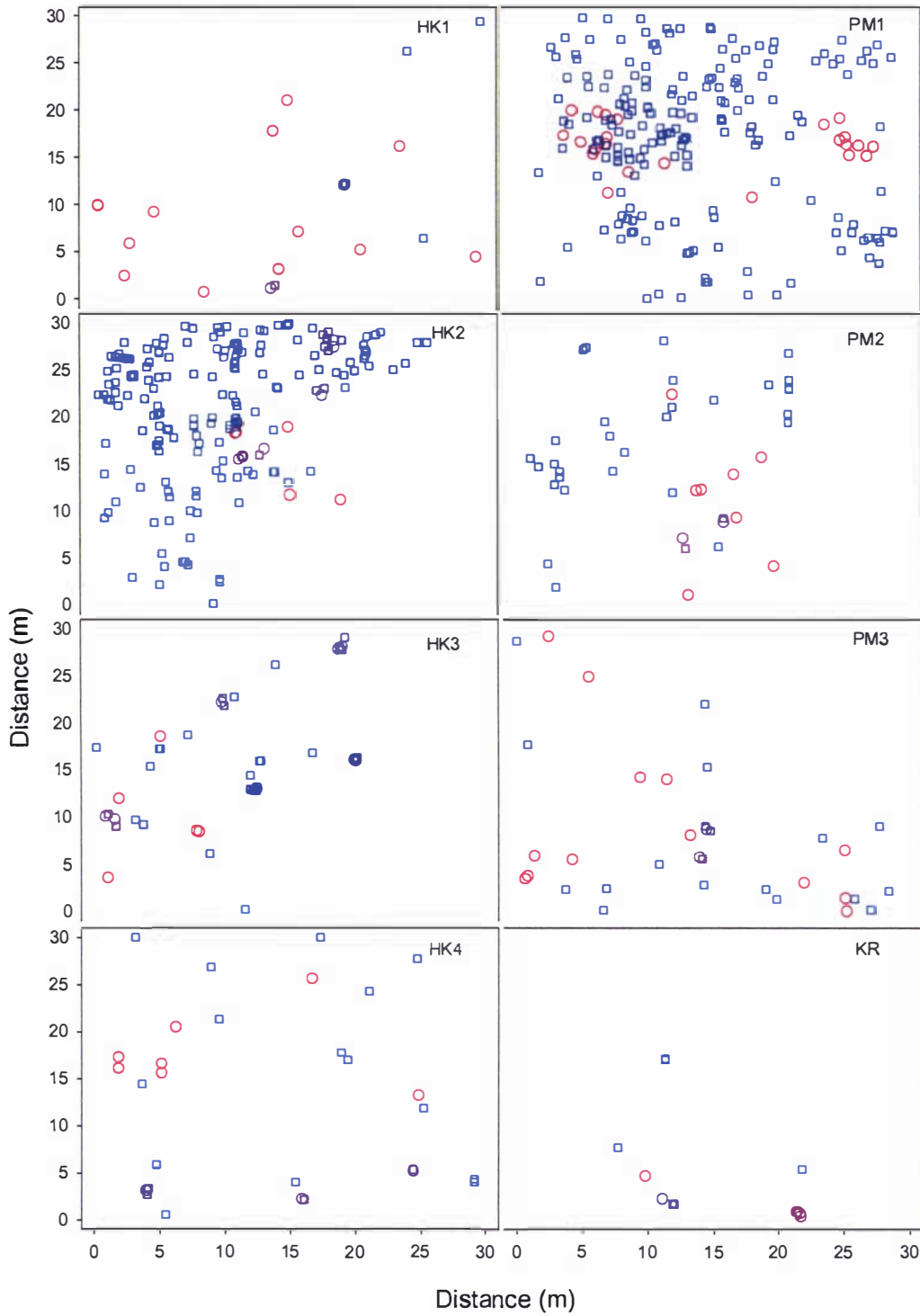
Fig. 4.3 Distribution and frequency of *Coprosma spathulata* individuals in eight 900 m² study plots in the Waikato region, 2003. ○, female plants, □, male plants.

Fig. 4.4 Distribution of natural fruit set in *Coprosma spathulata* from eight study plots in the Waikato region, 2003. The numbers of monitored flowers per plot are in parentheses.
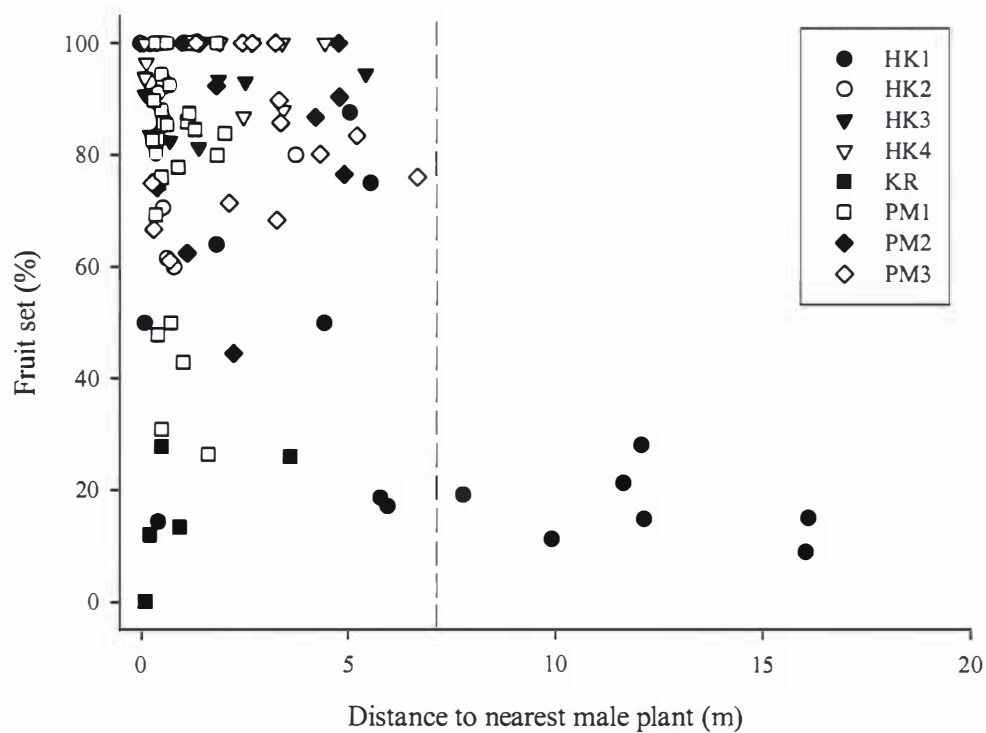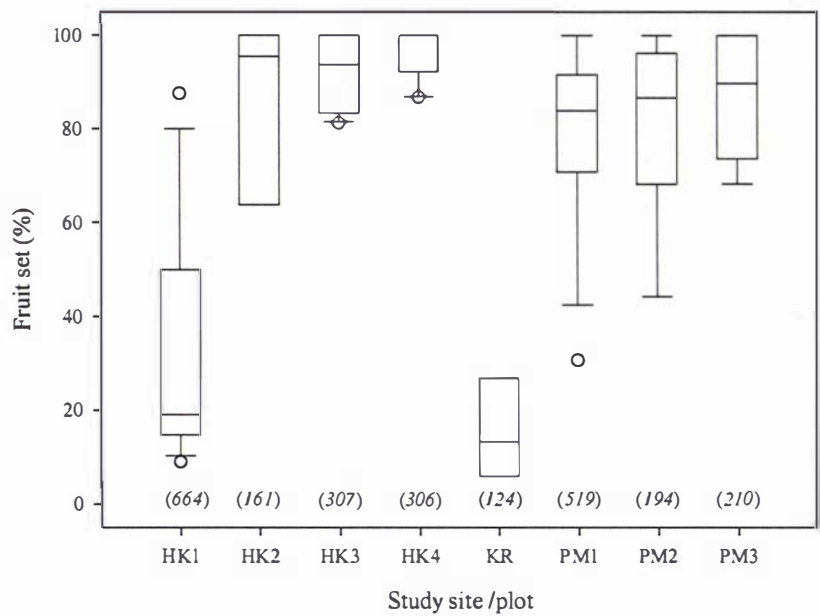
Fig. 4.5 Relationship between fruit set and distance to the nearest male plant in eight populations of *Coprosma spathulata*. The dashed line represents the implied pollen travel distance threshold for effective pollination.

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Table 4.1 Population size, male plant proportion (%), number of male flowers, the number of monitored female plants, mean fruit set per plot, and results of *t*-tests on the relationship between natural fruit set and male flower frequency from eight *Coprosma spathulata* study plots in the Waikato region, 2003. PM, Pukemokemoke Bush Reserve; HK, Hakarimata Scenic Reserve; KR, Karakariki Range.

Plot	Number of plants	Male plant proportion (%)	Number of male flowers/plot	Number of monitored female plants/plot	Mean fruit set (%)	d.f.	Fruit set vs male flower frequency (*F*-ratio, *P* value)
HK1	21	28.6	131	15	33.0	5	1.71, 0.416
HK2	192	91.1	2502	7	86.9	7	1.39, <0.001
HK3	45	68.9	931	8	93.3	10	21.61, <0.001
HK4	31	64.5	507	11	96.8	9	40.5, <0.001
KR	13	61.5	66	10	15.8	7	1.08, 0.257
PM1	259	72.2	3379	5	76.9	15	5.08, <0.001
PM2	45	71.1	640	16	82.7	8	1.76, <0.001
PM3	34	55.9	492	9	83.8	7	4.82, <0.001

Table 4.2 Means of distance from female plants to a pollen source (male plant), GLM results of relationship between natural fruit set and distance to a male plant, mean numbers of conspecific pollen grains on stigmas ± SE, and GLM results of relationship between natural fruit set and numbers of pollen grains on stigmas, in eight *Coprosma spathulata* study plots in the Waikato region, 2003.

Plot	Male plant distance (m)	d.f.	Natural fruit set vs male plant distance (*t*-ratio, *P* value)	Pollen grains per stigma±SE	Natural fruit set vs pollen on stigmas (*t*-ratio, *P* value)
HK1	7.66	14	73.6, <0.001	14.0±1.8	12.85, 0.013
HK2	0.86	10	0.04, 0.845	66.4±13.6	5.72, 0.190
HK3	1.22	12	0.15, 0.705	69.1±19.5	83.9, 0.245
HK4	1.85	10	1.15, 0.226	55.0±16.3	102.7, 0.031
KR	1.1	4	0.95, 0.320	4.8±2.1	5.95, 0.080
PM1	0.82	24	0.77, 0.382	30.8±5.2	2.27, <0.001
PM2	2.66	8	5.54, 0.022	16.8±2.6	5.44, <0.001
PM3	2.72	8	1.8, 0.177	29.8±3.9	1.4, <0.001

Table 4.3 Results of natural fruit set and results of *t*-tests (natural fruit set versus year), and GLM (natural versus crossed fruit set) analyses from two *Coprosma spathulata* plots during the 2002-03 flowering seasons. PLI, pollen limitation index.

Variable		Study site	
		HK1	PM1
Mean natural fruit set±SE	2002	8.2±2.8	58.9±6.4
	2003	33.0±6.6	76.9±4.2
Natural fruit set versus year (2002 vs 2003) (*F*; *P*)		8.63; 0.007	6.07; 0.018
Crossed fruit set (2002)±SE		66.6±7.2	91.1±2.9
Natural versus crossed fruit set (2002) (*F*; *P*)		228.2; <0.001	15.6; 0.001
PLI (2002)		0.88	0.35

Table 4.4 Number of seedlings, estimated vegetation cover classes, and tree basal area in eight *Coprosma spathulata* study plots in the Waikato region.

Plot	Number of seedlings	Canopy cover (%)	Shrub layer (%)	Ground cover (%)	Tree basal area (m^2/ha$^{-1)}$)
HK1	139	85	60	45	43.4
HK2	136	90	50	30	44.8
HK3	89	85	25	35	54.5
HK4	187	95	20	20	32.9
KR	34	80	95	40	45.1
PM1	85	90	45	30	43.7
PM2	16	90	45	30	46.6
PM3	35	80	45	45	53.6

Table 4.5 Results of GLM analysis of relationship between fruit set and male flower frequency, percent canopy, shrub and ground cover amongst 8 *Coprosma spathulata* study plots in the Waikato region, 2003. d.f. for *t* = 7.

Variable	Estimate of coefficient	SE	*t*-ratio	*P* value
Constant	-746.2	99.5	-7.50	<0.001
Male flower frequency	-0.0013	0.000	-14.52	<0.001
Canopy cover	6.889	0.914	7.54	<0.001
Shrub cover	12.36	1.71	7.27	<0.001
Ground cover	3.717	0.465	7.99	<0.001

Table 4.6 Presence of native shrub and tree species in eight, 900 m² *Coprosma spathulata* study plots in the Waikato region. Plant species are listed in order of the most frequent to the least frequent.

Species	HK1	HK2	HK3	HK4	KR	PM1	PM2	PM3	Frequency
Kunzea ericoides	x	x	x	x	x	x	x	x	8
Phyllocladus trichomanoides	x	x	x	x	x	x	x	x	8
Cyathea dealbata		x		x	x	x	x		5
Dacrydium cupressinum		x	x	x		x	x		5
Knightia excelsa		x	x	x	x	x			5
Leucopogon fasciculatus	x		x	x		x	x		5
Myrsine australis				x	x	x	x	x	5
Podocarpus totara			x		x	x	x	x	5
Melicytus ramiflorus					x	x	x	x	4
Pseudopanax crassifolius	x		x	x			x		4
Hedycarya arborea					x	x	x		3
Olearia rani			x	x	x				3
Weinmannia racemosa	x		x	x					3
Beilschmiedia tawa						x	x		2
Cyathodes juniperina						x		x	2
Nestegis lanceolata							x	x	2
Prumnopitys ferruginea	x					x			2
Agathis australis	x								1
Brachyglottis repanda							x		1
Coprosma arborea							x		1
Coprosma lucida	x								1
Geniostoma rupestre								x	1
Litsea calicaris					x				1
Macropiper excelsum								x	1
Melicope simplex								x	1
Nothofagus truncata	x								1
Prumnopitys taxifolia								x	1
Streblus heterophyllus							x		1
Total number of species	9	5	9	10	10	13	15	12	

4.5   Discussion

The results of this study show that in general, anemophily in an understory shrub of evergreen forests is an effective pollination syndrome, but prone to Allee effects at low male density. At one of the sites (plot KR), pollen limitation was evident and poor fruit set is apparently exacerbated by a dense shrub layer. In addition, although the gender ratio in this plot was male-biased, the total number of male flowers was low, a factor reflected by low pollen numbers on stigmas. Each male *C. spathulata* anther produces an estimated 30 000 pollen grains (see Chapter 9), a result that falls within the range found by Molina et al. (1996), who recorded a range of 2600 to 104 600 pollen grains per anther in ten anemophilous tree species. Released pollen is not intercepted solely by

receptive stigmatic surfaces of conspecifics; tree trunks, branches, and leaves of all species in the environment will pose physical barriers to transport, and a significant fraction of grains transported will be lost by impaction on these structures, with perhaps one in 1000 grains reaching the female organ of the target plant (Whitehead 1983; Niklas 1987). In many Northern Hemisphere wind-pollinated, deciduous tree species such as *Alnus, Betula, Ulmus, Fraxinus,* and *Fagus*, anthesis occurs before leaf development, a strategy that is conducive to pollen transport (Dowding 1987). The presence of a more dense, and species diverse shrub layer in plot KR may be filtering and intercepting airborne pollen grains. Increased plant species richness and density is part of the natural forest successional process, that, with time, could contract the *Coprosma spathulata* population at this site unless some form of disturbance reduces the density of the vegetation, particularly in the shrub layer. The relatively high frequency of seedlings in this plot, compared with the small population size, indicates recent population recruitment.

All but one of the study plots were male-biased, although gender ratios were variable amongst the plots. Equal or male-dominated populations appear the most common form of gender bias in the New Zealand dioecious flora (Wild & Zotov 1930; Godley 1964; Shore 1969; Rivers 1971; Lloyd 1974; Lloyd & Webb 1977; Webb & Lloyd 1980). Male-biased sex ratios (58.4%) were recorded in *Coprosma repens* from Wellington's south coast (Gordon 1959). Further investigation is required to establish if male-biased gender ratios are common in the genus *Coprosma* in New Zealand.

Relatively low fruit set in the plot with the female-biased population (plot HK1), particularly during the 2002 flowering season, was most likely due to a combination of insufficient pollen and the distance from female plants to the nearest male. Pollen limitation and limited pollen dispersal, although known to be important in animal-pollinated species, may be playing an equally important role in some wind-pollinated species (Koenig & Ashley 2003). Knapp et al. (2001) and Sork et al. (2002) found that oak trees (*Quercus douglasii, Q. lobata*) were at risk of reproductive failure as a consequence of reduced population densities and insufficient pollen supply due to isolation of individuals after habitat fragmentation. In *C. spathulata*, close female-male proximity (< 7 m) produced high fruit set in six of the eight plots. In plot HK1 the mean

fruit set in females more than 7 m from the nearest male was 17%, compared with 47% for those that were less than 7 m distant, indicating the importance of male proximity. Despite the low fruit set in two populations, *C. spathulata* populations appeared relatively stable at the time of the surveys; there was no indication of large-scale decline of the species in any of the plots. The presence of seedlings in all the plots suggests potential population recruitment, even where fruit set was low (e.g., plot HK1). This indicates past reproductive success and suitable habitat for germination and establishment. Longer term monitoring would enable an assessment of seedling survival and fecundity.

An interesting 'bet-hedging' strategy may be behind the longevity of female flowers bagged in cellophane that therefore remained unfertilised. This strategy was also noted in the wind-pollinated, dioecious species *Macropiper excelsum* (see Chapter 2), and it may be prevalent in other anemophilous species to maximise fertilisation opportunity by increasing the time that stigmas are presented for pollen capture. In a study of flower longevity, Primack (1985) recorded a mean female flower longevity of 8.2 days in an unnamed New Zealand grassland *Coprosma* species under natural conditions, and also found that female flowers generally last longer than males in gender dimorphic species. Male and female flowers in dioecious species have different roles in the reproductive process, and whereas the usefulness of male flowers is over as soon as the pollen is shed, a female flower must remain receptive until compatible pollen has reached the stigma (Primack 1985). Further studies of other wind-pollinated species would help determine if extended flower longevity is a common strategy.

The significant interannual variation in natural fruit set between the two monitored years (in plots PM1 and HK1) could be the result of greater flower densities during 2003. Pollen dispersal may also have been enhanced by the activities of field helpers and me during the flowering season of 2003, as I observed small clouds of pollen being released when unavoidably brushing past male plants, and pollen dispersal could also be initiated by lightly tapping flowering branches. It is possible that this species employs a dual pollen dispersal syndrome in *Coprosma spathulata*, i.e. animal and wind. In the past, many native birds foraged in the shrub layer and on the forest floor (Atkinson & Millener 1990), and their movement through the vegetation may have assisted pollen dispersal in wind-pollinated species. The variation in fruit set in *C. spathulata* between

the two monitored years could be attributed to differing climatic conditions among years, or perhaps mast flowering. Masting has been reported from a number of anemophilous tree species (Kelly 1994; Riveros et al. 1998; Kelly et al. 2001; Yasaka et al. 2003). To investigate fully whether *C. spathulata* is subject to this phenomenon, long-term monitoring of fruit set would be required.

Coprosma spathulata flowers display typical wind-pollination characteristics as described by Faegri and van der Pijl (1979) and Proctor et al. (1996): the flowers are unisexual, anthers are well exposed and hang on long filaments, stigmas are large and well-exposed, and pollen grains are produced in great quantities (see chapter 9). These characteristics are probably shared by all *Coprosma* species in New Zealand. Relatively simultaneous flowering of males and females and the extended longevity of unfertilised female flowers ensure a high likelihood of fertilisation, provided adequate pollen is available.

Low wind velocities within forest, and high and frequent rainfall are conditions that indicate anemophily would be an inefficient means of pollen dispersal in tropical regions (Nicklas 1985b; Bush 1995), and wind pollination in species-rich, wet evergreen, lowland plant communities was assumed to be rare (Whitehead 1969). New Zealand's lowland native forests are primarily evergreen, and although not as species-rich as their tropical counterparts, frequent rainfall and high humidity are experienced virtually all year round, particularly in northern North Island. For example, the mean annual rainfall of the Waikato region is approximately 1200 mm (NIWA 2004), a volume that is included in Regal's (1982) 'wet forest' category. During the *C. spathulata* flowering season of 2003, the mean rainfall during August and September was 117 mm and 102 mm respectively, and relative humidity was 87% and 81.5% (NIWA 2004). However, Bawa and Crisp (1980) reported no adverse effects on the fecundity of a wind-pollinated understory tree in Costa Rica and also described other examples of wind-pollination in this forest type. The characteristics of native forest environments in New Zealand would superficially indicate their unsuitability for efficient wind-pollination. However, habitat specificity on ridges ensures that *C. spathulata* is positioned to take maximum advantage of available wind for pollen dispersal, and it would appear that *C. spathulata,* and other *Coprosma* species in the

indigenous flora that occur in forests, are evidently not routinely disadvantaged by being wind-pollinated.

4.6   Acknowledgements

4.7   References

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Chapter 5

The influence of female-biased gender ratios on reproduction in *Melicope simplex*, a small-leaved, divaricating species from New Zealand

5.1   Abstract

Melicope simplex is a small-leaved, dioecious shrub/small tree that produces small, whitish flowers that are typical of the simple, unspecialised structures characteristic of the New Zealand flora. The flowers are gender size-dimorphic, with males larger than females. Female flowers contain vestigial anthers, produce eight ovules per ovary, but rarely produce more than four seeds per fruit. Gender ratios were female-biased in all of the 14 study plots investigated and female plants ranged from 56.5% to 95.2% of the population. The results of pollination treatments at two sites in the Waikato region in 2002 showed moderately high degrees of pollen limitation. In 2003, natural fruit set in the 14 sites ranged from 3.9% to 50.1%, with successful fruit set influenced primarily by a combination of male plant density, male floral frequency and distance to the nearest male plant. Male and female flowers produced similar amounts of nectar. Pollinator activity and pollen deposition was primarily diurnal, and flies were the most abundant potential pollinators caught on sticky traps. Population size-class structures show population stability with recruitment evident at all sites.

5.2   Introduction

One of the distinctive features of the New Zealand flora is the high frequency of gender dimorphism (Heine 1937; Carlquist 1966; Godley 1979; Lloyd 1985a; Webb & Kelly 1993; Webb et al. 1999). Dioecy (plants with unisexual flowers on separate plants) is considered to have evolved from hermaphrodism, and is the most common form of gender dimorphism worldwide (Proctor et al. 1996). Approximately 6% of the world's angiosperm species are dioecious (Sakai & Weller 1999), although the incidence of dioecy varies considerably in different regional floras (Sakai et al 1995a). For example,

dioecy occurs in 2.8% of Californian flowering plants (Sakai et al. 1995a), less than 5% of the British flora, in about 20% of Mediterranean shrub flora, and in up to 25% of tropical rain forest trees (see Proctor et al. 1996). It is also common in island floras, e.g., Hawaii where 14.7% of angiosperm species are dioecious (Bawa 1979; Sakai et al. 1995a). In an analysis of the occurrence of gender dimorphism in 363 indigenous seed genera of New Zealand, dioecism was the most common form, occurring in 17.9% of the genera (Webb et al. 1999).

The evolution of dioecy in populations has occurred independently in many floras and in diverse taxa, and hypotheses on the selective forces promoting dioecy include those that suggest dioecy has evolved as a mechanism to avoid inbreeding depression, as well as those that propose resource allocation, sexual selection, and ecological factors are important evolutionary drivers of separation of the sexes (Lloyd 1985a; Sakai et al. 1995a). Lloyd (1985b) suggested the separation of sexes may have evolved as a means of preventing geitonogamous self-pollination when there is an absence of specialised pollinators. Many dioecious species are of large stature, e.g., trees and shrubs, and because of large floral displays, geitonogamous selfing in ancestral hermaphroditic populations might have been important in the evolution of dioecy (Barrett 2002). The fitness consequences of outcrossing and selfing, and the allocation of resources to male and female function are considered key factors that promote transitions from hermaphrodism to dioecy and other forms of gender dimorphism (Barrett 2002). In an investigation of 971 Hawaiian indigenous angiosperm species, Sakai et al. (1995b) found the incidence of sexual dimorphism in the flora was the result of autochthonous evolution as well as colonisation by dimorphic species.

Gender ratios in dioecious species can vary widely between and within species and populations, and may be equal or biased toward males or females. Equal or male-dominated populations appear the most common form of gender bias in the New Zealand dioecious flora (Wild & Zotov 1930; Godley 1964; Shore 1969; Rivers 1971; Lloyd 1974; Lloyd & Webb 1977; Webb & Lloyd 1980). Godley (1964) investigated 16 native, dioecious species in natural populations and found 11 were male-biased, three species, *Plagianthus betulinus, P. divaricatus* and *Coprosma grandifolia*, had more or less equal proportions of the sexes, and two *Aciphylla* species, *A. aurea* and *A. scott-thomsonii*, were slightly (51.6–64.3%) female-biased. In a review of 45 dioecious

species, Delph (1999) found female-biased population in 15% of the species. Although the frequency of dioecy has been recorded in many floras, studies into the effect of female-biased gender ratios on fruit set are few, and appear to be non-existent in female-biased species (see Foweraker 1932; Godley 1964; Primack & Lloyd 1980; Rottenberg 1998; Decker & Pilson 2000; Gauquelin et al. 2002).

The greater allocation of resources to reproduction can render females more susceptible to environmental stress, resulting in sex-biased mortality (Stehlik & Barrett 2005). Additional explanations for biased sex ratios include local mate competition, group selection, the spread of selfish genetic elements, differences in the cost of rearing the two sexes, and sex-biased mortality after parental investment (see Geber 1999; Stehlik & Barrett 2005). For example, environmental conditions were considered to play an important role in governing sex ratios in dioecious *Rumex nivalis*: low-altitude populations were strongly female-biased, whereas in high-elevation populations, sex ratio bias was not pronounced, suggesting low male mortality in populations at high altitude (Stehlik & Barrett 2005). Delph (1999) reviewed sexual dimorphism in life history traits and found that in most species, the cost of reproduction was greater for females relative to males, and the higher cost had driven certain life history characteristics to be dimorphic.

Reproductive success in dioecious species is dependent on pollinator activity and pollen availability, and could be affected by gender ratios and male plant density. Rathke and Jules (1993) suggested the reproductive success of plants was more likely to decline where there was pollinator specialisation and/or self-incompatibility, compared with generalised pollinators. Pollen limitation in plants is a common phenomenon and is demonstrated empirically when female plant fertility in open-pollinated controls is lower than in flowers that have had supplemental pollination (Burd 1994). Burd (1994) examined studies of 258 species, of which 62% were reported to experience significant pollen limitation. Pollen limitation is influenced by a suite of factors including size and number of flowers displayed, floral longevity, ovule number, mating and pollination system, habitat type, the demography of populations, and local climatic conditions. Larson and Barrett (2000) investigated studies on 240 species to establish associations between pollen limitation and various ecological and life-history traits, and found that self-incompatible, non-autogamous species of forested habitats were more likely to be

pollen limited than those with contrasting traits (Larson & Barrett 2000). Chronic pollen limitation could constrain plant populations to the extent that local extinctions occur (Ashman et al. 2004).

There is a variety of reasons why plant populations may be small, sparse, or declining, but once at low levels, Allee effects (positive density dependence) can accelerate or exacerbate the problems in small populations and in species that benefit from the presence of their conspecifics (Ashman et al. 2004). Populations that rely on animal pollinators are more likely to experience Allee effects because low-density populations are often less attractive to pollinators and flowers may receive fewer conspecific pollen (Ashman et al. 2004). Plant species with self-incompatibility mechanisms, such as dioecy, can be also be affected by insufficient numbers of compatible mates when populations are small (Ashman et al. 2004). Any incompatibility system reduces the pool of potential pollen donors, and increases the probability of inadequate fertilisation (Burd 1994).

Seed production and seedling establishment are vital for long-term population persistence; however, for most plant species, the critical recruitment threshold levels required for population maintenance are unknown (Bruna 2003). Plant reproductive systems and strategies play a major role in the abundance and diversity of taxa in fragmented ecosystems (Lovejoy et al. 1986; Saunders et al. 1991), and seed output represents a direct, quantifiable measure that integrates numerous interacting factors, and provides an index of community integrity or 'health' (DeMauro 1993; Aizen & Feinsinger 1994). In New Zealand, fragmentation of continuous lowland forest tracts has occurred since human occupation about 1000 years ago, and has continued over the past 150 years since European settlement. Some lowland North Island forests that formerly occupied extensive areas have been reduced to isolated remnants, primarily as a consequence of clearing for agriculture and urban development (Young & Mitchell 1994; Ministry for the Environment 1997). For dioecious species, reduced population size as a consequence of fragmentation could alter gender ratios and affect reproductive success, particularly if there is a disproportionate reduction in pollen abundance. Bond (1994) classifies dioecious species as at greatest risk of reproductive process failure if there is dependence on pollinator and seed disperser specialisation.

This study describes an investigation into *Melicope simplex*, an endemic, dioecious, small-leaved shrub/small tree. The reproductive biology of New Zealand's native shrubs remains poorly documented (see Newstrom & Robertson 2005), and there is uncertainty about the status of breeding systems in many species. *Melicope simplex* is an example that typifies this. Hooker (1867) described the flowers of the genus *Melicope* as 'more or less unisexual' but did not describe individual species. Thomson (1892) reported hermaphrodism in *M. simplex*, and also described cleistogamic flowers from a South Island population. Cheeseman (1925) described the flowers as 'often unisexual'. Allan (1961) described them as perfect or unisexual, although recognised that their sexual expression had been little studied. Eagle (1975) illustrated separate male and female flowers, whereas Moore and Irwin (1978) discussed the features of separate male and female flowers but illustrate a 'bisexual' flower. Webb et al. (1999) list the breeding system of the genus *Melicope* as dioecious and gynodioecious.

This study investigates the breeding system and reproductive success of *Melicope simplex* in populations of differing density and gender ratios. The following questions were addressed. (i) What is the breeding system of this species? (ii) Are gender ratios consistent among geographically separated populations? (iii) Is reproductive success affected by the proportion or proximity of males in the population? (iv) Are populations declining or stable?

5.3   Materials and methods

Study species

Melicope simplex (Rutaceae) is an endemic, small-leaved, divaricating shrub up to 4 m tall, occurring throughout both North and South Islands, usually on the margins of coastal and lowland forest (Allan 1961). It is most commonly found within 30 m of forest edges, along the sides of tracks, and in other areas of higher light such as canopy gaps. The family Rutaceae is widely distributed with over 1000 species in 100 genera (Allan 1961). In New Zealand, the family Rutaceae comprise three species in two genera, *Phebalium nudum*, *Melicope simplex* and *M. ternata* (Allan 1961). Webb et al. (1999) described the breeding systems of the genus *Melicope* as dioecious and gynodioecious. In spring *M. simplex* produces small, greenish to white flowers in few- or many-flowered cymes (Allan 1961). The *Melicope* fruit is a dry, 4-celled capsule

(Webb & Simpson 2001), which splits when mature and the black, glossy seeds emerge and hang from a very fine, short filament (Fig. 5.1) – a characteristic that suggest the seed is dispersed by wind. However, the relatively large size of the seed, 3.1–3.8 mm long (Webb & Simpson 2001), and a lack of obvious aerodynamic features suggest seed dispersal distance by wind would be restricted. Burrows (1996) suggested the seeds probably roll after reaching the ground, and may be carried internally by birds. In seed germination trials, only 2% of *M. simplex* seeds germinated within the first year, but there was a flush of germination in the third and fourth years of the trial (Burrows 1996).

Floral characteristics

To establish flower-size dimorphism between gender, two flowers from 53 female plants and two flowers from 39 male plants were collected from one study site (Paengaroa Scenic Reserve). Flower diameter was measured, and a two-sample (unpaired) *T*-test was used to determine any significant difference.

Nectar standing crop was sampled from flowers of both genders at two of the study sites, Gordon Gow Scenic Reserve and Paengaroa Scenic Reserve, to measure the quantity of nectar per flower. Because the quantity of nectar was barely visible, and too small to be taken up with a micropipette, small flags of filter paper were used to collect nectar from each flower. At least 60 flower buds on 14 female and four male plants were initially bagged until anthesis, the standing crop of nectar was collected, and the flowers were then either rebagged or left exposed to pollinators (natural). Nectar was collected from approximately 10 flowers per plant from both groups 24, 48, and 72 hours after the initial standing crop sampling. Plans to continue nectar sampling after the 24-hour treatment had to be cancelled at the Paengaroa site because of persistent rain. Due to the small nectar volume in this species, sugar content was measured using the anthrone method as described by Kearns and Inouye (1993) and expressed as sucrose equivalents.

In addition, female flowers at these two sites were exposed to either nocturnal or diurnal pollinators. Initially, branches of flower buds on 15 plants at two study sites, Gordon Gow Scenic Reserve and Paengaroa Scenic Reserve, were bagged with fine mesh fabric

bags until the majority of flowers had reached anthesis. All remaining buds and senesced flowers were removed from these branches. The monitored flowers were bagged and allocated into nocturnal or diurnal groups, with nocturnal flowers unbagged at dusk and left unbagged until dawn, and diurnal flowers unbagged at dawn and rebagged at dusk. At least 10 flowers for each exposure for each plant were unbagged for three separate exposure periods: one, two and three nights or days. At the completion of each exposure period, stigmas were collected from each plant, set in slides with Fuchsin gel (Beattie 1971), and the pollen grains attached to each stigma were counted using a light microscope. To compare the frequency of potential pollinators between sites with differing male densities, 19 "Trappit" yellow sticky traps were positioned in female plants amongst flowers at 6 sites and left for 3 days. Two traps were also placed among flowers on male plants. After collection, the traps were stored in a freezer, and at a later date, all attached insects were counted and classified into invertebrate orders.

Reproductive success

An initial investigation to determine the breeding system and reproductive success of *Melicope simplex* was undertaken during the flowering seasons of 2002 at two study sites in the Waikato region of the North Island, Gordon Gow Scenic Reserve and Pukemokemoke Bush Reserve (see Chapter 2).

Further investigations were undertaken during the 2003 flowering season to assess the influence of male plant frequency on reproductive success. Fourteen 900-m^2 study plots were established at eight geographically separated study sites of varying size in the North Island (Fig. 5.2; Table 5.1). The position of all *M. simplex* individuals in each plot was recorded with tape measures placed along the X and Y axes of the plot, with a third tape used to measure the distance from the axis tapes. Plant gender, height, and numbers of flowers on all flowering plants were recorded. For plants with more than 100 flowers, the number of flowers was estimated by counting the flowers on lower branches and multiplying this by the total number of branches. For taller plants with over 1000 flowers, estimates were made. To measure natural fruit set, female plants in each plot were identified with numbered tags, and flowers and buds were counted on tagged branches. Each monitored female was given a 'light effect' score ranging from 1

for plants with low light under a completely closed canopy to 5 for plants in full sunlight. The number of monitored female individuals varied among plots, and varied depending on female plant frequency and flower numbers. In general, the proportion of female plants tagged and monitored decreased with the number of female plants. For example, natural fruit set was monitored in 30% of the population (21 plants) in plots with the greatest number of female plants. In low frequency plots (< 15 female plants), 77–100% of the female population were monitored. Fruit and seed set were counted after fruit maturation, but before seeds were shed.
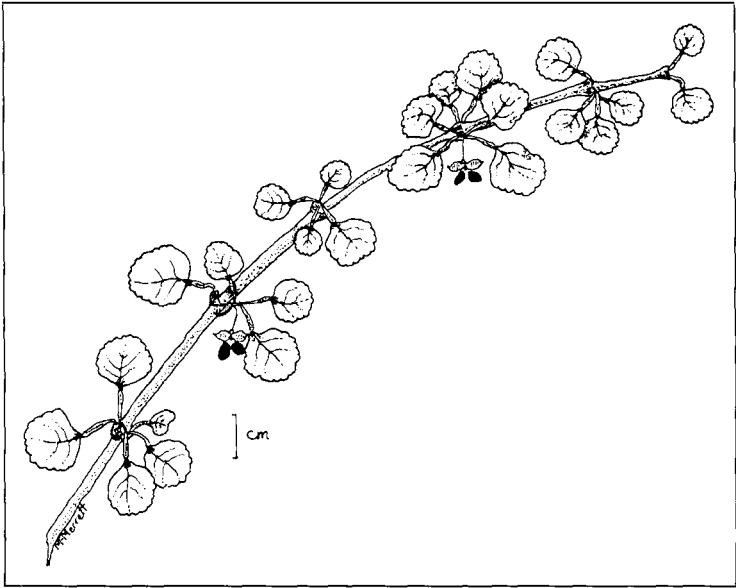
Fig. 5.1 Illustration of *Melicope simplex* with seeds.

Table 5.1 Location details, study site and plot identification, and site size (ha) of 14 *Melicope simplex* plots in the North Island.

Number of plots per site	Study site	Site code	NZMS 260 grid reference	Size of study site (ha)
2	Gordon Gow Scenic Reserve	GG	T14/498840	7.4
1	Karewarewa Scenic Reserve	KR	T22/613451	3.5
1	Mt Huia Scenic Reserve	MH	T22/557489	3.9
1	Mangaweka Scenic Reserve	MW	T22/480139	32
3	Paengaroa Scenic Reserve	PR	T21/436696	102
2	Pukemokemoke Bush Reserve	PM	S14/194991	40
3	Taihape Domain	TD	T21/511675	80
1	Vinegar Hill Domain	VH	T22/355378	20.6

Population structure

To assess population recruitment and persistence, population size-class structures were measured in each plot by counting the frequency of plants in the following height classes: adult (flowering individuals), saplings (> 1 m but not flowering), juveniles (30 cm – 1 m), and seedlings (< 30 cm).
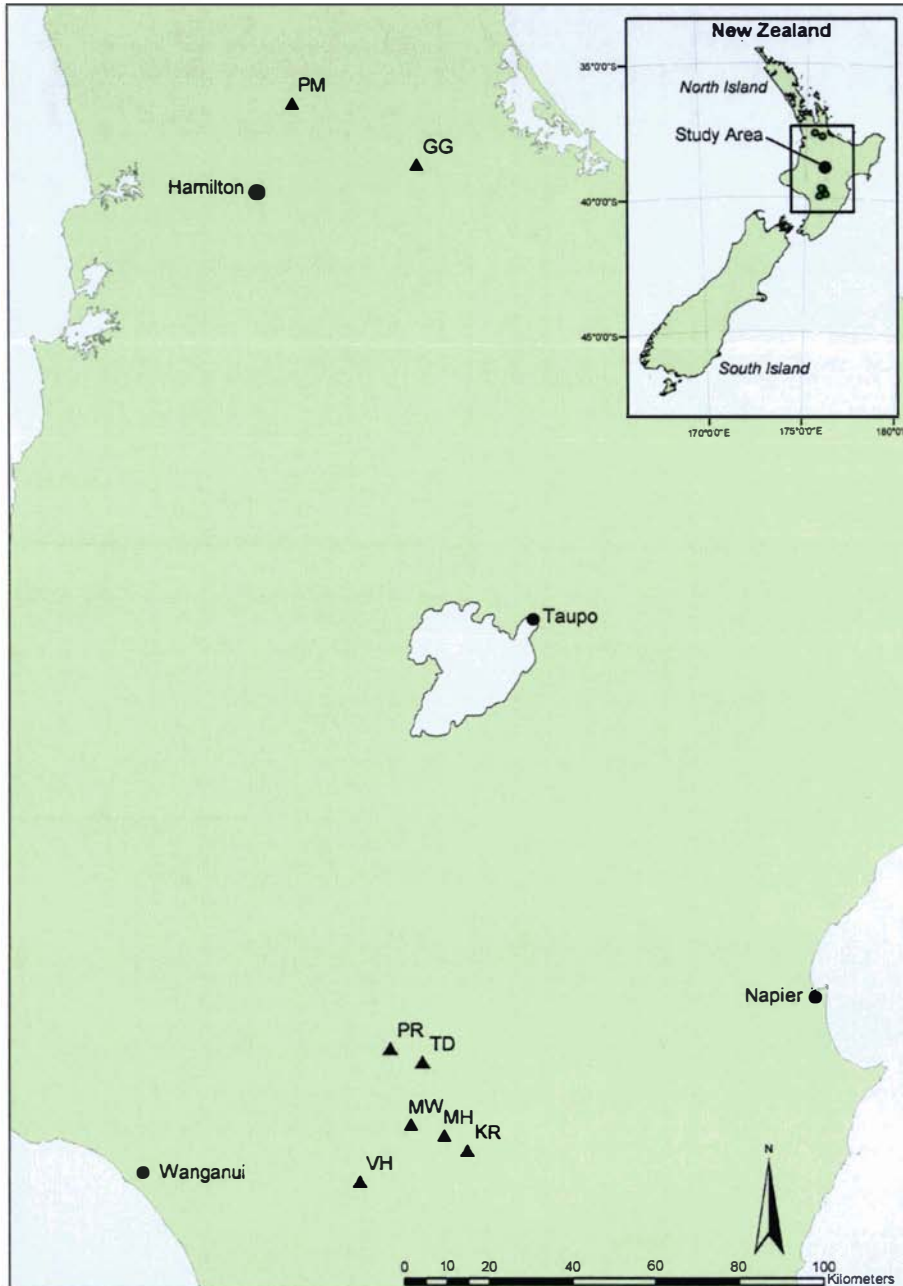
Fig. 5.2 Location of *Melicope simplex* study sites in the North Island

Data analysis

To compare fruit set between pollination treatments for the 2002 data, the numbers of fruit for the numbers of buds on each plant from each site were analysed using a generalised linear model (GLM) with binomial error distribution and logit link function, and F-test significance tests, with a separate analysis for each site, as described in Chapter 2. Pollen limitation in the 2002 treatments was calculated using a pollen limitation index as follows. PLI $= 1 - (P_N/P_X)$, where P_N is the percent fruit set of naturally pollinated, control flowers and P_X is the percent fruit set by plants that received supplemental cross pollen (Larsen & Barrett 2000). PLI of > 0.75 indicates a high degree of pollen limitation (following Newstrom and Robertson (2005), and PLI $= 0$ indicates no pollen limitation.

For the 2003 data, the of percent natural fruit set, as the proportion of fruit set from the tagged flowers on individual plants, and plant means were calculated for each site, and box plots were generated from individual plant fruit set for each plot. The box plots depict the median (central line in each box), 25% and 75% quartiles (upper and lower limits of each box), 90th and 10th percentiles (indicated by the whiskers), and outliers, (indicated by open circles). To evaluate factors influencing reproductive success, a GLM, as described above, was used to identify relationships between natural fruit set, pollen travel distance, male plant frequency, light effect score, and the number of male and female flowers using individual plants for each site. Analysis of variance (ANOVA) was used to compare nectar concentration (in sucrose equivalents) between treatments, duration and plant gender. A scatter plot matrix and multiple linear regression analyses were used to evaluate relationships between mean percent fruit set, light index, invertebrate numbers, and female and male flower frequency for each plot with sticky trap data.

5.4   Results

Floral characteristics

M. simplex flowers during spring (September to November) and produces cream, sometimes pinkish, flowers in small clusters from leaf axils on separate plants (Fig. 5.3), and often in great numbers. Female flowers had vestigial stamens, a single pistil,

and although the ovary consistently held eight ovules, most frequently, a maximum of four seeds were produced. In male flowers, anthesis is two-staged, with two whorls of four anthers maturing sequentially; the outer whorl maturing first. There was floral dimorphism between gender, with a significant difference between female and the larger male *M. simplex* flowers (Fig. 5.4). The mean diameter for female flowers was 7.6 mm, ranging from 5.4–9.5 mm, and male flowers were significantly larger at 8.7 mm ($P <$ 0.001), and ranged from 7.7–10.0 mm. No deviation from dioecy was noted during this study. Dioecism is also present in the genus *Melicope* in Hawaii (Sakai et al. 1995a). However, observation of small (< 20 individuals) natural populations of *Melicope ternata* in the Coromandel Peninsula area, and of planted individuals in the Waikato region and in Palmerston North indicate *M. ternata* is hermaphroditic.

Nectar appeared to be exuded in very small quantities from the base of the ovary in female flowers, and from the base of the anthers/vestigial ovary in male flowers. The mean nectar standing crop quantities from bagged flowers were higher than natural (non-bagged) flowers at both sites (Fig. 5.5). The ANOVA results of nectar quantities show a significant difference between the two treatments (natural and bagged flowers) ($P < 0.001$), suggesting insects were cropping nectar from the natural flowers (Table 5.2).

For pollen grain counts, a total of 617 stigmas were collected from Paengaroa Scenic Reserve flowers and 239 from Gordon Gow Scenic Reserve. There was a significant difference in the number of pollen grains present on flowers exposed to different durations ($P = 0.018$), and between diurnally and nocturnally exposed stigmas ($P < 0.001$) (Fig. 5.6; Table 5.3). Paengaroa flowers had almost five times as many pollen grains per stigma compared with the Gordon Gow stigmas (Fig. 5.6). At Paengaroa, there was an increase in pollen deposition with increased exposure duration to pollinators, both diurnally and nocturnally (Fig. 5.6). Pollen grain numbers were low on the nocturnally exposed flowers, and of these ($n = 327$ & 122), pollen was present on only 20% and 7% of stigmas at Paengaroa and Gordon Gow respectively. Of the diurnally exposed flowers ($n = 290$ & 117), there were pollen grains present on 76% of stigmas from Paengaroa, but on only 23% from the Gordon Gow site.

Fig. 5.3 *Melicope simplex* flowers. A, female; B, male.

Fig. 5.4 Distribution of flower diameter of male and female *Melicope simplex* from Paengaroa Scenic Reserve, 2003. The numbers of flowers measured for each gender are in parentheses.
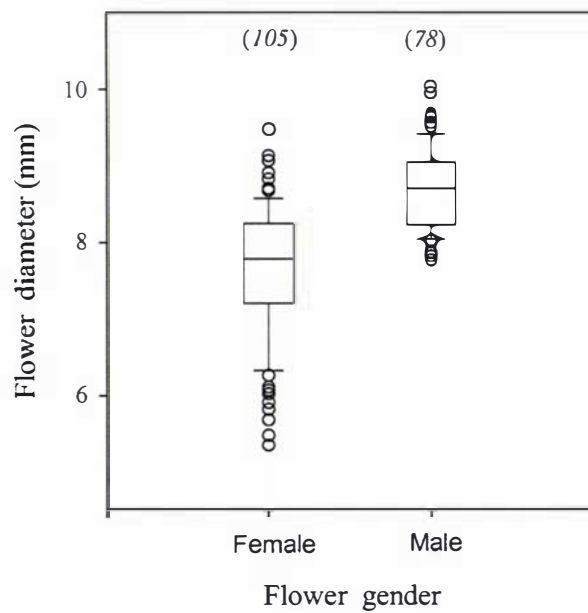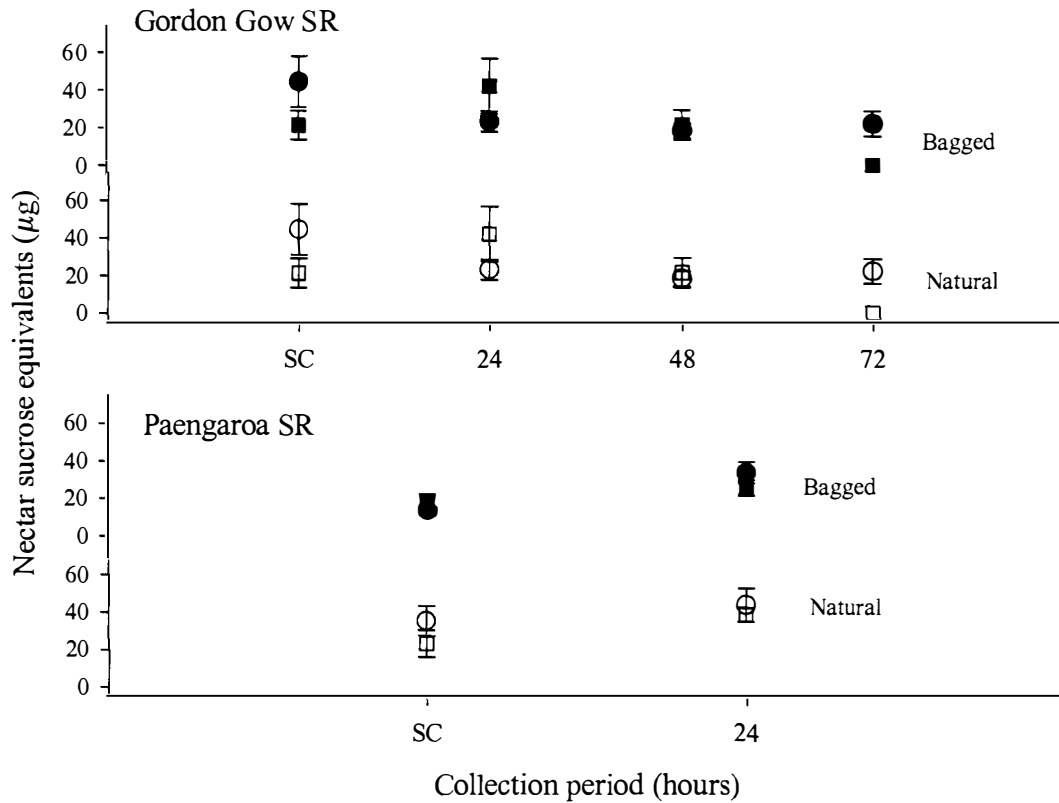
Fig. 5.5 Mean nectar quantities (μg) from *Melicope simplex* flowers of both genders from Gordon Gow Scenic Reserve and Paengaroa Scenic Reserve, at four collection times during the 2003 flowering season (October and November). SC, standing crop; ○●, female flowers; □■, male flowers.

Table 5.2 ANOVA results of nectar quantity in each plant gender at various collection times and two treatments (natural and bagged) of *Melicope simplex*, 2003.

Variable	d.f.	F-ratio	P value
Time	3	0.43	0.731
Gender	1	2.14	0.143
Treatment	1	24.73	<0.001
Time vs gender	3	0.72	0.541
Time vs treatment	3	1.11	0.344
Gender vs treatment	1	1.52	0.219
Residual	396	-	-

Table 5.3 ANOVA results of the number of pollen grains on stigmas over three diurnal and nocturnal pollinator exposure durations of *Melicope simplex*, 2003.

Variable	d.f.	Number of pollen grains vs variable	
		F-ratio	*P* value
Duration	2	4.61	0.010
Time (day/night)	1	68.02	<0.001
Duration vs time	2	4.03	0.018
Residual	853	-	-
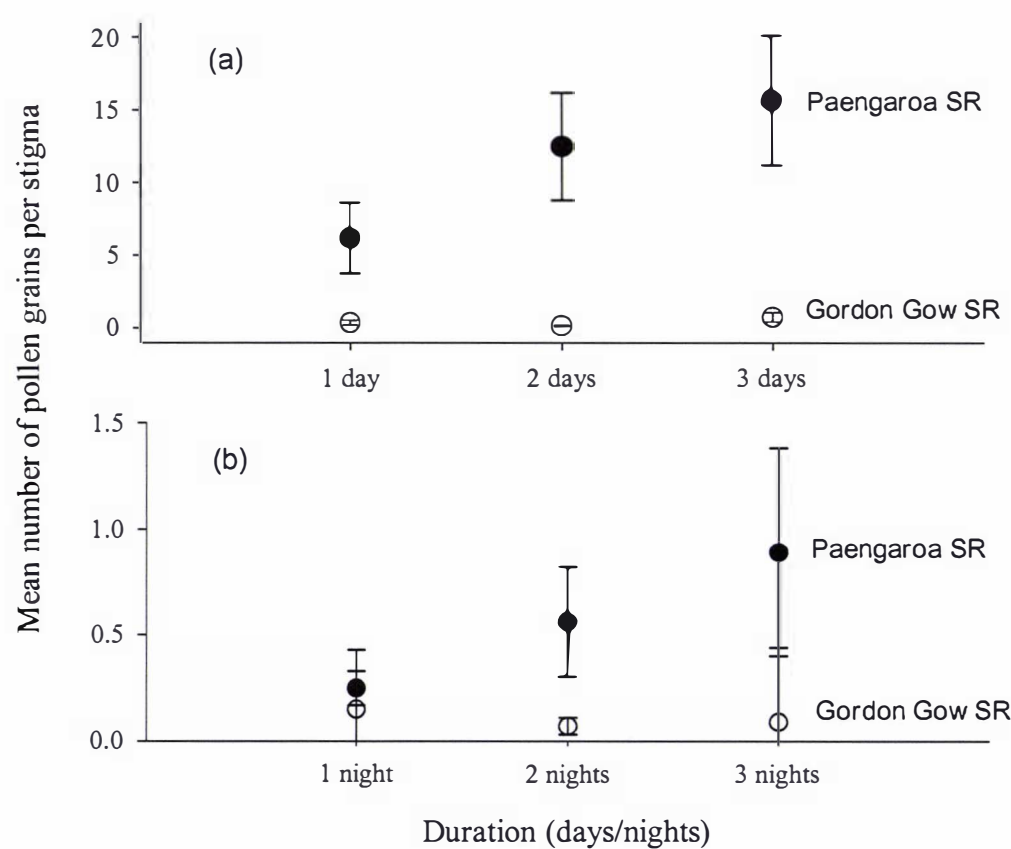
Fig. 5.6 Mean number of pollen grains per stigma at (a) three day and (b) night exposure periods during the 2003 (October and November) flowering season from two *Melicope simplex* study sites. Note the change of scale in the Y-axis of the two panels.

Flies (Diptera) were the most abundant invertebrate order collected from sticky traps, comprising 50.5% from female flowers and 35.6% from male flowers (Table 5.4). For female flowers, bugs (Hemiptera), and bees and wasps (Hymenoptera) were the next most abundant, at 16.7% and 13.7% respectively (Table 5.4). For male flowers, the second most frequent order was beetles (Coleoptera) and thrips (Thysanoptera), each at 25.3% (Table 5.4). At individual sites, flies were the most common potential pollinators, ranging from 36% to 72.7% of collected invertebrates (Table 5.5). Bees and wasps were relatively common at four of the plots, MH, PR1, TD3, and KR, ranging from 18.6% to 22.3%, but comprised less than 4% in plots MW and VH. There was no relationship between invertebrate frequency and natural fruit set, or male and female flower numbers (not shown).

Table 5.4 Total numbers, and proportion of the total numbers of invertebrates on sticky trap assessments traps hung amongst male and female *Melicope simplex* flowers, 2003.

Invertebrate order	Total number	Invertebrate frequency (%)	
		Female flowers	Male flowers
Araneida (spiders)	30	2.8	0
Coleoptera (beetles)	136	10.5	25.3
Diptera (flies)	577	50.5	35.6
Hemiptera (bugs)	187	16.7	6.9
Hymenoptera (bees, wasps)	152	13.7	4.6
Lepidoptera (butterflies, moths)	11	0.9	1.1
Neuroptera (lacewings)	1	0	1.1
Plecoptera (stoneflies)	1	0.1	0
Thysanoptera (thrips)	66	4.1	25.3
Unknown	7	0.6	0
Total	1168		

Table 5.5 Percentage of total numbers of invertebrate orders captured on sticky traps from among male and female *Melicope simplex* flowers from 6 *Melicope simplex* study plots, 2003.

Order	KR	MH	MW	PR1	TD3	VH
Araneida	0.43	0.34	0.34	0.34	0.68	0.43
Coleoptera	0	4.20	0.17	2.65	2.74	1.88
Diptera	4.71	8.90	4.79	5.22	5.39	20.38
Hemiptera	3.51	3.94	0.60	1.03	1.28	5.65
Hymenoptera	2.48	4.11	0.26	1.71	3.34	1.11
Lepidoptera	0.09	0.17	0.26	0.26	0.09	0.09
Neuroptera	0	0	0	0.09	0	0
Plecoptera	0	0	0	0	0.09	0
Thysanoptera	0	0.34	0.17	3.00	1.37	0.77
Unknown	0	0.09	0	0.17	0	0.34

Reproductive success

Mean natural fruit set from the 2002 pollination treatments (see Chapter 2) was less than 20% at both sites, with a significant difference between natural and crossed treatments at both sites (Table 5.6). PLI of 0.72 and 0.70 indicate moderately high levels of pollen limitation in 2002 (Table 5.6). There was also a significant difference in natural fruit set between 2002 (15.8%) and 2003 (34.7%) at Gordon Gow Scenic Reserve (Table 5.6), and in 2003 between the two sites, with a mean of 34.7% at Gordon Gow and 18.7% at Pukemokemoke. No fruit were set in either male or female bagged flower buds.

GLM analysis showed a significant relationship between natural fruit set, mean plant height and the distance nearest male plant, male plant frequency, but no relationship between natural fruit set and the number of flowers per plant, nor the proximity to higher light levels (Table 5.7).

In total, 6801 flowers were monitored for natural fruit set amongst the 14 plots of 2003. The means of fruit set ranged from a low of 3.9% (plot TD2) to a high of 50.1% at plot PR3 (Fig. 5.7; Table 5.8). The mean distance from a female plant to the nearest pollen source ranged from 2.3 m in plot PM2 to 20.3 m in plot GG1 (Table 5.8). Amongst the plots there was a significant relationship between fruit set and distance to the nearest

male plant in plots GG2 and PR1 ($P < 0.01$), showing higher fruit set in females that were in close proximity to male plants in these two plots (Fig. 5.8; Table 5.8). These results suggest male proximity is one of the contributors to pollen limitation in some of the plots. Low fruit set ($< 10\%$) was evident in plots KR, MW, TD2 & TD3), and appears to be affected by low male plant frequency, low male flower numbers, and distance between male and female plants (Table 5.7, 5.8). The number of seeds per fruit among the plots ranged from 1.3 at plot KR to 2.6 at plot GG1 (Table 5.8).

Table 5.6 Results of natural fruit set and results of t tests (natural fruit set versus year), and GLM (natural versus crossed fruit set) analyses from two *Melicope simplex* sites during the 2002–03 flowering seasons. PLI, pollen limitation index.

		Study site	
Variable		GG	PM
Mean natural fruit set (%)	2002	15.8	11.6
	2003	34.7	18.7
Natural fruit set vs year (2002, 2003) (*t*-ratio; *P* value)		8.00 ;0.004	1.44 ;0.253
Mean crossed fruit set, 2002 (%)		56.2	39.2
Natural vs crossed fruit set (2002) (*t*-ratio; *P* value)		57.7; <0.001	18.1; <0.001
PLI (2002)		0.72	0.70

Table 5.7 Results of GLM analyses of relationships between the means of natural fruit set and the distance to nearest male plant, male plant height, male plant frequency, and male flower numbers from 14 *Melicope simplex* study plots, 2003. d.f. for $t = 13$.

Variable	Estimate of coefficient	SE	*t*-ratio	*P* value
Constant	-1.675	0.0823	-20.36	<0.001
Distance	0.008	0.0062	1.29	0.196
Male plant frequency	0.072	0.0054	13.36	<0.001
Male flower numbers	0.0001	0.0000	6.89	<0.001

Table 5.8 Percent female plant frequency, number of monitored flowers, mean fruit set (%), significance of natural fruit set versus distance to the nearest pollen (male plant), and mean number of seeds per fruit at 14 *Melicope simplex* study sites.

Site/ plot	Female plant frequency (%)	Number of monitored flowers	Mean fruit set±SE	Mean distance to nearest pollen (m)	Fruit set versus distance to nearest pollen (*F, P*)	Mean number of seeds per fruit
GG1	92.3	804	34.6±7.8	20.3	0.05, 0.821	2.6
GG2	56.5	542	34.9±8.3	5.0	8.70, 0.015	2.4
KR	91.7	347	8.9±2.4	11.9	0.08, 0.782	1.3
MH	83.3	632	42.0±5.3	4.0	0.13, 0.719	2.4
MW	95.2	205	4.8±2.8	4.5	0.02, 0.903	2.0
PM1	85.7	608	22.9±5.6	6.1	0.19, 0.673	1.7
PM2	71.4	614	14.4±3.7	2.3	0.01, 0.922	2.0
PR1	63.3	1051	38.5±4.3	3.0	19.23, <0.001	2.2
PR2	76.5	352	32.5±5.1	10.0	0.61, 0.456	2.4
PR3	81.8	350	50.1±7.5	5.7	0.48, 0.510	2.3
TD1	66.7	201	32.2±8.0	5.8	1.65, 0.289	2.0
TD2	85.7	214	3.9±0.7	9.7	0.89, 0.398	2.0
TD3	89.5	393	6.2±1.7	18.7	0.65, 0.439	1.5
VH	90.0	488	16.2±4.4	4.5	1.68, 0.222	2.1

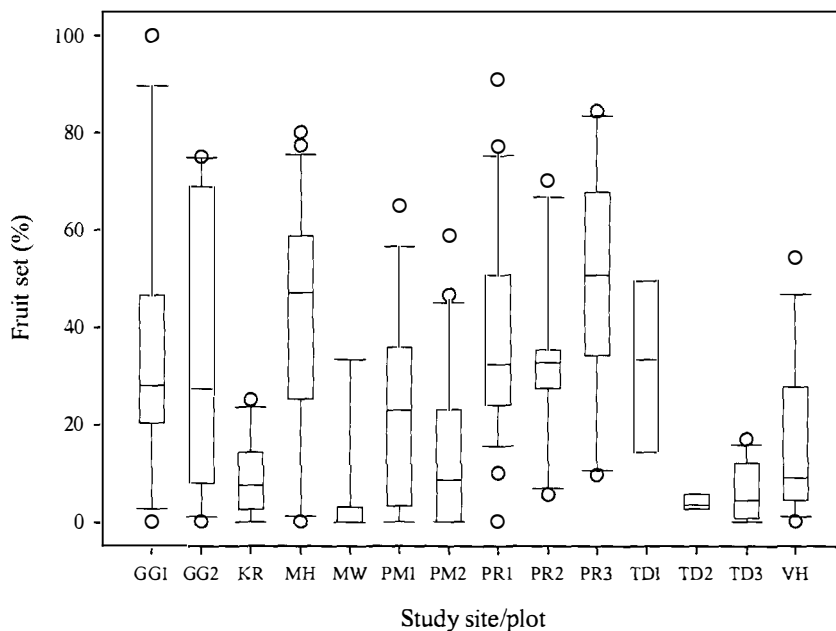**Fig. 5.7** Distribution of natural fruit set in *Melicope simplex* from 14 study plots in the North Island, 2003. The sites are listed alphabetically.
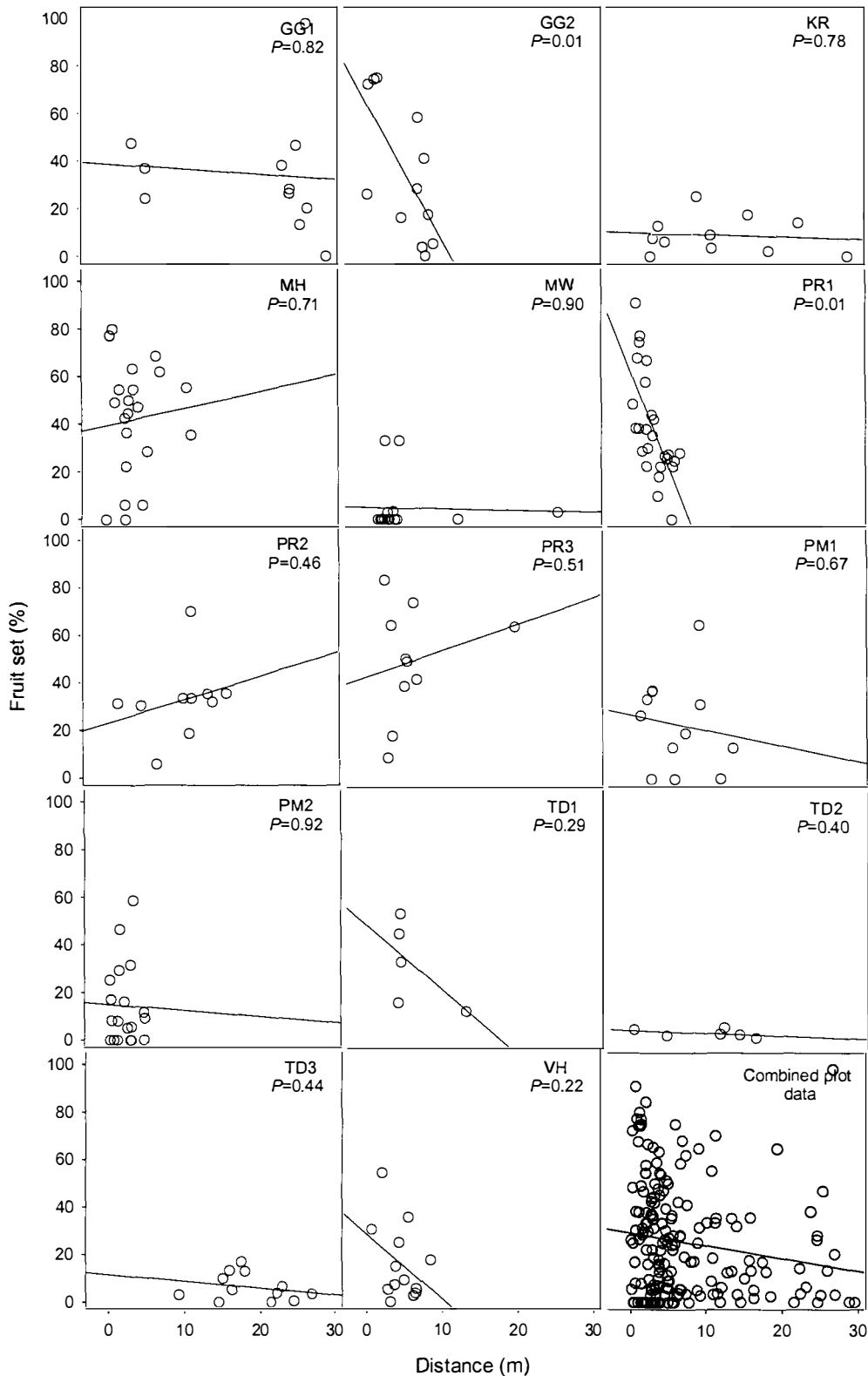
Fig. 5.8 Linear regression relationships between natural fruit set and the distance to the nearest male plant in the 2003 flowering season (October and November) from 14 *Melicope simplex* plots the North Island. Each symbol represents a monitored female plant.

Population characteristics and structure

Amongst the study populations, the total number of adult flowering plants (of both gender) ranged from a low of 7 in plot TD2 to 84 in plot MH (Fig. 5.9; Table 5.9). Gender ratios were female-biased in all plots, with the female proportion ranging from 56.5% in plot GG2, to 95.2% in plot MW (Table 5.8).

The mean height of female and male plants amongst flowering plants from all plots was similar, at 4.3 m to 4.4 m, and ranged from 1.0 m to 11.0 m (Table 5.9). At individual plot level, there was a significant difference in plant height between females and males only in plot GG2 ($P = 0.02$) where male plants were taller than females. There was considerable variation in flower numbers among plots, with the mean number of female flowers per plot ranging from 223 in plot TD2 to 4183 plot GG1 (Table 5.9). For male plants the mean number of flowers per plot ranged from 100 in plot MW to 5800 in plot PR2 (Table 5.9). In general, taller plants would be older and would be expected to produce more flowers. There was a significant difference in flower frequency between genders only in plot PR1 ($P = 0.05$).

Population size classes at most of the *M. simplex* study plots showed a typical reverse J curve; the only exception being plot GG2 (Table 5.10). Adult frequency ranged from 0.8% in plot TD1 to 40% in plot PM1. Juvenile frequency ranged from 4.4% in plot TD3 to 42.7% in plot GG1, and seedlings ranged from 33.6% in plot MH to 92.7% in plot TD3 (Table 5.10). Seedlings occurred most commonly near an adult female, and particularly on bare ground at forest edges or in higher light areas such as canopy gaps.
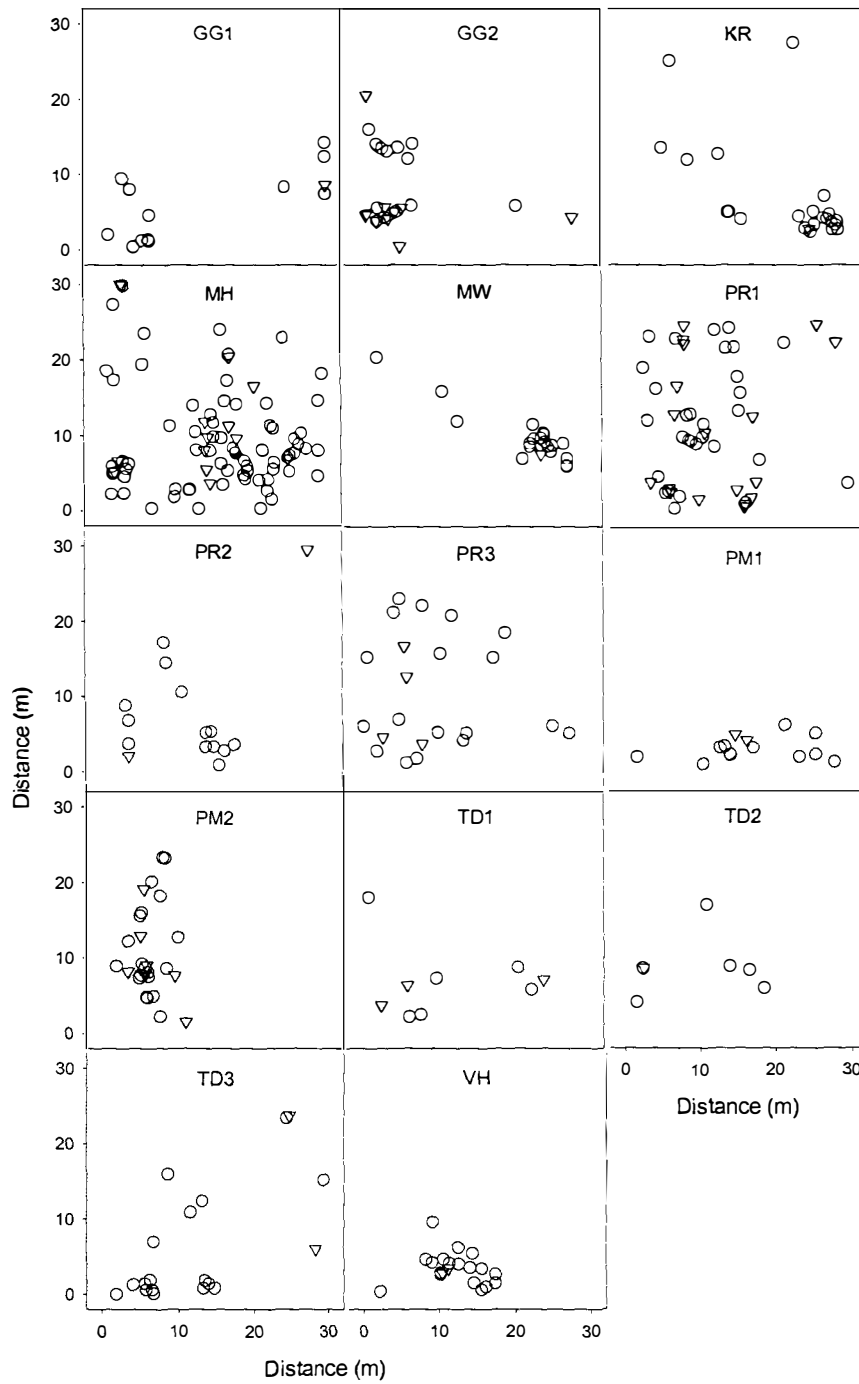
Fig. 5.9 Distribution and density of *Melicope simplex* flowering adult plants at 14 study plots in the North Island. (o), female plants; (∇), male plants.

Table 5.9 Number of adult plants per plot, mean plant height and number of flowers per monitored plant at 14 *Melicope simplex* plots in the North Island, 2003. *, $P < 0.05$ = significant difference in height between gender.

Site/plot	Number of adults	Mean plant heights (m)		Mean number of flowers per plant.	
		Female	Male	Female	Male
GG1	13	4.7	3.5	4183	3000
GG2	23	3.4*	4.7*	539	695
KR	24	4.6	4.5	1244	225
MH	84	4.3	4.3	263	295
MW	21	3.1	3.5	467	100
PM1	14	3.3	4.0	1559	1100
PM2	28	3.6	3.1	337	370
PR1	49	4.4	4.8	666*	1976*
PR2	17	4.8	4.1	1118	5800
PR3	22	5.3	4.5	282	550
TD1	9	4.8	6.4	2185	3250
TD2	7	5.0	2.6	223	150
TD3	19	5.1	5.3	567	1150
VH	20	4.2	3.6	1831	1755

Table 5.10 Population size-class distribution of *Melicope simplex* at 14 study sites in the North Island, 2003.

Site/plot	Number of plants	Population size-class structure (%)			
		Adults	Non-flowering	Juveniles	Seedlings
GG1	96	13.5	0	42.7	43.8
GG2	70	32.9	5.7	22.9	38.6
KR	159	15.1	2.5	27.7	53.5
MH	324	25.9	13.3	25.6	33.6
MW	261	8	14.9	29.9	47.1
PM1	35	40	0	20	40
PM2	279	10	14.7	15.1	60.2
PR1	686	7.1	2.2	19.1	71.6
PR2	84	20.2	3.6	10.7	65.5
PR3	105	21	1.9	29.5	46.7
TD1	1192	0.8	1.7	17.7	79.9
TD2	385	1.8	2.1	18.4	77.7
TD3	974	2	0.9	4.4	92.7
VH	395	5.1	2.3	14.7	77.7

5.5   Discussion

M. simplex flowers show typical simple, unspecialised structures that lack complicated adaptions for precise modes of pollination, including radial symmetry, freely exposed pollen and stigmas, exposed nectar, non-showy flower colour, and separate sexes – characteristics typical of flowers of the New Zealand flora (Lloyd 1985a). The flower size-dimorphism and vestigial anthers of *M. simplex* are not unusual in gender dimorphic taxa (Lloyd & Webb 1977; Delph 1996; Delph et al. 1996). In many dioecious species, male flowers are often larger than females, produced in greater abundance, and vestigial organs of the other sex may be present (Delph 1996; Proctor et al. 1996; Eckhart 1999). For example, Delph et al. (1996) found flower size dimorphism in 97% of 102 families containing unisexual flowers. Smaller female flowers may be a consequence of suppressed anther development associated with suppressed petal development (Proctor et al. 1996), or increased attraction for pollinators, or a response to hormonal signals (Delph 1996).

Melicope simplex is unusual in having what appears to be a consistent, female-biased gender distribution in geographically separated populations. However, the proportion of males, although never reaching 50%, was highly variable, ranging from 4.8% to 43.5%. Variation in sex-ratio is quite common in gender-dimorphic species (Lloyd 1973; Delph 1990; Barrett 1992; Ashman 1999), and in some species sex bias can vary within and between seasons (Decker & Pilson 2000). Female-biased sex ratios are less common in dioecious species, but are occasionally reported. For example, in New Zealand, Ladley et al. (1997) found female-biased populations of the native mistletoes *Ileostylus micranthus* and *Tupeia antarctica,* but no apparent gender-related difference in fruit set in *I. micranthus* plants. In a review of life history traits in 45 dioecious species, Delph (1999) found 15.5% with female-biased populations. Female-biased populations of *Juniperus thurifera*, a dioecious bush or tree, have been reported from the mountains of the Mediterranean (Gauquelin et al. 2002), but reproductive success was not examined.

Several theories have been proposed to explain the evolution of biased sex-ratios, including frequency-dependent selection, local competition between males, local resource competition, inbreeding depression, life history traits, environmental gradients, and selection on attractive traits (Colwell 1981; Charnov 1982; Charlesworth & Toro

1982; Delph 1999; Campbell 2000; Ashman & Diefenderfer 2001). Reproduction is generally considered to be more costly for females and the cost of the relatively higher reproductive effort could lead to delays in reproductive maturity, less frequent flowering, less growth, and/or death at a younger age (Lloyd & Webb 1977; Delph 1999). Decker and Pilson (2000) established that variation in gender ratio (female-biased in 1995–96, and male-biased in 1997–98) in *Croton texensis* was genetically determined, and suggested that in some plant species at least, population sex-ratios were determined by a combination of genetic and environmental factors. Stehlik and Barrett (2005) found that variation in female-biased sex-ratios in populations of *Rumex nivalis* were influenced by altitude, with a higher frequency of males in higher altitude populations. Investigating the evolution of female-biased gender ratios in *M. simplex* was beyond the scope of this thesis, but presents an opportunity for future research.

Although hermaphrodism or bisexuality has been reported in *M. simplex* (Thomson 1892; Cheeseman 1925; Allan 1961; Moore & Irwin 1978), there was no sign of deviation from dioecy in the 14 plots of this study. In dioecious species, reproductive success is dependent on pollen availability and abundance, and pollinator activity. In female-biased populations, provided pollinator activity is at acceptable levels, pollen availability would seem to be the most crucial factor that could limit seed set, particularly in populations with a low proportion of male plants and/or flowers.

Determining the factor/s that could contribute to poor fruit set in *M. simplex* has required comparing female fecundity with male plant and male flower density, and the following three variables emerged as the most likely contributors: 1. low male plant density, 2. low floral numbers in male plants, and 3. distance to the nearest male. At least two of these variables in combination seem to result in poor fruit set in *M. simplex*. For example, there were only 1 or 2 male plants in five of the six plots with low fruit set. The exception was plot PM2 where although the frequency of male plants was relatively high ($N = 8$), the mean number of flowers per plant was relatively low (370). Low male plant floral numbers in conjunction with greater distances to pollen appear to be the contributing factors in low fruit set in females in three of the study plots, KR, TD2, and TD3. Plot TD2 had the lowest fruit set of all 14 plots, probably the consequence of only one male plant in the plot concomitant with a low numbers of flowers. The plots with the highest natural fruit set (plots GG2, MH, PR1, & PR3), had

either a high density of plants of both gender (plots MH, PR1, & PR3) or a high proportion of males (plot GG2). In a study of reproductive success in two dioecious mosses, Bisang et al. (2004) found fertilisation success was male limited in female-dominant populations, and Somanathan and Borges (2000) reported reduced fruit set with increased distance to male plants in the dioecious *Diospyros montana*.

Pollinator activity and pollen deposition was primarily diurnal, as evidenced by low pollen grain numbers on stigmas that were exposed to nocturnal pollinators. The nectar rewards for insect visitors was similar in both male and female flowers, and probably attracts a range of potential pollinators. Flies, the most abundant of the invertebrates caught on sticky traps, are an insect group with considerable diversity in New Zealand, and of the indigenous plants whose pollinators have been recorded, are the most frequent insect visitors (Heine 1937; Primack 1983). The higher frequency of thrips in the vicinity of male flowers suggests they may be feeding on pollen, but not necessarily pollinators. Norton (1984) captured thrips on female flowers of mahoe (*Melicytus ramiflorus*), kaikomako (*Pennantia corymbosa*), and five-finger (*Pseudopanax arboreus*), and suggested they could be involved in the pollination of some dioecious species; however, the extent to which they contribute to the pollination dynamics of lowland forests was unknown (Norton 1984).

Fluctuation in pollinator numbers could contribute to fluctuation in fruit set between years. Burd (1994) considered pollen limitation to be a common phenomenon that occurred in many vascular plants because the pollination environment was not constant. Inadequate pollen receipt was considered the primary cause of low fecundity in perennial plants (Burd 1994). In the case of *M. simplex*, inadequate pollen receipt is more likely a consequence of insufficient available pollen rather than low numbers of pollinators. In addition, the morphological features of *M. simplex* flowers offer little in the way of protection of the sexual organs from rain, and because this species flowers during spring, the likelihood of rain is relatively high. Persistent rain during flowering would dilute nectar, and not only make the flowers less attractive to an insect pollinator, but probably reduce pollinator activity.

Chronic pollen limitation can have several outcomes for plant populations, to the extent of causing population extinction (Ashman et al. 2004). Determining how and whether

pollination limitation influences population dynamics is central to our understanding of the importance of pollen limitation in the maintenance of plant diversity (Ashman et al. 2004). Recording population size classes has enabled an assessment of population viability and persistence in *M. simplex*, and the results of this study show that although fruit set was low at some sites, the numbers of juvenile and seedling plants indicates most populations are stable, and likely to persist in the long term. In long-lived woody species, there may be no great necessity for high reproductive success because their longevity does not require frequent replacement (Bond 1994; Riveros et al. 1998). Replacement of an individual only occurs when suitable conditions become available for germination of seeds stored in the seed bank, and for subsequent seedling establishment (Riveros et al. 1998).

The differences in fruit set at two sites between 2 years shows that reproductive success can vary from year to year, and because *M. simplex* is a relatively long-lived species, low fruit set in any one year does not necessarily negatively impact on population viability in the longer term, as implied by these results. For example, by calculating the average number of female flowers per plant (1104) by the average fruit set (25%) from all plots, 276 seeds could be produced from each female plant per year, an amount that seems more than adequate for replenishing the seed bank. Burrows (1996) suggested relatively long-term seed banks could probably develop in *M. simplex*. Long-lived seeds enable species to persist over time until conditions suitable for germination become available, and species can re-establish after their apparent demise (van der Valk 1992). Suitable habitat conditions in areas of higher light appear to promote seed germination in *M. simplex*, and it would seem highly likely that this species has benefited from habitat fragmentation in the past by taking advantage of, and colonising, newly created edges. Also, its presence in naturally created canopy gaps that occur as a result of tree falls due to senescence or storm events, suggests this species could be classified as 'early successional' in the New Zealand context.

The dynamics of the reproductive process, pollen flow and germination of an individual species are integral components of a much larger natural population ecosystem dynamic, and the maintenance of a certain species density and age structure are processes found at the end of a more extensive sexual reproduction/plant replacement chain (Riveros et al. 1998). This investigation into the reproductive biology of *Melicope*

simplex provides useful insight into the multiple factors that influence successful fertilisation in a female-biased dioecious shrub/ species, and illustrates the complexity of interactions that can occur at the species level in native ecosystems.

5.6   Acknowledgements

5.7   References

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Chapter 6

Gender distribution and population structure in *Pimelea arenaria* (Thymelaeaceae), a gynodioecious sand dune species from New Zealand

6.1   Abstract

Pimelea arenaria sens. str. is one of a small suite of native species that occur on unconsolidated sand dunes and in dune hollows of the North Island of New Zealand. It grows exclusively on sand dunes, and forms a multi-stemmed, low-growing, spreading shrub up to 3 m in diameter. Its breeding system is gynodioecious, and during spring and summer it produces abundant small, white flowers in clusters at the apex of branches. Recruitment failure was evident at most of the 18 sites surveyed, although fertilisation success was relatively high, averaging 47% for females and 68% for hermaphroditic plants. The proportion of females in populations was higher in the northern half of the North Island (15.9% to 45.5%), compared with populations from Kawhia southwards (0% to 12.7%). Females were absent from three southern west coast populations. Although most populations surveyed are not under immediate threat, lack of recruitment in the long term could affect population persistence, with small populations particularly vulnerable.

6.2   Introduction

Coastal sand dunes are one of the most disturbed and highly modified ecosystems in New Zealand, with many now converted to pasture or plantation forestry, or developed for housing and roads. In addition, a number of exotic plant species have been deliberately planted for dune stabilisation, 'beautification', and nitrification, e.g., marram (*Ammophila arenaria*), ice plant (*Carpobrotus edulis*), gazania (*Gazania linearis*), and tree lupin (*Lupinus arboreus*). The impact of habitat degradation and modification has resulted in the decline in abundance of coastal native species and, in some cases, local extinctions have occurred (Gabites 1993). Of the 187 coastal plants listed in the most recent compilation of New Zealand's threatened and uncommon

plants, 52 are species of beach-habitats, including the gynodioecious species *Pimelea arenaria* (de Lange et al. 2004).

One of the distinctive features of the New Zealand flora is the high frequency of gender dimorphism (Heine 1937; Carlquist 1966; Godley 1979; Lloyd 1985; Webb & Kelly 1993). Gynodioecy is a form of gender dimorphism involving the production of female (pistillate) and hermaphrodite (pistillate and staminate) flowers on separate plants in the same population (Sakai & Weller 1999). Gynodioecy is regarded as an intermediate evolutionary step from hermaphrodism to dioecy, involving male-sterile individuals arising within a population of hermaphrodites (Delph & Lloyd 1996; Webb 1999). The evolutionary pathway from hermaphrodism to gynodioecy and often on to dioecy is the best documented transition to gender dimorphism, although in many genera gynodioecy appears to be a persistent breeding system in its own right (Webb 1999). In New Zealand's flora, gynodioecy is the second most common form of gender dimorphism, occurring in 7.2% of indigenous genera (Webb et al. 1999).

Gender ratios in gender dimorphic species can vary between and among populations (Foweraker 1932; Godley 1955, 1963, 1964; Burrows 1960; Webb & Lloyd 1980; Connor 1984; Webb 1999). For example, in a study of sex expression in natural populations of four *Pimelea* species, Godley (1979) found variation in the frequency of each gender among populations, but also reported variation in ratios at different times of the flowering season. Burrows (1960) also reported variation in gender ratios (from 1:1 to 10:1, hermaphrodite: female) among species, populations, and also seasonally in four *Pimelea* taxa.

In gender dimorphic species, female flowers are often smaller than their hermaphroditic counterparts (Proctor et al. 1996; Delph 1996; Delph et al. 1996). In *Pimelea,* the female flower is smaller than the hermaphrodite, and has smaller staminodes but a larger stigma (Burrows 1960). In some gynodioecious species, females produce more seed than hermaphrodites whereas in others, hermaphrodites can produce more seed than females (Bawa 1980). Because all ovules have the potential to be cross-fertilised in females, there may be genetic advantages in the offspring of female plants, compared with offspring of self-compatible hermaphrodites (Bawa 1980). Lloyd (1980) describes the sexual behaviour of plants in terms of their "functional gender", which is an

estimate of their maleness or femaleness, based on the relative paternal and maternal investment into pollen and ovule production before anthesis.

Successful pollination is usually the main mechanism by which seeds are formed so it is a vital link in the persistence of most plant populations (Proctor et al. 1996). Plant reproductive systems and strategies play a major role in the abundance and diversity of taxa in modified ecosystems (Lovejoy et al. 1986; Saunders et al. 1991), and since breeding systems significantly influence genetic diversity, identifying these is an appropriate initial step of investigation because seed output represents a direct, quantifiable measure that integrates numerous interacting factors, and provides an index of community integrity or 'health' (DeMauro 1993; Aizen & Feinsinger 1994).

This study investigates population structure, gender ratios, natural fertilisation success, and vascular plant associations in populations of *Pimelea arenaria* in the North Island of New Zealand.

6.3   Materials and methods

Study species

Pimelea arenaria sens. str. Cunn. (Thymelaeaceae) is a low growing shrub (< 50 cm tall) that occurs on coastal sand dunes (Fig. 6.1), and was described by Allan Cunningham from specimens collected from sandy ridges at the mouth of the Hokianga River, Northland (Allan 1961). In New Zealand the family Thymelaeaceae is represented by two genera: *Kelleria* (previously *Drapetes* Heads 1990), and *Pimelea*. The genus *Pimelea* comprises about 80 species of shrubs or subshrubs that are mainly distributed in Australia and New Zealand, but with a few species on Lord Howe Island and in Timor (Allan 1961). There are 19 named species and 11 taxonomically indeterminate *Pimelea* taxa in New Zealand (Landcare Research 2005).

Pimelea arenaria is one of a relatively small suite of native plants that occur exclusively on unconsolidated sand dunes (Wardle 1991). While Allan (1961) described its distribution as 'throughout', its geographic range has declined over the last century. *P. arenaria* sens. str. has been recorded from numerous beaches in the North Island, but is listed in the 'Gradual Decline' category of the most recent lists of threatened and

uncommon plants (de Lange et al. 2004). Populations from at least ten North Island beaches have apparently become extinct during the last century including, Taipa, Piha, Hahei, Port Waikato, Lyall Bay, Plimmerton, Red Rocks, Seatoun, and Waikanae (pers. obs.; Esler 1975; Milne & Sawyer 2002). New populations have been located at East beach (Rangaunu Bay, Northland, 2002), and Kawhia (Hamish Kendal *pers. comm.* 2001).

The breeding system of the genus *Pimelea* is reported as gynodioecious (plants which produce female and hermaphrodite flowers) (Burrows 1960). *P. arenaria* produces large numbers of small, white flowers in terminal clusters (Fig. 6.2), over a relatively long flowering season from September for 6–9 months. Flowering occurs in pulses: clusters of buds develop at the stem apex, and after flowering and senescence, new vegetative buds form at the edge of old flower clusters, which in turn develop flower buds. Senesced flowers retain their petals, which form a protective barrier around the developing ovule. Fertilisation in hermaphrodite flowers occurs through autonomous selfing (see Chapter 2). Fruit maturation in *P. arenaria* is relatively rapid, i.e. from flower to ripe fruit in 4 to 6 weeks. Mature fruit colour was mostly purplish-black (Fig. 6.3) but pink and red fruits have also been noted. Each fleshy fruit contains only one seed.

Fig. 6.1 An adult *Pimelea arenaria* growing at Spirits Bay, Northland, 2001.

Fig. 6.2 Female (F) and hermaphroditic (H) flowers of *Pimelea arenaria* from Matarangi, 2001.
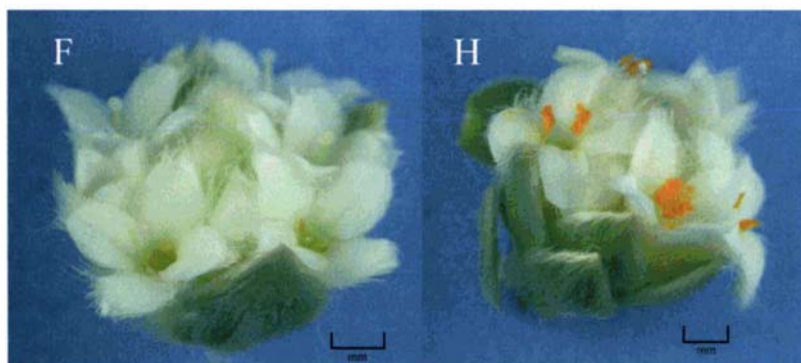
Fig. 6.3 Fruit of *Pimelea arenaria* from Matarangi, 2001.

Study sites

Herbarium records (AK; CHR; WAIK) and literature reviews (Partridge 1992) were used to identify populations of *P. arenaria* throughout the North Island of New Zealand. Eighteen geographically separated populations were identified as study sites (Fig. 6.4), and from February 2001 to December 2002, dune systems were extensively searched for natural populations of *P. arenaria*. At each site all the *P. arenaria* plants located were classified into three size classes: seedlings (up to 5 lateral branches), juveniles (non-flowering but larger than seedlings), adults (flowering plants), and plant gender was noted for the flowering individuals. All individuals in smaller populations (< 100) were recorded, but in larger populations a subset of plants was recorded by running transects across the dunes noting all the plants that were encountered along with these lines until

at least 120 individuals were included to ensure adequate population representation. Fertilisation success was determined by collecting senesced inflorescences of both genders from each site. The number of flowers and maturing ovules in each inflorescence from each site was recorded. At each site, the vascular plant species growing in association with *P. arenaria* were recorded.
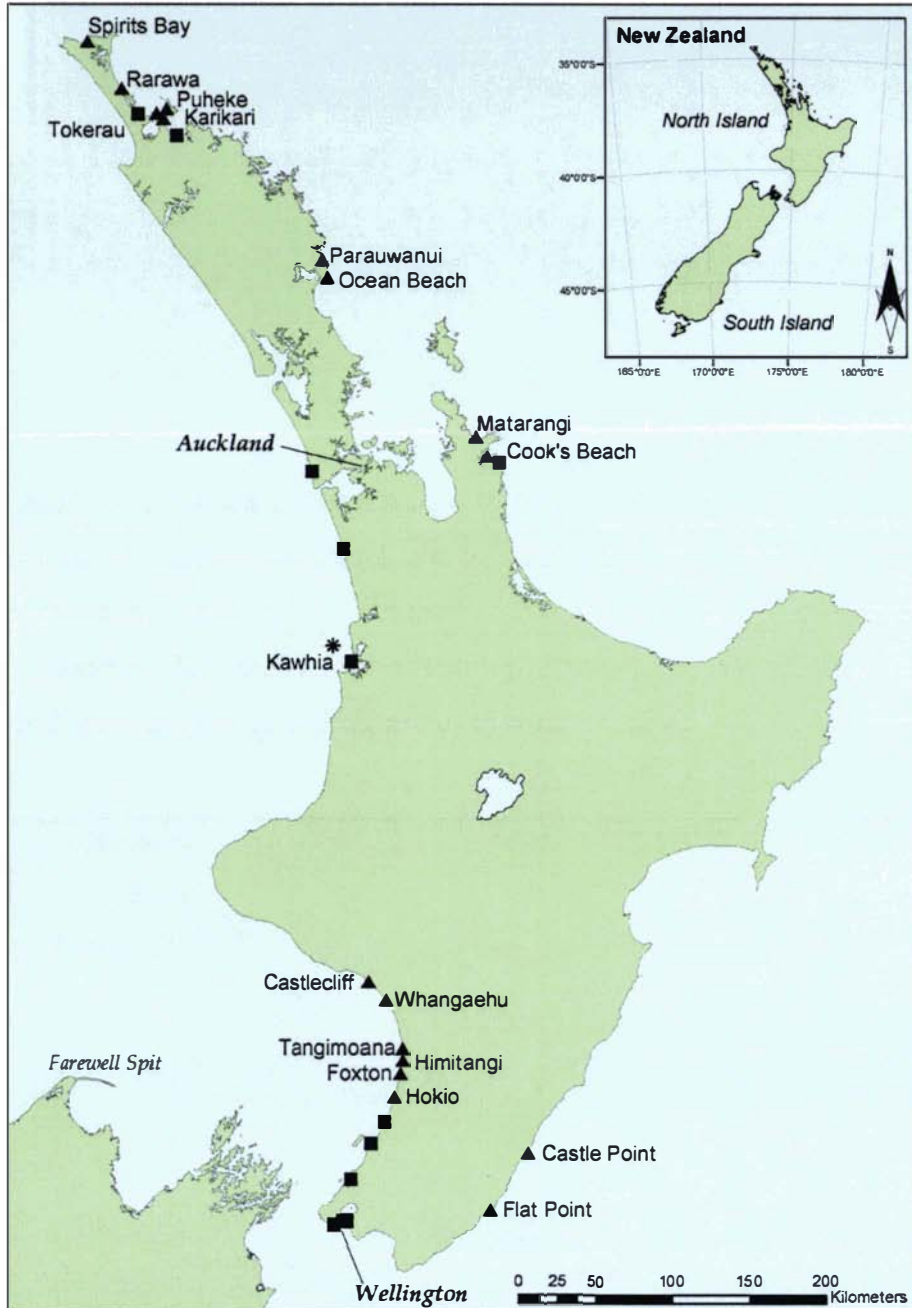

Fig. 6.4 Location of *Pimelea arenaria* study sites (▲), and extinct populations (■), Kawhia*, study site and new population.

Data Analysis

For each population, population means were calculated for fertilisation success per flower cluster, and the number of flowers per inflorescence. Box plots were generated from individual plant fertilisation success for both female and hermaphroditic plants. The box plots depict the median (central line in each box), 25% and 75% quartiles (upper and lower limits of each box), 90th and 10th percentiles (indicated by the whiskers), and outliers, (indicated by open circles). *T*-tests were used to compare fertilisation success between genders at each study site. Regression analyses were used to establish relationships between the proportion of female plants, the number of flowers per cluster, and the fertilisation success in each population with latitude.

6.4   Results

Pimelea arenaria was found exclusively on sand dune ecosystems, mostly on unconsolidated sand of dune faces, dune ridges, and in dune hollows behind the foredune. Sand dune height and profile varied amongst the 18 study sites, from low and shallow (< 1 m), at Spirits Bay, to steep and high (c. 20 m) at Kawhia, with numerous variations between these extremes.

Most *P. arenaria* adult plants found were less than 50 cm in height, and in some large plants the almost-circular canopy extended to approximately 3 m dia. Stems were commonly partially buried by sand, and occasionally, at some sites, the extensive root system was exposed as a result of erosion of the foredune. Population structures showed adult plants the dominant size-class at all sites (Fig. 6.5). No seedlings were recorded from seven of the eighteen beaches (Tokerau, Ocean Beach, Castlecliff, Tangimoana, Foxton, Castle Point and Flat Point), and were in low numbers (< 20%) at all other sites except Hokio (Fig. 6.5). Most of the seedlings at Hokio were clustered near a single adult plant. No juveniles were recorded from Ocean Beach or Castle Point, and this size-class comprised less than 20% of populations at all other sites except Spirits Bay (Fig. 6.5). The larger populations were all in the far North, from Karikari to Spirits Bay (Table 6.1).

Population gender ratios were variable amongst the study sites, albeit hermaphrodite-

biased at all sites. At sites with both female and hermaphrodite plants, the proportion of females ranged from a high of 41.6% at Cook's Beach, to a low of 2.7% at Himitangi (Table 6.1). Female plants were not found at Castlecliff, Tangimoana or Whangaehu (Table 6.1). Occasionally plants of both genders were seen growing intertwined, but separate primary stems were not readily identifiable because of sand burial. There was a significant relationship ($P < 0.001$) between latitude and female frequency, with a higher frequency of females in populations north of Kawhia (Fig. 6.6; Table 6.1).

The mean number of flowers per inflorescence in female plants was variable among the sites, ranging from 3.8 at Himitangi to 15.6 at Ocean Beach, and in hermaphrodites ranging from 2.9 at Castlecliff to 18.5 at Ocean Beach (Table 6.1). There was a significant correlation ($P < 0.001$) between latitude and the number of flowers per inflorescence, with more flowers in each inflorescence in populations north of Kawhia (Fig. 6.7; Table 6.1).
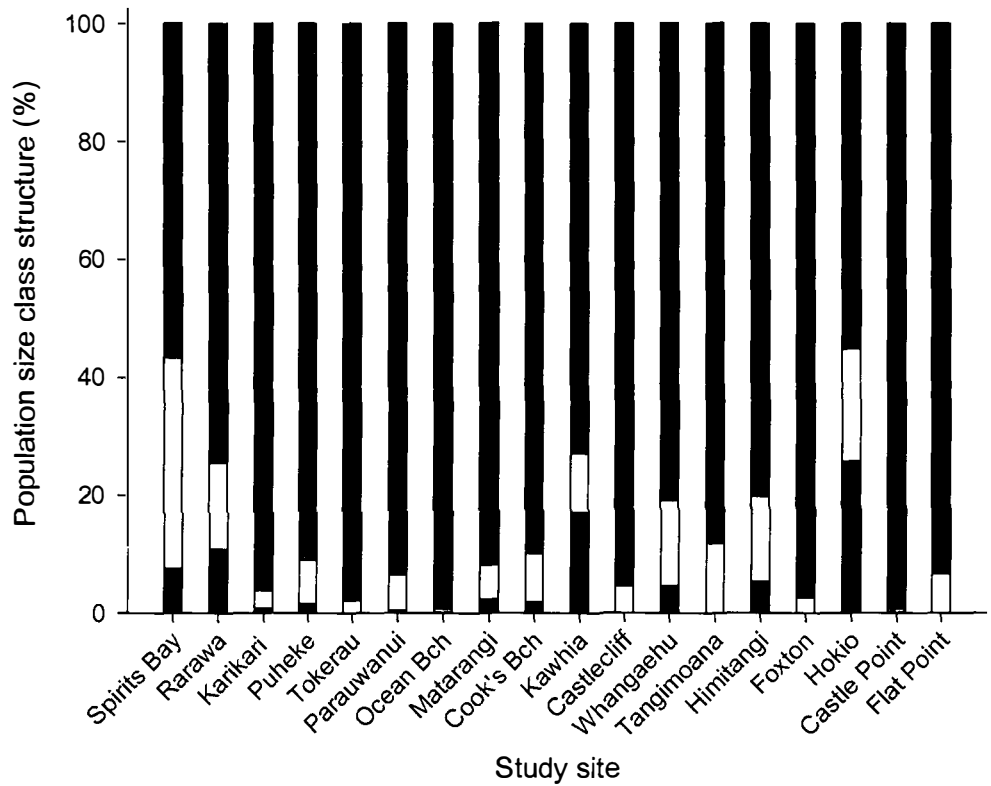

Fig. 6.5 Population size-class structure of 18 North Island *Pimelea arenaria* populations, arranged from north (Spirits Bay) to south (Flat Point), 2001-2002. Black, seedlings; light grey, juveniles; dark grey, adults.

Table 6.1 Study site, surveyed population size, female frequency, mean number of flowers per cluster, and fertilisation success for female and hermaphrodite flowers, and results of two-sample *t* tests comparing female and hermaphrodite fertilisation success of *Pimelea arenaria* at 18 study sites in the North Island, 2001-2002, listed from north to south. F, female; H, hermaphrodite

Study site	Number of flowering plants	Proportion female (%)	Mean number of flowers/cluster		Mean fertilisation success (%)			
			F	H	F	H	Female vs hermaphrodite (*t, P*)	d.f.
Spirits Bay	337	22.5	8.2	7.8	56.4	38.1	3.67, <0.001	94
Rarawa	200	26.8	9.7	10.9	53.7	54.5	-0.13, 0.896	101
Karikari	315	23.1	8.9	9.1	71.5	52.5	3.65, <0.001	108
Puheke	235	36.9	10.4	10.0	60.2	57.4	0.46, 0.648	97
Tokerau	141	35.5	10.5	10.2	51.0	43.6	1.46, 0.147	107
Parauwanui	188	32.4	13.9	12.8	74.1	75.2	-0.29, 0.773	110
Ocean Beach	13	30.8	15.6	18.6	71.4	58.8	1.67, 0.106	106
Matarangi	162	37.6	7.7	8.1	71.3	63.9	1.85, 0.066	109
Cook's Bch	99	41.6	6.6	6.9	46.1	72.5	-5.21, <0.001	85
Kawhia	173	11.9	4.5	4.5	80.3	83.6	-0.94, 0.347	132
Castlecliff	88	0.0	-	2.9	-	87.3	-	-
Whangaehu	84	0.0	-	3.8	-	69.0	-	-
Tangimoana	17	0.0	-	4.5	-	97.0	-	-
Himitangi	91	2.7	3.8	4.0	26.5	79.9	-5.04, <0.001	94
Foxton	118	0.9	4.0	4.3	15.0	82.3	-12.88, <0.001	88
Hokio	143	12.7	4.7	4.7	59.1	82.2	-3.40, 0.002	109
Castle Point	71	12.7	4.2	4.2	40.3	72.3	-5.32, <0.001	115
Flat Point	76	5.6	3.8	3.8	74.7	58.5	2.79, 0.006	107
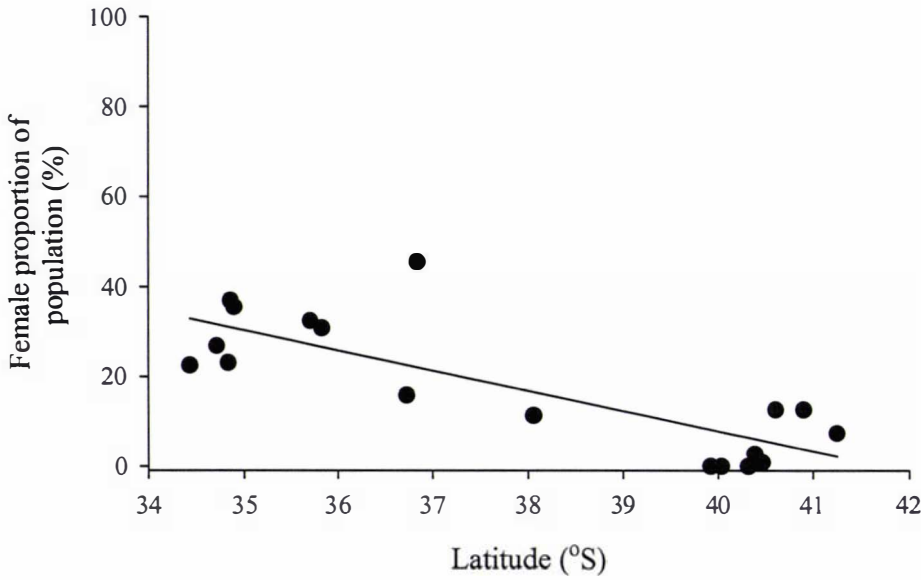
Fig. 6.6 Relationship between female frequency per population and latitude in 18 populations of *Pimelea arenaria* in the North Island, 2001-2002. Each symbol represents one population. The regression results for these data are: $F_{2,17} = 29.9$, $R^2 = 63.0$, $P < 0.001$.
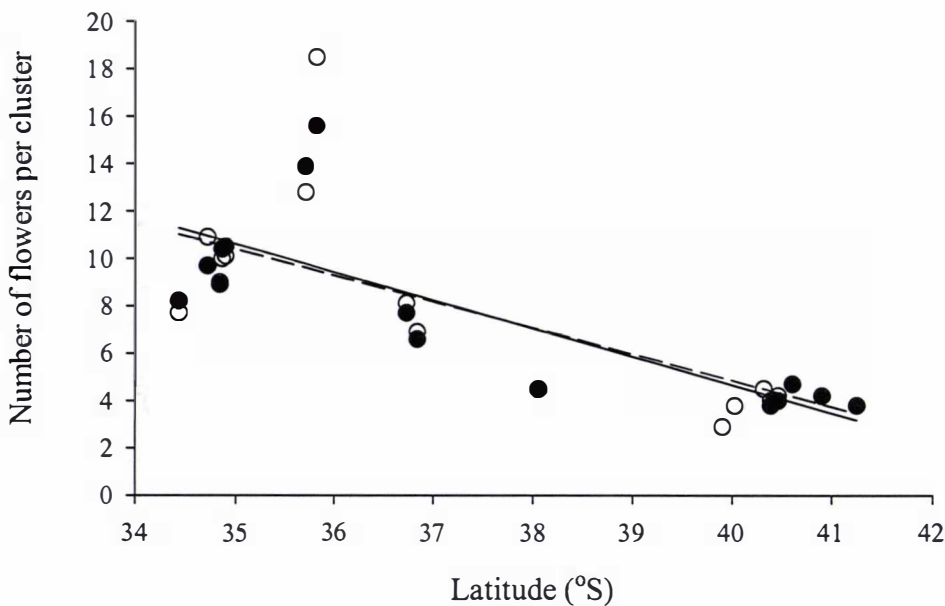
Fig. 6.7 Relationship between latitude and numbers of flowers per cluster for female (●, dashed line), and hermaphroditic (○, solid line) plants from 18 populations of *Pimelea arenaria* in the North Island, 2001-2002. The regression results for these data are: female, $F_{2,17} = 28.3$, $R^2 = 61.6$, $P < 0.001$; hermaphroditic, $F_{2,17} = 22.2$, $R^2 = 55.5$, $P < 0.001$.

Mean fertilisation success in female plants ranged from a high of 80% at Kawhia to 15% at Foxton (Fig. 6.8; Table 6.1). In hermaphrodite plants, fertilisation success ranged from 97% at Tangimoana to 38% at Spirits Bay (Fig. 6.9; Table 6.1). There was a significant difference in fertilisation success between female and hermaphroditic plants at eight of the study sites (Table 6.1). At three of these, Spirits Bay, Karikari, and Flat Point, female inflorescences produced a higher proportion of seeds than hermaphrodites, whereas at five of the sites, Cook's Beach, Himitangi, Foxton, Hokio and Castle Point, hermaphrodite inflorescences produced more than females (Table 6.1). There was a significant relationship between latitude and fertilisation success in female plants; populations with a higher component of female plants had significantly higher levels of fertilisation success($P < 0.02$) (Fig. 6.10; Table 6.1). There was a significant relationship between female plant frequency and fertilisation success in hermaphrodites ($P < 0.01$), with increased fertilisation success in hermaphrodites in populations with fewer female plants (Fig. 6.11).

At nine (50%) of the beaches surveyed in this study, the number of exotic, naturalised, vascular plant species either equalled, or out-numbered, the native flora (Table 6.2). All vascular plant species noted growing in association with *P. arenaria* from the 18 study sites are listed in Table 6.3. Some of the exotic species, particularly marram (*Ammophila arenaria*), tree lupin (*Lupinus arborea*), pampas (*Cortaderia selloana*), and wilding pines (*Pinus pinaster, P. radiata*) are likely to negatively affect the habitat requirements of *P. arenaria*; overtopping and shading by pampas, tree lupin and wilding pines, and the dense swards formed by marram dominate the substrate and displace other species. At some popular beaches, e.g., Parauwanui, Cook's Beach, deliberate plantings of exotic garden plants have become naturalised, and have the potential to displace native species. For example, the exotic ice plant, *Carpobrotus edulis*, forms a dense, spreading groundcover that excludes virtually all other species. At more isolated beaches with little or no human habitation nearby, e.g., Spirits Bay, Rarawa, Puheke, and Flat Point, there were fewer exotic species and proportionally more native taxa (54–70%) than at more populated sites (Table 6.2). The most widespread exotic taxa were those with wind-dispersed seeds e.g., *Conyza* sp., *Crepis capillaris*, *Lagurus ovatus*, and *Senecio elegans*.
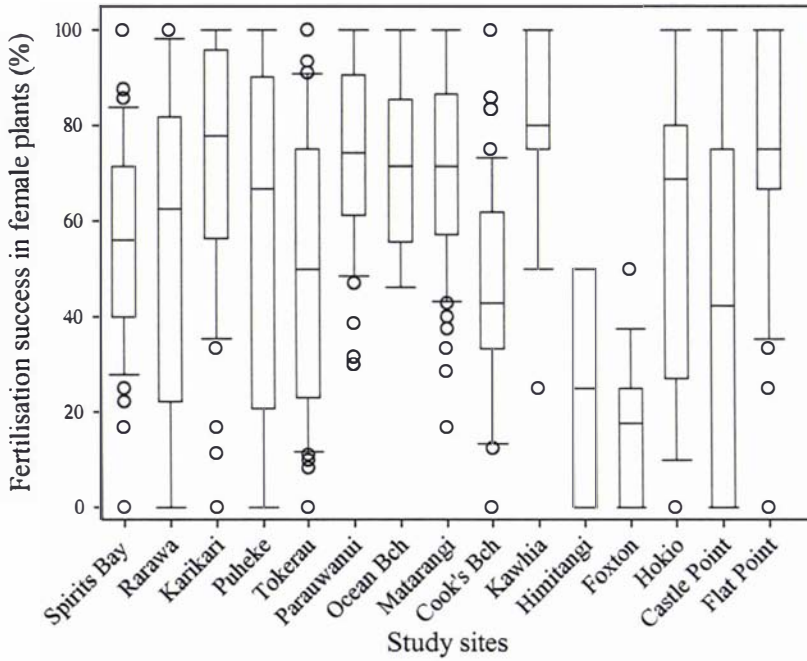
Fig. 6.8 Distribution of fertilisation success in female individuals of *Pimelea arenaria* from 15 populations in the North Island, 2001-2002, arranged from north (Spirits Bay) to south (Flat Point).
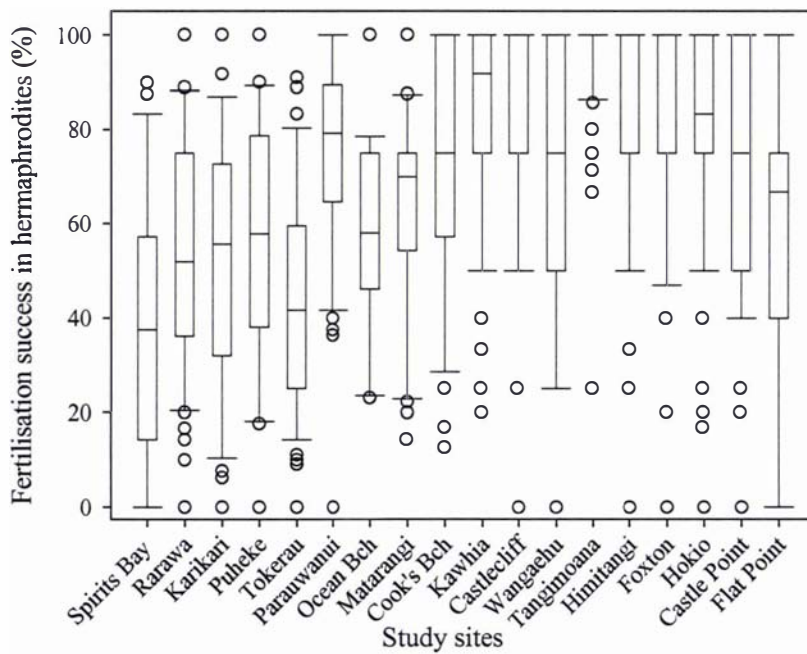
Fig. 6.9 Distribution of fertilisation success in hermaphroditic individuals of *Pimelea arenaria* from 18 populations in the North Island, 2001-2002, arranged from north (Spirits Bay) to south (Flat Point).
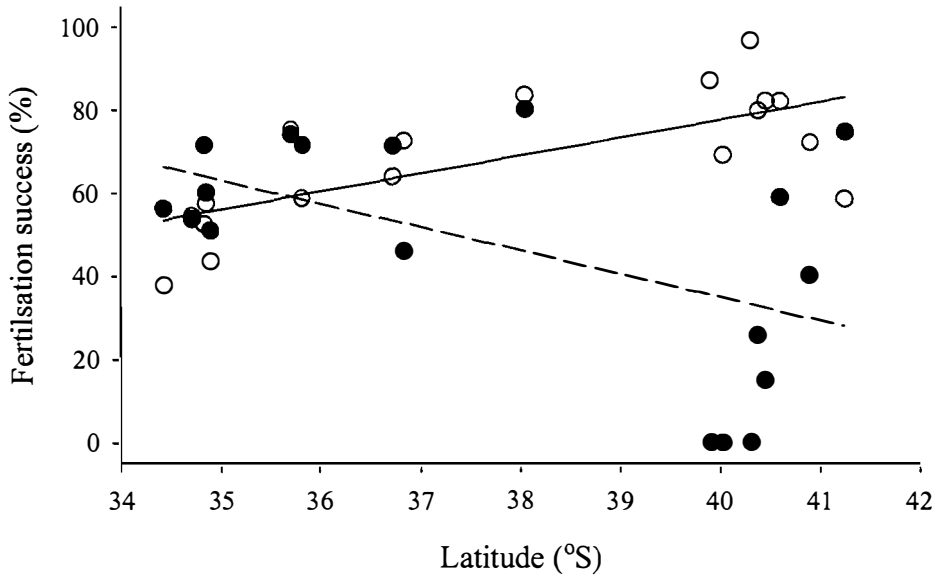
Fig. 6.10 Relationship between latitude and fertilisation success for female (●) and hermaphroditic (○) plants and in 18 populations of *Pimelea arenaria* in the North Island 2001-2002. The regression results for these data are: female, $F_{2.17} = 6.3$, $R^2 = 23.6$, $P < 0.02$; hermaphrodite, $F_{2.17} = 16.3$, $R^2 = 47.4$, $P < 0.001$. Dashed line, females; solid line, hermaphrodites.
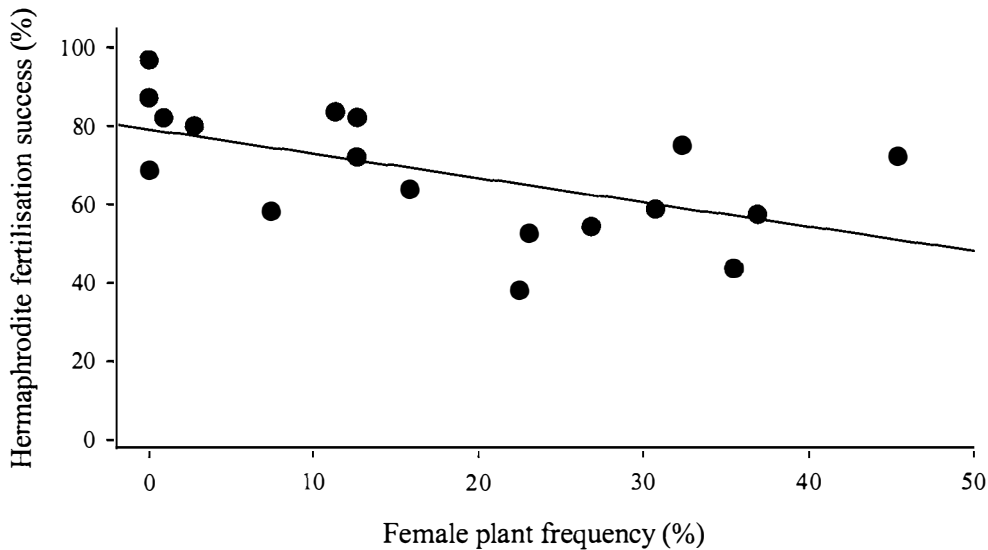
Fig. 6.11 Relationship between mean percentages of hermaphrodite fertilisation success and percent female plant frequency in 18 populations of *Pimelea arenaria* in the North Island, 2001-2002. The regression results for these data are: $F_{2.17} = 8.0$, $R^2 = 29.2$, $P < 0.01$.

Table 6.2 Numbers of native and exotic species that were recorded growing in association with *Pimelea arenaria* at 18 study sites in the North Island, 2001-2002.

Study site	Vascular plant species	
	Native	Exotic
Spirits Bay	12	5
Rarawa	11	9
Karikari	7	5
Puheke	10	6
Tokerau	10	10
Parauwanui	7	9
Ocean Beach	5	3
Matarangi	10	8
Cook's Beach	7	29
Kawhia	9	12
Castlecliff	10	15
Whangaehu	13	17
Tangimoana	8	10
Himitangi	7	6
Foxton	6	12
Hokio	6	4
Castle Point	5	10
Flat Point	7	3

Table 6.3 Vascular plant species found growing in association with *Pimelea arenaria* at 18 study sites in the North Island, 2001-2002. * indicates exotic species.

	Species		Species
*	*Acacia* sp.	*	*Leontodon taraxacoides*
*	*Agapanthus orientalis*		*Leptocarpus similis*
*	*Agave* sp.	*	*Lilium formosanum*
*	*Aloe* sp.	*	*Lotus pedunculatus*
*	*Ammophila arenaria*	*	*Lotus suaveolens*
*	*Anthoxanthum odoratum*	*	*Lupinus arboreus*
*	*Arctotheca calendula*	*	*Lycium ferocissimum*
	Austrofestuca littoralis	*	*Malus domestica*
*	*Briza maxima*	*	*Medicago lupulina*
*	*Cakile edentula*	*	*Medicago sativa*
	Calystegia soldanella	*	*Melianthus major*
	Carex testacea	*	*Meliolotus indicus*
*	*Carpobrotus edulis*	*	*Muehlenbeckia complexa*
*	*Catapodium rigidum*	*	*Oenothera glazioviana*
*	*Conyza* sp.	*	*Opuntia vulgaris*
	Coprosma acerosa	*	*Orobanche minor*
	Coprosma repens	*	*Osteospermum fruticosum*
*	*Cortaderia selloana*		*Oxalis rubens*
	Cortaderia splendens		*Ozothamnus leptophylla*
	Cortaderia toetoe	*	*Pennisetum clandestinum*
*	*Cotoneaster glaucophyllus*		*Pimelea arenaria*
*	*Crepis capillaris*	*	*Pinus pinaster*
*	*Crocosmia croscosmiiflora*	*	*Pinus radiata*
*	*Cynodon dactylon*	*	*Polygala myrtifolia*
	Desmoschoenus spiralis	*	*Rumex* spp.
	Dichelachne crinita	*	*Senecio elegans*
	Dodonaea viscosa	*	*Senecio glastifolius*
*	*Erigeron karvinskianus*	*	*Senecio jacobaea*
	Ficinia nodosa	*	*Solanum linneanum*
*	*Foeniculum vulgare*	*	*Sonchus oleraceus*
*	*Galium aparine*		*Spinifex sericeus*
*	*Gazania linearis*		*Tetragonia tetragonioides*
*	*Holcus lanatus*	*	*Ulex europaeus*
*	*Hypochoeris radicata*		*Zoysia minima*
	Lachnagrostis billardierei		
*	*Lagurus ovatus*		

6.5   Discussion

Pimelea arenaria is one of a small suite of native shrubs that occur on the relatively unconsolidated sand of dunes and dune hollows. It is gynodioecious, and produces large numbers of flowers on both female and hermaphroditic plants over a relatively long flowering season.

Lloyd (1976) describes gynodioecy as a variable breeding system exhibiting a wide range of conditions from 'equisexual', when females are rare and males have considerable ovule contribution, to the other extreme where females are common and males have low ovule contributions. Female frequency in the 18 populations of *Pimelea arenaria* of this study ranged from 0 to 45%, showing large variation within this species between geographically separated populations. Variation in gender distribution is not unusual in gynodioecious species; for example, Lloyd (1976) reported female frequencies ranging from 15 to 51% in 12 taxa, and Burrows (1960) reported female frequencies ranging from 37 to 49% in four New Zealand *Pimelea* species. All but one of the study populations with gender ratios of less than 25% females had a significant difference in seed production between genders, but in five of the populations, females had lower seed set than hermaphrodites. In the seven populations with female frequency greater than 25%, only one population had a significant difference in fertilisation success. Delph and Lloyd (1996) suggested that inbreeding depression and significantly higher seed production in females were contributing factors in maintaining high female frequency in populations of the gynodioecious *Hebe subalpina*; inbreeding depression was caused by selfing in the hermaphrodites (Delph & Lloyd 1996). Although female plants are typically at low frequencies in natural populations of gynodioecious taxa, gender ratios sometimes approach 1:1 when the seed fecundity of hermaphrodite plants is so low that the population is essentially dioecious (Webb 1999). Further investigations would be required to determine whether *Pimelea arenaria* was experiencing inbreeding depression.

Interesting differences in the number of flowers per cluster between the northern North Island and southern North Island populations of this study shows a clinal variation that could suggest some form of selective gradient – larger flowers per inflorescence associated with reduced hermaphrodite fertilisation success in the northern populations. This suggests a more outcrossing strategy compared with the southern populations where there are fewer flowers per inflorescence, females are rarer, and hermaphrodite fertilisation success is higher, presumably through autogamy. In a meta-analysis of reproductive traits in gynodioecious species, Shykoff et al. (2003) found that females produced more flowers, set more fruits, and produced more seeds that were larger and germinated better than those of hermaphrodites of the same populations, giving females an advantage. Primack and Lloyd (1980) studied the proportion of male and

hermaphrodite flowers on individuals of the andromonoecious species manuka (*Leptospermum scoparium*), and found the percentage of hermaphrodite flowers increased with increased nutrients. Examination of these processes was beyond the scope of this study and would involve further investigation, but because of the variability of gender ratios, this species would be an interesting inclusion in research into evolutionary processes of gender dimorphism.

Only occasional mature fruit were seen during the surveys for this study, despite relatively high reproductive success in most populations, suggesting active fruit removal by frugivorous or predatory animals. At Spirits Bay, however, several clusters of fruit were present on one plant, but they were close to an active paper wasp nest (*Polistes* sp.), which had apparently deterred frugivory. Birds are likely fruit-eaters and seed dispersers of *P. arenaria* and the following taxa were seen in dunes during the surveys: sparrows (*Prunella modularis* or *Passer domesticus*), pipit (*Anthus novaeseelandiae*), skylark (*Alauda arvensis*), blackbird (*Turdus merula*), and thrush (*Turdus philomelos*). Lizards may be dispersers of fruit (Whitaker 1987; Wotton 2002), but were not observed during this study. The insects most commonly seen working *Pimelea arenaria* flowers, and presumed to be pollinators, were copper butterfly (*Lycaena* sp.), common blue butterfly (*Zizina labradus*) (Fig. 6.12), and the honey bee (*Apis mellifera*). Dawson et al. (2005) reported fly visitation.

Despite high fertilisation success in all the populations surveyed, poor recruitment was evident at most sites. In larger populations the lack of recruitment is not of immediate concern, although long-term monitoring of population dynamics may help resolve unanswered questions about recruitment. Large numbers of seeds are apparently produced, e.g., a plant with a diameter of 1 m could have approximately 2000 terminal stems, and if a minimum of four flowers per cluster reproduced at 50% fruit set, approximately 4000 seeds per plant per season could be produced. Seed viability does not appear to be a factor influencing recruitment failure as Dawson et al. (2005) found *P. arenaria* seed germinated readily in controlled conditions. It is possible that specific environmental conditions promote seed germination. For example, heavy rain over several hours or days may cause ponding in dune hollows and depressions on foredunes, which may promote seed germination. There is the possibility that seeds and/or fruit are ingested by mice or rats, or that germination is occurring but seedlings are being

browsed. While no sign of browsing was noted on adult plants, seedlings may be palatable, especially to rabbits (*Oryctolagus cuniculuc cuniculus*) or hares (*Lepus europaeus occidentalis*). Rabbits and hares favour dunelands and eat a wide range of plants and seeds (King 1990). In addition, the shells of the common brown snail (*Cantareus asperus*) were noted on the many of the dune systems, and may browse *Pimelea* seedlings. Most seedlings have not developed methods of deterring browsers and are susceptible to browse compared with adult plants (G. Barker *pers. comm.*). Further study is required to determine the specific causes of poor recruitment.

Fig. 6.12 Copper butterfly (left) and common blue butterfly (right) working *Pimelea arenaria* flowers.

The vegetation of sand dunes follows a sequence from a small number of native, early successional species which colonise unconsolidated sand of the foredune, e.g., *Spinifex sericeus*, *Desmoschoenus spiralis*, *Calystegia soldanella*, and *Oxalis rubens.*, to more abundance and diversity of the later successional species on the more consolidated sand of backdunes, e.g., *Muehlenbeckia australis*, *Coprosma acerosa*, *Ficinia nodosa*, and *Ozothamnus leptophylla*.

Based on observation of study sites with the least anthropogenic development (e.g., Spirits Bay), the common native sand binding plants of foredunes, e.g., spinifex and pingao, form open, spreading plant communities that are constantly changing in response to alteration in dune profile caused by periodic sand deposition and erosion. Native sand-binding species, including *P. arenaria*, require 'active' dune systems with wind-blown sand serving as a form of habitat restoration. The growth of indigenous sand dune species such as pingao (*Desmoschoenus spiralis*) and spinifex (*Spinifex*

sericeous) is actually stimulated by sand deposition (Hesp 2000), and it seems likely this also applies to *P. arenaria*. Coastal sands are extremely nutrient-poor and contain only small amounts of essential mineral salts for plant nutrition, particularly nitrates and phosphates; however, the presence of nitrogen-fixing bacteria may supplement the nitrogen economy of the dune system (Rieley & Page 1990). There is also evidence suggesting some dune plants obtain phosphorus by the cleavage of inorganic phosphorus directly from organic substrates through the exudation of phosphatase in the vicinity of specialised lateral roots, which may promote plant growth in dune substrates that are phosphorus-deficient (Rieley & Page 1990). The loss of neighbouring native forests with an associated loss of organic material from leaf-fall may have had an effect on the nutrient balance of sand dune ecosystems, but further investigations would be required to establish if there is any link between recruitment failure in *P. arenaria* and substrate nutrients.

The areas of bare sand amongst spinifex and pingao provide habitat for colonisation by the most common native foredune species such as *Calystegia soldanella*, *Lachnagrostis billardierei*, *Oxalis rubens*, *Ozothamnus leptophylla*, and *Pimelea arenaria* (Bergin & Herbert 1998; Bergin 1999, 2000). With an increasing interest in dune protection and restoration, sand binding species, mainly pingao, have been planted at many sites where they once occurred, restoring locally extinct populations (Bergin & Herbert 1998; Bergin 1999, 2000; Milne & Sawyer 2002). While marram planting has been used extensively for dune stabilisation, it forms dense swards that displace and exclude all other species. Many dune systems are dominated by marram and, because of these dense swards, are no longer considered 'active' dune systems (Hilton 2003).

Naturalised exotic plant species dominate on most sand dunes (Johnson 1993) and all the dunelands surveyed in this study had a component of exotic flora, outnumbering native taxa at many of the sites. The impact of exotic species is variable, and depends on growth habit, abundance and density. Control of some of these, particularly wilding pines, could be important for future maintenance of *P. arenaria* populations. Dense ground-covering species such as *Carpobrotus edulis* and *Gazania linearis* are also likely to reduce potential habitat for seed germination and colonisation by native species. Monitoring of such interactions is required to determine the precise effects of exotic species presence and abundance on the persistence of populations of native taxa.

Although some *P. arenaria* populations have become extinct, it still persists at many sites in the North Island, and some populations are quite large. The most likely cause of the local extinctions appears to be habitat loss, especially where development such as roads and housing have occurred close to the foredune, particularly at beaches with narrow dune systems.

There is a high level of interest in the importance of dune ecosystems, and restoration planting of species such as pingao and spinifex has been conducted on a number of sand dunes throughout New Zealand (Bergin & Herbert 1998; Bergin 1999, 2000; Steward 2003). *P. arenaria* is a suitable candidate for restoration planting but widespread lack of recruitment in natural populations requires further investigation to determine the causes.

6.6   Acknowledgements

6.7   References

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Chapter 7

The breeding system of *Raukaua anomalus*, a small-leaved shrub from New Zealand[1]

7.1 Abstract

The breeding system of *Raukaua anomalus* was thought to be dioecious or monoecious. Evidence from nine geographically separated populations in the Waikato, the central North Island, and the Wairarapa regions shows this species to be hermaphroditic. The flowers are tiny, protandrous, and occur in clusters on short peduncles at the leaf axils. Flowering occurs sequentially, and each cluster may comprise buds, flowers, and fruit at the same time. *R. anomalus* is self-compatible, but within-flower dichogamy largely prevents autonomous selfing. The smallness of the flowers and the complete abscission of the anthers, filaments, and petals after the male phase are probably the reasons for the previous uncertainty about its breeding system.

Keywords Araliaceae; *Raukaua anomalus*; breeding system; hermaphrodite; dioecy; monoecy; protandry

7.2 Introduction

Raukaua anomalus (Araliaceae) is an endemic shrub; in a recent taxonomic review, it was one of three *Pseudopanax* species included in a reinstatement of the genus *Raukaua*, resulting in a new combination, *R. anomalus* (Mitchell et al. 1997). It was previously known as *Panax anomalum* (Hooker WJ 1843; Hooker JD 1867; Kirk 1899; Cheeseman 1906), *Nothopanax anomalum* (Cheeseman 1925), *Neopanax anomalum* (Allan 1961; Laing & Blackwell 1964), and *Pseudopanax anomalus* (Moore & Irwin 1978; Poole & Adams 1990; Smith-Dodsworth 1991; Wilson & Galloway 1993).

Raukaua anomalus is a small-leaved divaricating shrub, sensu Kelly (1994), which grows to approximately 3 m tall (Mitchell et al. 1997), the only small-leaved shrub in the family in New Zealand (Wilson & Galloway 1993). It is distributed throughout the three main New Zealand islands from 35°S southwards, occurring in forest margins and lowland forest, scrub, and shrubland (Allan 1961; Wilson & Galloway 1993; Mitchell et al. 1997).

The specific breeding system of *Raukaua anomalus* was not described by Hooker (1867), Cheeseman (1906, 1925), Allan (1961), Laing and Blackwell (1964), Poole and Adams (1990), Smith-Dodsworth (1991), or Wilson and Galloway (1993), although the breeding system of the various genera to which this species has been assigned was described as polygamous (Hooker 1867), unisexual or polygamous (Kirk 1899), monoecious or dioecious (Allan 1961; Smith-Dodsworth 1991), and dioecious (Poole & Adams 1990). Mitchell et al. (1997) described the *Raukaua* genus as having an "undetermined sexuality, though possibly hermaphroditic or andromonoecious". Kirk (1899) specifically described male and female flowers of *Panax anomalum*, and Eagle (1975) illustrated separate male and female flowers, suggesting dioecy or monoecy. In a review of gender dimorphism in indigenous New Zealand seed plants, Webb et al. (1999) classified the genus *Pseudopanax* as dioecious, but *Raukaua* was not listed.

Raukaua anomalus is one of several native shrub species being investigated in relation to breeding strategies and reproductive success, and it was during field-based pollination experiments, followed by a review of the literature, that it became evident there was some confusion about the breeding system of this species.

This study describes the breeding system of *Raukaua anomalus* from nine geographically separated populations in the North Island.

7.3 Materials and methods

Flowers and flower buds were collected from nine study sites in the North Island (Fig. 7.1; Table 7.1). The numbers of plants used in three of the study populations were very small (< 10) but comprised the entire population: Gordon Gow Scenic Reserve

(Waharoa), Mangakotukutuku gully (Hamilton), and Wairere bush (Gordonton). The last site, a small kahikatea remnant, had a single adult plant.

Under a dissecting microscope, at least five buds from each plant were dissected, anthers were examined for the presence of pollen, and the number of ovules counted. To determine the reproductive strategy, pollination treatments were carried out on tagged plants in two populations, Gordon Gow Scenic Reserve and Mamaku Plateau, and treated as follows: 1. Controls (natural): natural pollinators allowed, stems in bud were tagged and buds counted. 2. Manual supplementary cross-pollination (crossed): during anthesis pollen from different plants was collected on a small artist's paintbrush and applied to stigmas. 3. Manual self-pollination (selfed): flower buds were bagged and, during anthesis, pollen was applied directly onto stigmas with pollen collected from the same plant, and flowers rebagged. 4. Pollinators excluded (bagged): flower buds were counted then stems were enclosed in fine, mesh, fabric bags, tied with coloured twist ties, and left bagged with no further treatment. For the purposes of this study, buds were not bagged individually, precluding differentiating between passive geitonogamous and autonomous pollen transfer.

The percent fruit set for individual plants, and their means and standard errors were calculated for each treatment. Box plots were generated from individual plant fruit set for each treatment. The box plots depict the median (central line in each box), 25% and 75% quartiles (upper and lower limits of each box), 90th and 10th percentiles (indicated by the whiskers), and outliers (indicated by open circles). To compare fruit set between pollination treatments, the number of fruit for the number of buds on each plant from each site were analysed using a Generalised Linear Model with binomial error distribution and logit link function, and F-test significance tests, with a separate analysis for each site.
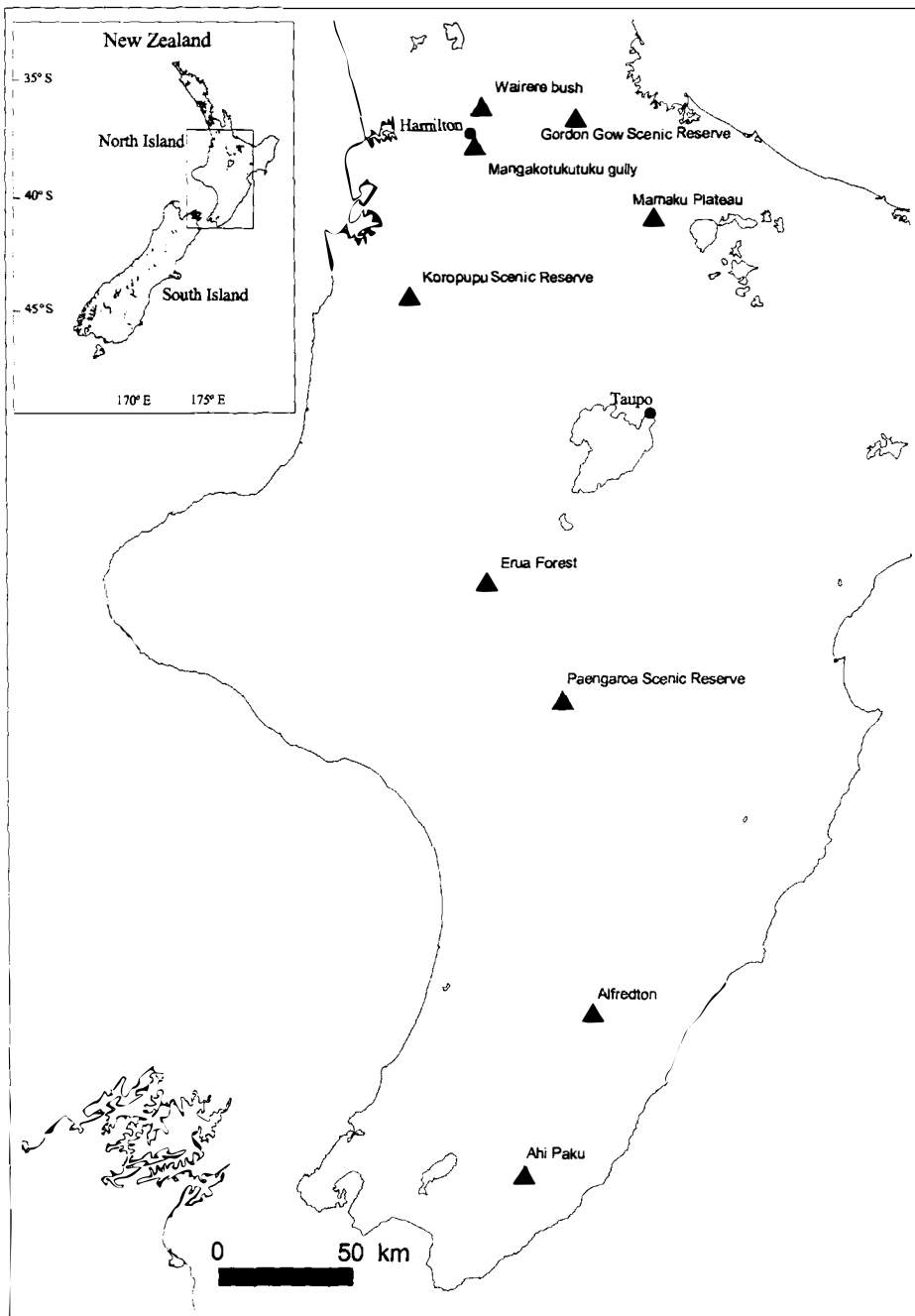
Fig. 7.1 Location of *Raukaua anomalus* study sites (▲) in the North Island.

Table 7.1 Location, collection date, number of *Raukaua anomalus* plants, and dissected buds from nine study sites in the North Island.

Study site	NZMS 260grid reference	Flower collection date	Number of plants	Number of buds
Ahi Paku	S27/291947	15 Jan 04	20	51
Alfredton	T25/545543	14 Jan 04	19	44
Gordon Gow Scenic Reserve	T14/497841	15 Jan 02	7	35
Koropupu Scenic Reserve	R16/891177	23 Feb 03	22	110
Mamaku Plateau	U15/783472	24 Jan 02	10	50
Mangakotukutuku gully	S14/123737	9 Jan 03	3	30
Erua Forest	S19/174269	18 Feb 03	15	90
Paengaroa Scenic Reserve	T21/437647	18 Feb 03	10	225
Wairere bush	S14/148880	15 Jan 02	1	10

7.4 Results

Raukaua anomalus flowers are tiny (2–3 mm dia.) and occur in clusters on short peduncles at the leaf axils. Flowering occurs sequentially, and each cluster may comprise buds, flowers, and fruit at the same time. The flowers are hermaphroditic, dichogamous (temporal separation in presentation of pollen and stigmas), and protandrous (anthers mature before stigmas become receptive). During the male stage, which lasts 36–48 hours, the five anthers are exserted; after anthesis, the anthers, filaments, and petals completely abscise (Fig. 7.2). The style forms what appears to be a central column during the male phase, but comprises two style branches closely abutted. After abscission of the male organs and the petals, the upper surface of the receptacle becomes shiny, possibly indicating stigma receptivity, and the two style branches elongate and separate to form a V (Fig. 7.2). The stigmatic surface is a tiny flattened area on the inner side of the tip of each style branch. Sepals and styles persist on the mature fruit. The number of ovules per ovary is consistently two, and there was no variation in breeding system among the populations studied.

Fig. 7.2 Cluster of *Raukaua anomalus* flowers from Gordon Gow Scenic Reserve, January 2003. **A,** male phase; **B,** female phase.
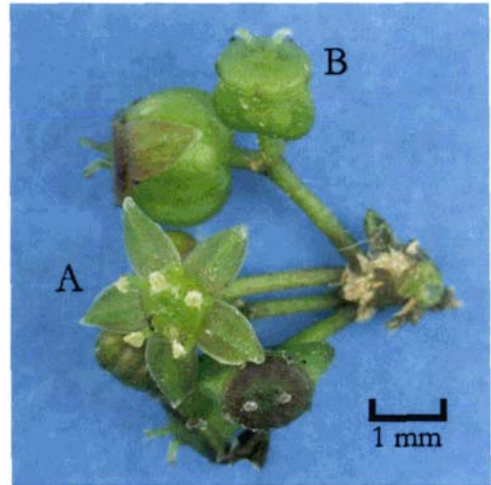
The results of pollination treatments show mean natural fruit set of 39.6% at Gordon Gow Scenic Reserve and 17.2% from the Mamaku Plateau site. There was, however, considerable within-population variation at both sites, ranging from 0 to 80% (Fig. 7.3). The mean fruit set of crossed flowers was higher than natural at both sites, although analysis showed this to be significant only at the Mamaku site, and indicates pollen limitation (Fig. 7.3; Table 7.2). Mean fruit set from the selfed treatment was 26.7% at Gordon Gow Scenic Reserve and 30.3% at Mamaku Plateau, showing self-compatibility, but poor fruit set (14% and 9.4% respectively) in the bagged treatments suggests that this species is largely pollinator dependent.

Table 7.2 Significance tests of pollination treatments on fruit set of *Raukaua anomalus* at two sites from binomial GLMs. Significance: *, $P < 0.05$; ***, $P < 0.001$. NS, not significant.

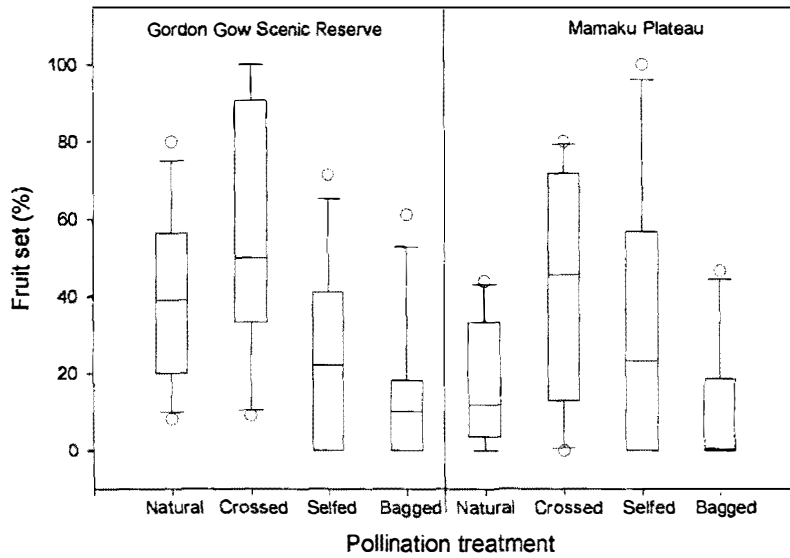| Pollination treatments	Gordon Gow Scenic Reserve	Mamaku Plateau
Natural versus crossed	NS	*
Natural versus selfed	NS	NS
Natural versus bagged	***	NS
Selfed versus crossed	NS	NS

Fig. 7.3 Distribution of fruit set in four pollination treatments of *Raukaua anomalus* from two study sites.

7.5   Discussion

Raukaua anomalus produces clusters of tiny, hermaphroditic, dichogamous flowers in leaf axils. In dichogamy, self-pollination is prevented if pollen is shed at a time when the stigma is not receptive (Faegri & van der Pijl 1979), and when there is no overlap in the presentation of the pollen and stigma, dichogamy is considered complete (Lloyd & Webb 1986). In the case of *R. anomalus*, the benefits of dichogamy appear to be counteracted by the close proximity of flowers at both gender stages, increasing the likelihood of geitonogamous fertilisation.

In protandry, the anthers typically mature one to a few days before stigmas are receptive (Proctor et al. 1996), and although there is a variety of means of disposing of the used stamens or anthers, the commonest method is abscission at the base of the filament (Lloyd & Webb 1986). After anthesis, complete abscission of male organs and petals occurs in *R. anomalus*, leaving only the female organs and the sepals, which both persist on mature fruits. Lloyd and Yates (1982) suggested protandry and numerous other features of cross-pollinated flowers function as an outcrossing mechanism that decreases the frequency of selfing and also as a means of avoiding interference between paternal and maternal functions. In the most comprehensive study of protandry in New

Zealand's hermaphroditic plants, Thomson (1881) noted it in 37% of the 235 species he investigated.

Protandry is more common among insect-pollinated plants, is characteristic of flowers with more advanced evolutionary features, and is also common among plants with a physiological self-incompatibility system (Proctor et al. 1996). Successful fruit set in hand-pollinated and bagged flowers suggests *R. anomalus* does not have any physiological barrier to prevent self-fertilisation but depends on separation of male and female stages to reduce autonomous selfing.

Hermaphrodism has not previously been reported in the five New Zealand genera in the family Araliaceae. Their breeding systems have variously been described as polygamo-dioecious (mainly dioecious, but some plants bear a few flowers of the opposite sex, or sometimes a few perfect flowers) (*Stilbocarpa*), dioecious or polygamo-dioecious (*Meryta*), dioecious or polygamous (unisexual and bisexual flowers on the same plant) (*Schefflera*), dioecious or monoecious (*Neopanax*, now *Pseudopanax*), and dioecious (*Pseudopanax*) (Allan 1961).

Raukaua anomalus produces hermaphroditic flowers in populations at various sites in the North Island, and it is likely this species has the same breeding system throughout its range.

7.6   Acknowledgements

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Chapter 8

Gynodioecy in *Teucridium parvifolium* (Verbenaceae), a threatened, small-leaved shrub from New Zealand[2]

8.1   Abstract

Flowers of *Teucridium parvifolium* were examined *in situ* to investigate its breeding system, which was previously thought to be hermaphroditic. Gynodioecy was found in two of the six North Island populations investigated. There was a significant difference in the size of flowers from female and hermaphrodite plants at each site, but no difference in flower size within each gender among the sites. Female flowers have vestigial, non-functional anthers on short filaments positioned near the base of the corolla.

Keywords   *Teucridium parvifolium*; Verbenaceae; gynodioecy; hermaphrodite; New Zealand; small-leaved; endemic

8.2   Introduction

Gynodioecy is a form of gender dimorphism involving the production of female (pistillate) and hermaphrodite (pistillate and staminate) flowers on separate plants in the same population (Sakai & Weller 1999). The two most common forms of gender dimorphism in New Zealand's indigenous seed genera are dioecy (17.9%) and gynodioecy (7.2%) (Webb et al. 1999). Gynodioecy is regarded as an intermediate evolutionary step from hermaphrodism to dioecy, involving male-sterile individuals arising within a population of hermaphrodites (Delph & Lloyd 1996; Webb 1999). The evolutionary pathway from hermaphrodism to gynodioecy and often on to dioecy is the most common, and best documented, transition to gender dimorphism, although in many genera, gynodioecy appears to be a persistent breeding system in its own right (Webb 1999). Gender ratios in gender dimorphic species can vary between and among

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[2] Published as Merrett MF 2005. Gynodioecy in *Teucridium parvifolium* (Verbenaceae), a threatened, small-leaved shrub from New Zealand *New Zealand Journal of Botany* 45. 613–617.

populations and have been reported in the New Zealand flora (Foweraker 1932; Godley 1955, 1963, 1964; Burrows 1960; Webb 1981; Connor 1984). In dioecious species, female flowers are often smaller than their hermaphroditic counterparts (Proctor et al. 1996; Delph 1996).

Teucridium parvifolium (Verbenaceae) is an attractive, small-leaved endemic shrub that occurs in coastal to lowland forests and shrubland with populations described as "local" in both the North and South Islands (Allan 1961). Flower descriptions (Cheeseman 1925; Allan 1961; Wilson & Galloway 1993) and illustrations (Eagle 1975; Smith-Dodsworth 1991) suggest the breeding system of *T. parvifolium* to be hermaphroditic, and it was not listed among gender dimorphic taxa by Webb et al. (1999).

During the flowering season of 2002–03, flowers on a plant at Paengaroa Scenic Reserve were seen to lack the typical, exserted, well-developed anthers of hermaphroditic flowers. This suggested the possibility of this species having a gynodioecious breeding system.

This study describes an investigation into the breeding system and floral morphology of *Teucridium parvifolium* in six populations in the North Island.

8.3   Materials and methods

Teucridium parvifolium is one of only two New Zealand species in the family Verbenaceae (Allan 1961). It is a much-branched, soft-wooded species that grows to approximately 1.5 m tall, and produces attractive, white, zygomorphic flowers in leaf axils from November to January (Allan 1961; Wilson & Galloway 1993; Poole & Adams 1994). The fruit is dry and comprises four nutlets surrounded by a persistent calyx (Allan 1961; Webb & Simpson 2001). The species is listed in the Gradual Decline category of the most recent lists of New Zealand's threatened and uncommon plants (de Lange et al. 2004).

Teucridium parvifolium populations were visited at known locations in the North Island, and extensively searched for flowering individuals. In total, 242 flowering plants were examined *in situ* at six study sites during the flowering season of December and January

2003–04 (Table 8.1). Up to five flowers or flower buds were collected from each putative female plant and examined under a dissecting microscope for the presence of pollen and ovules.

From sites with both female and hermaphroditic plants, flower dimensions (outer corolla length and width) of fully open flowers were measured from a minimum of two flowers per gender per plant, and their means and standard errors were calculated for each gender from each study site. Box plots were generated from individual flower dimensions. The box plots depict the median (central line in each box), 25% and 75% quartiles (upper and lower limits of each box), 90th and 10th percentiles (indicated by the whiskers), and outliers, (indicated by open circles). *T*-tests were used to compare flower size between genders within populations, and within genders between populations.

Table 8.1 Location of study sites, the number of plants examined, and the female proportion of *Teucridium parvifolium* from six populations in the North Island.

| Study site	Ecological District	NZMS 260 grid reference	Number of plants and female proportion (%)
Alfredton Domain	Eastern Wairarapa	T25/513540	34 (0)
Carter Scenic Reserve	Wairarapa Plains	S26/255126	15 (0)
Koropupu Scenic Reserve	Waitomo	R16/891177	54 (0)
Paengaroa Scenic Reserve	Rangitikei	T21/438696	37 (18.9)
Vallance property	Eastern Wairarapa	T26/524230	34 (0)
Wallace Bush	Eastern Wairarapa	T25/515556	68 (10.3)

8.4   Results

Female plants, producing only pistillate flowers, were present in two of the six study populations, Paengaroa Scenic Reserve (18.9% female), and a forest remnant on the Wallace property (Wallace Bush) in the Wairarapa region (10.3% female) (Table 8.1). Specimens with pistillate flowers have been lodged in herbaria AK and CHR.

Perfect flowers on hermaphroditic *T. parvifolium* plants have a single style that forks near the apex, and four filaments with white, wheel-shaped anthers (Fig. 8.1). The exserted stamens are approximately the same length or slightly longer than the style. At anthesis, the anther splits around its circumference to release sticky pollen. In contrast, pistillate flowers have vestigial, brown, non-functional anthers on short filaments positioned near the base of the style (Fig. 8.1). Four ovules were consistently present in all examined ovaries from female and hermaphroditic flowers. An interesting feature was the presence of "pearl-like" spheres attached to the outside of both sides of anthers on both types of flowers (Fig. 8.2), which were also present on anthers before anthesis and after flower senescence. These "pearls" were also scattered among glandular hairs on the undersides of leaves, petioles, and young stems; on the outer surface of flower petals and sepals; and on the upper surface of the immature fruit. They were translucent or opaque, ruptured easily, and contained an oily-looking substance. The function and derivation of these are unknown. They may be a type of trichome, outgrowths of the epidermis such as hairs, scales, or water vesicles. Trichomes have a variety of functions including water and mineral absorption, salt secretion, and insect defence (Raven et al. 1992).

At both study sites, the width and length of flowers from female plants were significantly smaller than flowers from hermaphroditic plants (Fig. 8.3; Table 8.2). However, the two sites did not differ significantly when either gender were compared.

Fig. 8.1 Perfect flowers from hermaphrodite (H), and pistillate flowers from female (F) plants of *Teucridium parvifolium* from Paengaroa Scenic Reserve. A vestigial anther is slightly visible at the base of the corolla of the female flower (arrowed).
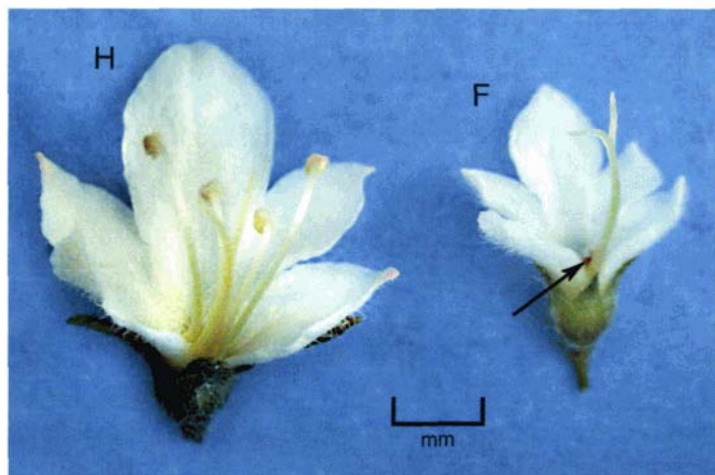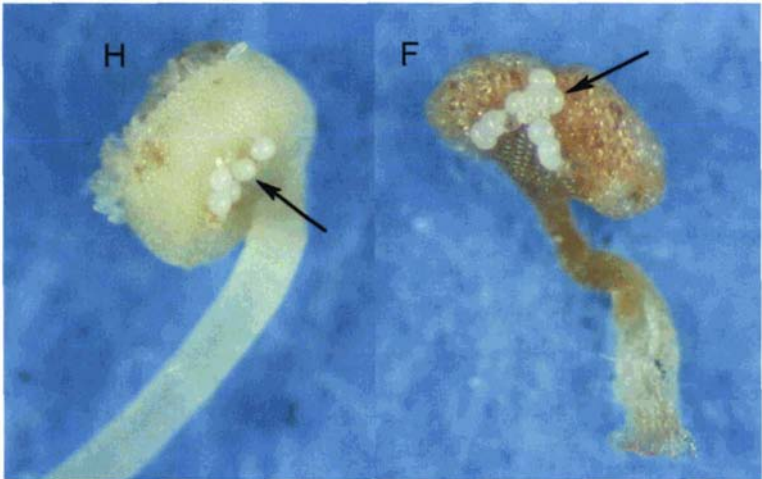
Fig. 8.2 Anthers from perfect flowers from hermaphrodite (H), and pistillate flowers from female (F) plants of *Teucridium parvifolium* showing "pearl-like" spheres (arrowed) attached to the outer surface.

Table 8.2 The results of significance tests to compare flower dimensions (length and width) of hermaphrodite (perfect) and female (pistillate) flowers from two populations of *Teucridium parvifolium*. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant.

Flower dimensions (mm)	Hermaphrodite versus female		Paengaroa Scenic Reserve versus Wallace Bush	
	Paengaroa Scenic Reserve	Wallace Bush	Female	Hermaphrodite
Length	***	**	NS	NS
Width	**	*	NS	NS
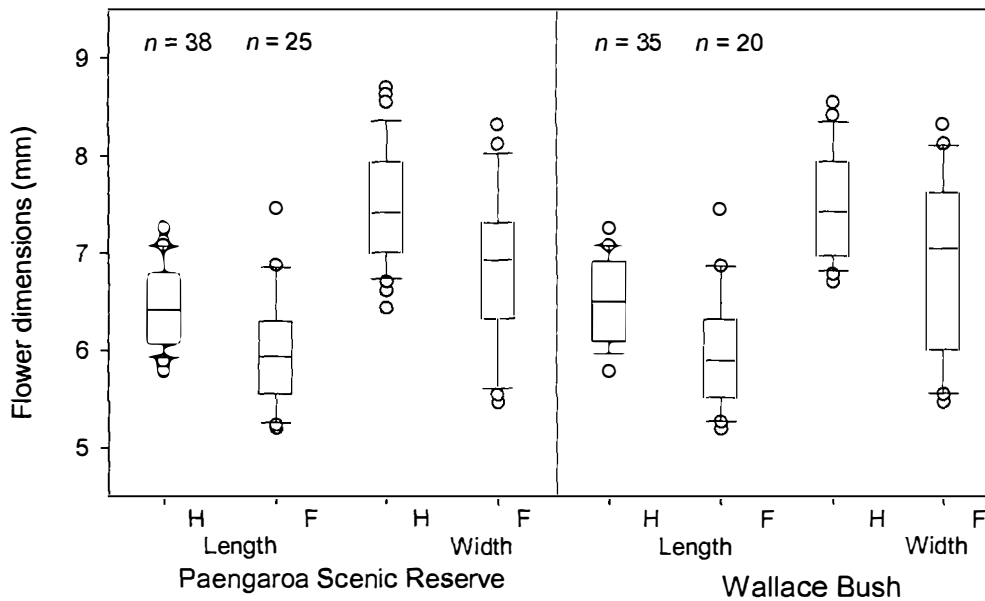
Fig. 8.3 Number of flowers and distribution of floral dimensions (length and width) of hermaphrodite (H) and female (F) flowers of *Teucridium parvifolium* from Paengaroa Scenic Reserve and Wallace Bush.

8.5   Discussion

Two of the six study sites examined contained more than 10% female plants, indicating that *Teucridium parvifolium* has a gynodioecious breeding system in at least some of its populations.

The difference in flower size between pistillate and perfect *T. parvifolium* flowers is not an unusual phenomenon in gynodioecious species. In a review of sexual dimorphism in perianth size, Eckhart (1999) found that perfect flowers were almost always larger than female flowers. In New Zealand, this feature has been reported in the genera *Fuchsia* (Godley 1955), *Pimelea* (Burrows 1960), *Gingidia* (Webb 1981), and several but not all of the gynodioecious species of *Hebe* (Delph 1996).

The family Verbenaceae comprises approximately 75 genera with over 3000 species, distributed mostly in tropical and subtropical regions (Heywood 1978). Within the family Verbenaceae, gynodioecy has been reported in *Rhaphithamnus venustus* from the Juan Fernández Islands (Sun et al. 1996; Anderson et al. 2001; Bernadello et al. 2001), and dioecy in *Citharexylum fruticosum* from South Florida (Tomlinson & Fawcett

1972), in three species of *Callicarpa* from the Bonin Islands (Kawakubo 1998), and in *Aegiphila mollis* and *A. parviflora* from Venezuela (Ramírez 2005). Gender dimorphism has not been reported in *Vitex lucens* (Godley 1971; Barrell et al. 1997), the only other New Zealand species in the family Verbenaceae. An investigation of South Island *T. parvifolium* populations would add to our knowledge of the extent of gynodioecy in this species.

The number of flowering *T. parvifolium* plants was variable among the sites, ranging from 15 to 68 individuals, which may have compromised my ability to find females at some of the sites. For example, the three populations with fewer than 35 plants contained only hermaphrodites. However, two of these study sites were relatively small and the populations were examined in their entirety. It is also worth noting that females were not found in the second-largest population, Koropupu Scenic Reserve (Table 1), suggesting population size is not the only factor influencing the presence of female plants. This finding highlights the need for close examination of flowers in multiple populations before a breeding system can be considered completely characterised for a species, and it is likely that additional gender dimorphic species will be uncovered in the New Zealand flora.

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Chapter 9

Do pollen-ovule ratios reflect breeding systems and successional stages in native shrubs of New Zealand?

9.1 Abstract

An investigation of pollen grain numbers, ovule numbers, and pollen-ovule ratios (P/Os) was undertaken to compare the P/Os of native shrubs of New Zealand with Cruden's (1977) breeding system and successional stage categories, and his (2000) sexual system theories. There was no significant relationship between P/Os and breeding systems, and there was no clear pattern among the species that would suggest P/Os were useful for identifying breeding systems. Also, there was no consistency between these results and Cruden's (1977) successional stage categories. The distinctive features of the New Zealand vascular plant flora could help explain the high P/Os. High numbers of pollen grains, or low numbers of ovules producing high P/Os, could be linked to floral characteristics, plant life form, pollinator size and efficiency, or environmental conditions, and/or could have evolved to compensate for a depauperate, and perhaps inefficient pollinating fauna, regardless of the breeding system or successional stage.

9.2 Introduction

Measuring the ratio of the number of pollen grains to ovules (P/O) in flowers has been proposed as a means to determine the breeding system of a species. In his classic study, Cruden (1977) established categories to demonstrate that pollen-ovule ratios (P/Os) were an indicator of a plant's breeding system. Based on a study of 90 species, Cruden (1977) assigned P/Os into five breeding system categories, from cleistogamy (self-fertilisation) to xenogamy (completely self-incompatible). Autogamous (self-compatible) species were shown to have substantially lower P/Os than xenogamous species (Cruden 1977). For example, cleistogamous species have P/Os of 5 ± 0.7 (mean $\pm$ standard error), whereas xenogamous species have P/Os of 5859 ± 936.5 (mean $\pm$

standard error). According to Cruden (1977) pollen-ovule ratios reflect pollination efficiency; the lower the pollen-ovule ratio, the more efficient the transfer of pollen, and pollination efficacy is a feature of self-compatibility and/or autogamy in species of early successional stages (Cruden 1977). In a follow-up to his earlier work, Cruden (2000) established that the P/Os of wind-pollinated species were generally higher than animal-pollinated taxa with the same sexual system, and in animal-pollinated species, dioecious and gynodioecious taxa had higher P/Os than hermaphrodites (Cruden 2000). An alternative explanation for the correlation between P/Os and breeding system has been proposed by the sex allocation theory, where the relative resources allocated to pollen decreases and that allocated to seeds increases as self-fertilisation increases (Charnov 1982; Queller 1984).

The indigenous flora of New Zealand has many unusual features including a prevalence of small, inconspicuous, structurally simple flowers, fleshy fruit, unspecialised pollinating fauna, gender dimorphism, and a low proportion of annuals concomitant with a high proportion of woody taxa compared with most temperate floras (Webb & Kelly 1993). Because of these differences in plant life history traits, the idea of testing relationships between P/Os of New Zealand shrubs and their breeding systems was considered. Robertson and Lloyd (1991) reported P/Os of six *Myosotis* species, but there have been no previous comparative studies of pollen-ovule ratios amongst a range of genera in the New Zealand shrub flora, nor between Cruden's results and breeding systems of New Zealand shrubs.

This investigation was initiated to compare Cruden's (1977, 2000) conclusions, which were based largely on annual and herbaceous taxa, with the P/Os of some indigenous shrub species. The specific objectives were to assess whether P/Os are a useful for identifying breeding systems and successional stages in the New Zealand flora.

9.3   Methods

A literature review was undertaken for published pollen-ovule ratios of indigenous shrub species. In addition, pollen and ovule numbers of sixteen shrub species, representing a range of reproductive systems were calculated (used in pollination treatments in Chapter 2). To measure pollen and ovule numbers, mature flower buds

were collected from approximately 10 plants from populations of each species, and preserved in 80:20 ethanol:glacial acetic acid. For estimating the number of pollen grains per flower, mature, undehisced anthers of each species from each site were squashed, and pollen grains were suspended in a solution of methylene-blue, 70% ethanol and detergent (Dafni 1992). The number of grains in a known volume of the suspension was counted, using a haemocytometer. This number was multiplied by the dilution factor and then by the number of anthers, following the methods of Dafni (1992) and Cruden (1977). Six pollen counts were made from each anther, and at least 5 buds of each species from each site were examined. For each species the pollen grains/methylene-blue mixture was stored a 1.5 ml centrifuge tube, and initially agitated for 60 seconds using a Vortex machine. The sample was agitated for 30 seconds before each sub-sample was removed to ensure adequate mixing. Pollen counts were repeated until standard errors were less than 10%. Standard errors were established by calculating the square root of the means of each pollen sub-set. The number of ovules per flower were counted by dissecting ovaries under a dissecting microscope. Following Cruden (1977), is was assumed that all ovules would develop into a seed if fertilisation occurred. The pollen-ovule ratios of hermaphroditic species were calculated by dividing the estimated number of pollen grains per flower by the number of ovules per flower. Where samples were taken from more than one population of an individual species, data were combined and means for each species were calculated. For gender dimorphic species, the pollen-ovules ratios were adjusted to compensate for population gender ratios. Each species was classified into the 'breeding systems' (out-crossing and selfing) described by Cruden (1977), sexual system (hermaphroditic, dioecy, gynodioecy), pollination vector (animal, wind). The criteria for assigning breeding and sexual systems were based on the results of pollination treatment experiments (natural, supplementary crossed, manually selfed and bagged) from natural populations of 16 of the species (Chapter 2),and from published data (Norton 1980; Webb 1994).

Successional stage categories, as described by Cruden (1977) were assigned to each species (Table 9.1). Three successional stages were identified based on habitat and life-history characteristics: 1. species of open sites, disturbed soil, with rapid seed germination, and relatively short lived (< 50 years); 2. species of partially open habitats such as forest edges and canopy gaps, with slower seed germination and establishment, and relatively long lived (> 50 years); and 3. long-lived species mostly occurring under

a canopy in a stable habitat with slow population change, and unreliable pollinators (e.g., activity negatively affected by rain, as defined by Cruden 1977).

The mean pollen and ovule counts per flower and standard errors for each category were calculated, and compared with Cruden's categories. For dioecious species, the mean pollen grain number was divided by the mean ovule number from equal numbers of flowers from each gender. For the gynodioecious species, only hermaphroditic flowers were considered for calculation of the P/O. Differences in species means of pollen grains per flower, ovule numbers per ovary and P/Os among the various breeding systems, sexual systems, pollination vectors, and successional stages were analysed using one-way analysis of variance (ANOVA). Traditionally P/Os were performed on hermaphrodite animal-pollinated species only, therefore dioecious and wind-pollinated species were excluded from the ANOVA analyses. One species (*Alseuosmia macrophylla*) was separated into two categories, (a & b) for breeding system analyses because of two different systems among the populations (see Chapters 2 & 3).

9.4   Results

In total, the data from 20 taxa were placed into the various breeding and sexual systems (see Chapter 2). Twelve of the study species are hermaphroditic, seven dioecious, and one gynodioecious (Table 9.1). Of the species with hermaphroditic flowers, one is obligately autogamous, five are facultatively autonomous, two facultatively xenogamous, and five xenogamous, as identified in Chapter 2 (Table 9.1). *Pomaderris hamiltonii* is hermaphroditic and apomictic (Harvey & Braggins 1985), and was classified into the obligate autogamous category for this study. Sixteen of the taxa are animal-pollinated, and four wind-pollinated (Table 9.1, 9.2). Of the four wind-pollinated taxa, one is hermaphroditic and three are dioecious (Table 9.1). In the successional stage classification, five of the species were classified as stage 1, eight as stage 2, and six as successional stage 3 (Table 9.1).

Mean pollen grain numbers per taxon ranged from a low of 3210±207 in the selfing *Teucridium parvifiolium* to 120 370±7488 in the dioecious, wind-pollinated *Coprosma spathulata* (Table 9.1). Mean ovule numbers ranged from 1 in two species, the gynodioecious *Pimelea arenaria* and the dioecious *Macropiper excelsum,* to 13 in

hermaphroditic *Alseuosmia pusilla* (Table 9.1). Mean P/Os ranged from 526±23 in hermaphroditic *Pseudowintera colorata* to 114 475±8831 in *M. excelsum* (Table 9.1).

Among the various breeding systems of the hermaphroditic species, mean pollen grain numbers ranged from 6238 in obligate autogamy to 14 570 for xenogamy (Table 9.2). Ovule numbers ranged from 3 in obligate autogamy to 11.5 in xenogamous species. Mean P/Os ranged from 1610±331 in xenogamy to 3630±345 in facultative xenogamy (Table 9.2). The distribution of log P/Os of the various breeding systems, pollination/sexual syndromes, and successional stages are illustrated in Fig. 9.1.

In the pollination syndrome/sexual system groups, hermaphroditic and dioecious animal-pollinated species had lower numbers of pollen grains, higher numbers of ovules, and lower P/Os than wind-pollinated hermaphrodites (Table 9.2). Means of pollen grain numbers, ovule numbers and P/Os of hermaphroditic animal-pollinated species were higher than those of the gynodioecious species (Table 9.2). In both hermaphroditic and dioecious species, the mean number of pollen grains, ovule numbers and P/Os were higher in wind-pollinated taxa than in animal-pollinated species (Table 9.2). ANOVA results show a significant relationship between the mean number of ovules per ovary and successional stage ($P < 0.001$), but no relationships between means of pollen grain numbers, ovules numbers, with breeding system, pollination syndrome, or successional stage (Table 9.3).

Table 9.1 Means and standard errors of pollen grain and ovule number per flower, pollen-ovule ratios (P/O), breeding system, sexual system, pollination vector and successional stage of 20 native shrub taxa. [1] data from Webb (1994); [2] data from Norton (1980). OA, obligate autogamy; FA facultative autogamy; FX facultative xenogamy; X, xenogamy; H, hermaphrodite; D, dioecious; G, gynodioecious; A, animal; W, wind. * SE not available.

Taxon	Number of flowers	Mean pollen grain number	Mean number of ovules	Mean P/O±SE	Breeding system	Sexual/ pollination system	Success-ional stage
A. macrophylla (a)	10	22 083	10.6	2096±128	X	HA	3
A. macrophylla (b)	12	22 168	10.8	2063±111	FX	HA	3
A. pusilla	15	10 175	13.1	810±63	FA	HA	3
A. quercifolia	23	13 021	13	1171±88	X	HA	3
A. turneri	18	12 977	9.1	1448±88	FA	HA	3
Coprosma spathulata	5	120 370	2	60 185±3744	D	DW	2
Coriaria arborea	10	68 519	5	13 704±1016	FA	HW	1
Corokia cotoneaster[1]	8	16 698	1.2*	16 698±*	X	HA	2
Cyathodes juniperina	6	12 114	5	2441±238	FX	DA	1
Leucopogon fasciculatus	15	10 710	2.3	4866±399	FX	DA	1
Macropiper excelsum	6	114 475	1.0	114 475±8831	D	DW	1
Melicope simplex	14	42 249	8.0	5281±466	D	DA	2
Melicytus micranthus	9	17 685	3.0	6054±593	D	DA	2
M. novae-zelandiae	10	100 370	2.0	50 185±2765	D	DW	2
Pimelea arenaria	5	8444	1	8444±1106	G/FA	GA	2
Pomaderris hamiltonii.	8	6238	3	2079±204	OA	HA	1
Pseudowintera axillaris[2]	5	4451	7.2	645±94	X	HA	3
Pseudowintera colorata[2]	4	4468	8.5	526±23	X	HA	3
Raukaua anomalus	14	9947	2	4974±341	FX	HA	2
Teucridium parvifolium	6	3210	4	803±52	FA	H/GA	2

Table 9.2 Means of numbers of pollen grain and ovule numbers per flower, and P/Os and standard errors for differing breeding and sexual systems, pollination vector, and successional stage of native shrub species of New Zealand. HO, hermaphroditic species only.

|	Number of species	Mean number of pollen grains	Mean ovule number	Mean P/O±SE
Breeding system (HO)				
Obligate autogamy	1	6238	3	2079±204
Facultative autogamy	4	9998	8	2601±482
Facultative xenogamy	4	15 588	6	3630±345
Xenogamy	5	14 570	11.5	1610±331
Sexual system				
Hermaphrodite				
Animal pollinated	10	12 893	9.1	1964±171
Wind pollinated	1	68 519	5	13 704±1016
Dioecious				
Animal pollinated	4	22 363	4.6	4910±282
Wind pollinated	3	109 162	1.7	70 935±6825
Gynodioecious				
Animal pollinated	1	8444	1	8444±1106
Successional stage (HO)				
1 (early)	1	6238	3	2079±204
2 (early-mid)	3	8337	2	5552±667
3 (mid)	6	14 517	11.3	1353±68
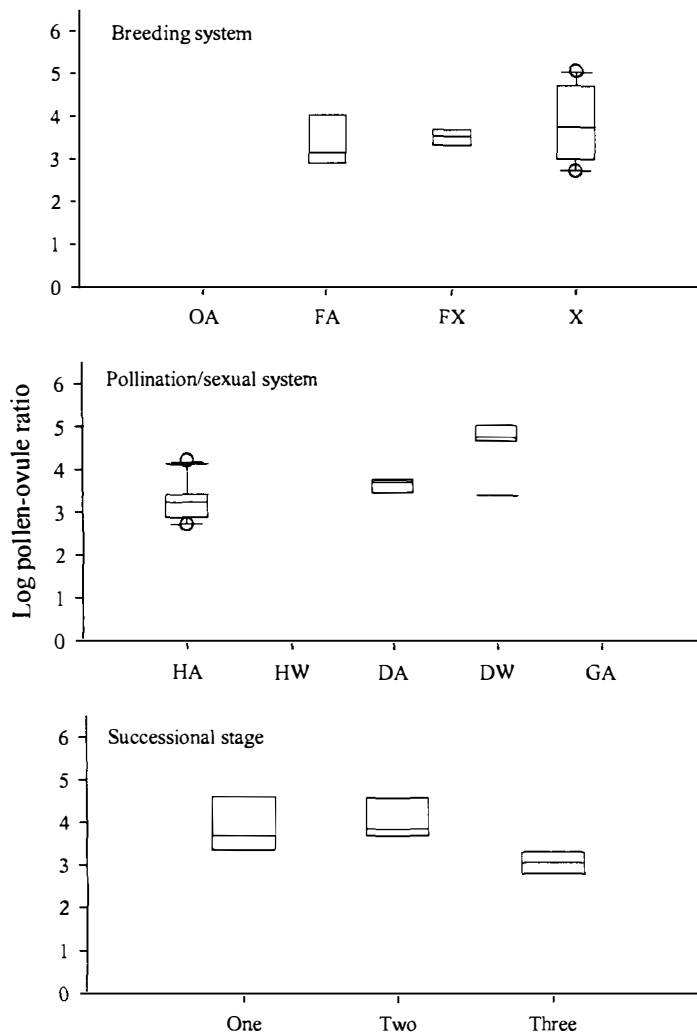
Fig. 9.1 Distribution of pollen-ovule ratios of breeding systems, sexual systems and pollination vector, and successional stage. OA, obligate autogamy; FA facultative autogamy; FX facultative xenogamy; X, xenogamy; DA, dioecy/animal; DW, dioecy/wind; HA, hermaphrodite/animal; HW, hermaphrodite/wind; GA, gynodioecy/animal.

Table 9.3 Significance results of ANOVA for testing differences in pollen grain number per flower, ovule number per ovary, and P/Os, between breeding systems, pollination syndromes, and successional stages of 20 shrub taxa. Wind pollinated and dioecious taxa were excluded from the breeding system analyses.

Variable	Pollen grains per flower	Number of ovules per ovary	P/O
Breeding system(F-ratio, P value)	0.74, 0.556	0.30, 0.825	0.07, 0.975
Pollination syndrome(F-ratio, P value)	1.72, 0.219	2.66, 0.134	0.13, 0.728
Successional stage (F-ratio, P value)	0.57, 0.585	24.4, <0.001	3.53, 0.074

9.5   Discussion

The shrub species of this study are representative of a range, albeit small, of breeding systems, sexual systems and successional stages of lowland forests of the North Island of New Zealand. However, the results showed limited representation in some of the categories analysed, a factor likely to influence the overall results. Two of the highly self-compatible species (*Alseuosmia pusilla, Teucridium parvifolium*) had comparatively low P/Os, but so too did two self-incompatible species (*Pseudowintera* spp.). P/Os of the breeding system categories of all the study species were manifestly different from those reported by Cruden (1977) (Table 9.4). There was no significant relationship between P/Os and breeding systems, and no clear pattern amongst these species that would indicate P/Os were useful for identifying breeding systems. Jürgens et al. (2002), reduced the breeding system categories to two groups, selfers and out-crossers, and found significantly higher P/Os in out-crossing species compared with selfing species. However, applying the same method to the data from this study did not produce any significant difference in P/Os.

Table 9.4 Comparison of Cruden's (1977) mean pollen-ovule ratios for the breeding system categories, and the results of this study.

Breeding system	Cruden's categories		Shrubs in this study	
	Mean P/O±SE	Log P/O±SE	Mean P/O±SE	Log P/O±SE
Cleistogamy	4.7±0.7	0.65±0.07	-	-
Obligate autogamy	27.7±3.1	1.43±0.05	2079±204	3.30±0.05
Facultative autogamy	168.5±22.1	2.15±0.06	2601±482	3.19±0.06
Facultative xenogamy	796.6±87.7	2.81±0.05	3630±345	3.51±0.04
Xenogamy	5859.2±936.5	3.65±0.06	1601±331	3.09±0.05

Cruden's (1977) theory that selfing species would have lower P/Os as a result of more efficient pollen transfer and/or less reliance on pollinator visits does not apparently apply to the apomictic species, *Pomaderris hamiltonii*, the only representative in the obligate selfing category. The P/O of this species was lower than the facultative autogamous category of this study, and many magnitudes greater than Cruden's (1977) cleistogamy or obligate autogamy P/Os.

Pollen-ovule ratios may not necessarily be useful, depending on the presence or lack of evolutionary constraints (Vasek & Weng 1988), although a number of studies have broadly confirmed the validity of P/Os as an indicator of breeding systems (e.g., Lord 1980; Philbrick & Anderson 1986; Plitmann & Levin 1990; Molano-Flores 2001). However, such correlations may be accounted for in terms of the sex allocation theory, where the relationship is derived from resource allocation to the number of pollen grains, number of ovules, and resource cost per pollen grain and per ovule (Gallardo et al. 1994). Further investigation would be required to determine whether the resource allocation theory explains pollen-ovule ratios in the New Zealand shrub flora.

In this study, the taxon *Alseuosmia macrophylla* is an interesting example of morphs from different study sites having similar pollen grain and ovule numbers and P/Os, but with different breeding systems (Table 9.1). Cruden (2000) reported different breeding systems among populations of the same species, but this was accompanied by substantial variation in pollen grain and ovule numbers between populations. Differences in P/Os among populations with the same breeding system may reflect pollinator efficiency or another selective force (Cruden 2000). Among species with equivalent breeding systems, P/Os can vary significantly, for example, among some plant families, the P/Os in xenogamous, animal-pollinated taxa can vary from 500:1 in Onagraceae to 200 000:1 in Boraginaceae (Cruden 2000). Other traits may influence P/Os in animal-pollinated plants such as duration of stigma receptivity, stigma area, stigma depth, pollen grain size, habitat, and the size of the pollen bearing area of the pollinator (Cruden 2000). Webb (1994) recorded quite different P/Os in two populations of *Corokia cotoneaster*, with the dissimilarity associated with different pollinator efficiencies. The P/Os of floras dominated by woody perennial species, such as in New Zealand, may be higher within the various breeding systems than floras with a high proportion of annual and herbaceous perennial species, possibly a consequence of differences in floral frequency, environmental conditions, and/or pollinator guilds and abundance. Jürgens et al. (2002) reported significantly higher P/Os in perennial life forms compared with annual species.

Further investigations, with the inclusion of more species in the various breeding system categories, and species with differing life forms would contribute toward a greater understanding of whether P/Os are a reflection of breeding systems in the New Zealand

flora. In particular, more representatives in the obligate selfing breeding system categories would enable a better determination of whether the P/O of *P. hamiltonii* is representative of this category.

The significantly higher P/Os in dioecious and gynodioecious species compared with the hermaphroditic taxa in this study supports Cruden's (1977) theory. Jürgens et al. (2002) also reported significantly higher pollen grain and ovule numbers and P/Os in gynodioecious species relative to hermaphroditic taxa. Higher P/Os of gender dimorphic species may reflect selection to maintain the available pollen pool above some critical level for pollination vectors (Cruden 2000).

The results from the P/Os of wind-pollinated species are consistent with Cruden's (2000) hypothesis. Wind-pollination (anemophily) is a relatively passive process controlled primarily by microclimate factors (Whitehead 1983), and its effectiveness, particularly in dioecious species, is dependent on the production of large numbers of pollen grains. Gender dimorphism is common in wind-pollinated taxa (Darwin 1877; Charlesworth 1993; Proctor et al. 1996), and three of the four wind-pollinated shrub species of this study are dioecious. Wind-pollinated floral structures are usually designed to maximise pollen entrainment and capture and their floral characteristics include large, well-exposed anthers often hanging on long filaments or in catkins; large, well-exposed and often finely divided and/or feathery stigmas; small, absent or insignificant perianth; the absence of attractants; and small pollen grains produced in great quantities (Faegri & van der Pijl 1979; Proctor et al. 1996). The four wind pollinated species all produced large numbers of pollen grains (68 519–120 370 per flower). These quantities are consistent with pollen grain numbers in wind-pollinated species of the Juan Fernádez Islands (Anderson et al. 2001). Cruden (2000) described wind-pollinated flowers as mostly having a single ovule, which in turn would influence their P/Os. However, three of the four wind-pollinated species in this study consistently have two or more ovules per ovary.

Various reproductive traits could affect P/Os. For example, *Coriaria arborea*, the only hermaphroditic wind-pollinated species in the study, produces drooping floral racemes of up to 30 cm long that comprise numerous small flowers each with five ovules, but as only one ovule matures into a fruit, the P/O is actually higher than that stated. The

dioecious species *Macropiper excelsum*, produces flowers with a single ovule, but the flowers are minute and occur densely packed and in vast numbers (> 200) on bracted spikes, and a single male spike can produce more than 39 million pollen grains.

In the successional stage analyses of P/Os, there was no parallel with Cruden's (1977) categories, and species of the later successional stage had lower P/Os than those of the early successional stage. These results may reflect incorrect classification of the study species, or there may be other attributes that are required to group New Zealand species into successional stages.

Some of the distinctive features of the New Zealand vascular plant flora include a high proportion of long-lived perennial and woody species and a low frequency of annuals (c. 6%) compared with most temperate floras, a preponderance of small flowers with simple structures, the relative infrequency of self-incompatibility, and a depauperate and unspecialised pollinating fauna (Webb & Kelly 1993). High P/Os in the New Zealand flora may have evolved to compensate for a depauperate, and perhaps inefficient pollinating fauna, regardless of the breeding system or successional stage of the species.

9.6   Acknowledgements

9.7   References

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Chapter 10

Synthesis

This chapter reviews the main objectives of the thesis, summarises the major research outcomes, and discusses future research for breeding systems and fragmentation effects. The research has identified gaps in the current knowledge of breeding systems in the indigenous shrub flora, and because of the complexity and variability of plant traits and interactions, the need for species-specific research is accentuated. This research is the first multi-species comparison of reproductive success in New Zealand's indigenous shrub flora. An investigation of this nature is especially relevant for threatened species for the design of recovery programmes or restoration projects.

This thesis research has added considerably to our knowledge of the breeding systems of lowland indigenous shrub species, and has identified factors that are likely to influence reproductive success (Chapter 2). Two of the study taxa that were presumed entomophilous show pollen characteristics that indicate anemophily (Chapter 2). This research has also illustrated the importance of maintaining plant-pollinator mutualisms (Chapter 3), and has ascertained the importance of relationships between male plant frequency and reproductive success in dioecious species (Chapters 4 and 5). Unusual distributions of sex-ratios have been identified in two gender dimorphic species (Chapters 5 and 6). Discrepancy in the current knowledge of the breeding systems of two species has been recognised (Chapters 7 and 8), and the value of using pollen-ovule ratios as a tool for identifying breeding systems in indigenous shrubs has been investigated (Chapter 9).

10.1   Review of thesis objectives

The main objectives of this thesis were (i) to identify the breeding systems and strategies of 16 indigenous shrub species, (ii) to determine whether reproductive success was affected by the proximity of conspecifics, (iii), to assess whether species with

certain breeding systems were more likely to be affected by fragmentation effects and (iv) assess whether populations were declining or stable.

10.2   Breeding systems and proximity of conspecifics

Of the sixteen species of this study, eight have proved to be hermaphroditic, seven dioecious, and one gynodioecious. One species, *Raukaua anomalous*, was previously thought to be dioecious, but during the course of this study was identified as hermaphroditic, and one of the hermaphroditic species, *Teucridium parvifolium*, has been found to have gynodioecism in two populations in the North Island. Six of the hermaphroditic species are animal-pollinated, and one is wind-pollinated. The three dioecious wind-pollinated species show indications of a dual (wind and animal)-pollination; in the past, the dispersal of *Coprosma spathulata* pollen may also have been animal assisted by movement amongst the undergrowth by foraging native birds. *Macropiper excelsus* and *Melicytus novae-zelandiae*, reported as entomophilous (Webb et al. 1999) show characteristics and pollen counts that suggest wind-pollination, and cryptic dioecy has been verified in the North Island in populations of *Cyathodes juniperina* and *Leucopogon fasciculatus*.

Plant breeding systems play a significant role in determining population viability and persistence in modified ecosystems. Each of the species in this study has its own set of traits that influence its presence, abundance, and population processes. These processes are the mechanisms that drive changes in both individual populations and entire communities (Hobbs & Yates 2003). Rathke and Jules (1993) suggested the reproductive success of plants was more likely to decline where there was pollinator specialisation and/or self-incompatibility. In the hermaphrodite group of species in this study, only two taxon were self-incompatible (Fig. 10.1), and one of these, *Alseuosmia macrophylla*, appears to have more specialised pollinators in some populations. For example, plants in the Mamaku population (*A. macrophylla* (a), Table 10.1), are mostly self-incompatible and thus reliant on pollinators. Bellbirds and moths were recorded visiting flowers at this site, and are presumed to be pollinators. Bellbirds in particular would be considered a 'more specialised' pollinator, however, relatively low fruit set in this population and a high pollen limitation index (Fig. 10.1) suggests some form of

breakdown in the plant-pollinator mutualism, despite visitation by bellbirds and moths. No other potential diurnal insect pollinators of *A. macrophylla* flowers at the Mamaku site were observed during the fieldwork for this study, nor recorded on video. It is possible that the loss of a primary pollinator, e.g., stitchbird, is having a negative impact on reproductive output. The three other study populations of *A. macrophylla* showed varying degrees of self-compatibility, which ensures at least some fruit set in the event of pollinator decline or collapse. While some plants can persist without sexual reproduction but through vegetative expansion, this is generally uncommon in long-lived shrub species. *A. macrophylla* is able to reproduce vegetatively when stems come in contact with the ground (pers. obs.) however, if clonal propagation was the principal method of reproduction, populations could become dominated by plants that are genetically identical, negligible fruit set due to self-incompatibility would be inevitable, and in the long term population viability could be compromised.

The only other hermaphroditic species showing self-incompatibility at two study sites was *Alseuosmia quercifolia* (Fig. 10.1), also indicating pollinator dependency. However, neither study population showed pollen limitation (Fig. 10.1). No bellbirds have been seen or heard at either of the *A. quercifolia* study sites, suggesting insects as the most likely pollinators. No diurnal insects were seen visiting flowers during the field work for this study nor during previous work with *A. quercifolia* (Merrett & Clarkson 2000; Merrett et al. 2002), which suggests moths as the most likely pollination vector. The role of moths as pollinators of the New Zealand flora has been not been investigated.

For the remaining hermaphrodite populations of this study, the combination of self-compatibility, and a PLI below 0.75 (Fig. 10.1) suggests adequate pollinator activity, which in turn suggests that natural fruit set is sufficient for population viability and persistence provided suitable seed dispersal methods and habitat conditions are available. The low proportion of hermaphroditic shrub taxa in this study with self-incompatibility mechanisms (22%) suggests that estimates of 80% self-incompatibility in the New Zealand flora (see Newstrom & Robertson 2005) may be an over-estimation. Currently the breeding systems of only approximately 6% of native shrubs (including this study) has been investigated, highlighting the lack of knowledge in this field.

Within all of the study populations, whether taxa were self-incompatible or self-compatible, there was no evidence that proximity to conspecifics had any negative effect on natural fruit set, indicating that potential pollinators were able to travel the distances between plants within the plots.

For gender dimorphic species, separation of the sexes ensures outcrossing, but fruit set is dependent on pollinator access and travel to a pollen source. Bond (1994) considers pollinator dependent dioecy the most highly ranked plant attribute that could contribute to extinction risk. In the gender dimorphic species in this study, high pollen limitation levels (>0.75) were evident in two populations, *Coprosma spathulata* HK and *Cyathodes juniperina* PM (Fig. 10.2). Because *C. spathulata* is wind pollinated, pollinator abundance or activity was not a factor contributing to low natural fruit set. In the first investigation of two *C. spathulata* populations, the HK population demonstrated that proximity and numbers of male plants were the main factors affecting natural fruit set. In the second investigation with eight populations, combinations of male plant proximity, male flower frequency and the density of the associated shrub and ground cover plants were important factors affecting natural fruit set. *C. spathulata* is unusual for a wind pollinated species because it grows under a canopy, a habitat that would seem unsuited to an anemophilous taxon. However, provided female plants are within approximately seven metres of a male plant, and the female:male plant ratio is adequate, natural fruit set is high. Bawa and Crisp (1980) also reported no adverse effects on fecundity in an understory, wind-pollinated tree in Costa Rica.

Further studies on populations of *Cyathodes juniperina* would need to be undertaken to determine the most important factors influencing fruit set. The PM population of this study had poor natural fruit set and a high PLI, but flowers were in low numbers on the plants there, probably because of low light levels under a closed canopy; affecting floristic attractiveness to potential pollinators.

The female-biased populations of *Melicope simplex* are interesting because this strategy is unusual in vascular dioecious taxa. Combinations of low male plant density, low numbers of male flowers, and increased distance to male plants affected natural fruit set in this species. However, plants produce many hundreds of flowers, and an adequate seed bank can be produced even when seed set is proportionally low. In a study of

female-biased populations of dioecious mosses fertilisation success was found to be limited, suggesting these populations could be more vulnerable (Bisang et al. 2004).

Overall, more gender dimorphic species had higher levels of pollen limitation than hermaphrodite species in this study, conforming to Bond's (1994) extinction risk assessment. Chronic pollen limitation can negatively impact on plant populations (Ashman et al. 2004), but for long-lived species such as shrubs, the effects may not be immediately evident, nor as crucial as for short-lived taxa. Wind-pollinated species proved to have the most efficacious pollination syndrome, and produced the highest fruit set regardless of breeding system.

Identifying variation in gender ratios in the gynodioecious *Pimelea arenaria*, as well as establishing new knowledge about the breeding systems of *Raukaua anomalus* and *Teucridium parvifolium* highlights the deficiencies of our current knowledge of the breeding systems of shrubs. Both *P. arenaria* and *T. parvifolium* are listed in the most recent classification of threatened plants of New Zealand (de Lange et al. 2004), thus having an understanding of their breeding systems is a first step in understanding factors that can influence any restoration or rehabilitation management plan.

Although the pollen-ovule ratios of the study species proved an unreliable means of identifying plant breeding systems, this investigation identified significant differences in P/Os in the New Zealand flora compared with the northern hemisphere flora (largely annuals and herbaceous perennials) that the P/O ratio theory was based upon (see Cruden 1977, 2000). The distinctive features of the New Zealand vascular plant flora such as few annual taxa, could help explain the high P/Os. High numbers of pollen grains, or low numbers of ovules producing high P/Os, could be linked to floral characteristics, plant life form, pollinator size and efficiency, or environmental conditions, and/or could have evolved to compensate for a depauperate, and perhaps inefficient pollinating fauna, regardless of the breeding system or successional stage. During the three years of fieldwork for this study, few insect pollinators were observed visiting flowers, particularly in the native forest habitats, and subsequent observations of insect pollinators reinforces the widely-held view that the pollinating fauna is depauperate. If this were the case, widespread self-incompatibility would be disadvantageous.

Table 10.1 Study species, breeding system, breeding strategy and potential threats to population viability and persistence.

| Species	Breeding system	Breeding strategy	Potential threats
Hermaphrodite taxa			
Alseuosmia macrophylla(a)	H	SI	Pollinator decline, herbivory, habitat loss
A. macrophylla(b)	H	SC	Herbivory, habitat loss
A. pusilla	H	SC	Herbivory, habitat loss
A. quercifolia	H	SI	Pollinator decline, herbivory, habitat loss
A. turneri	H	SC	Herbivory, habitat loss
Coriaria arborea	H	SC	Habitat loss
Pomaderris hamiltonii	H	SC	Habitat loss
Raukaua anomalus	H	SC	Habitat loss
Teucridium parvifolium	H	SC	Habitat loss
Gender dimorphic taxa			
Coprosma spathulata	D	-	Gender imbalance, habitat loss
Cyathodes juniperina	D	-	Habitat loss
Leucopogon fasciculatus	D	-	Habitat loss
Macropiper excelsum	D	-	Habitat loss
Melicope simplex	D	-	Gender imbalance, habitat loss
Melicytus micranthus	D	-	Habitat loss
Melicytus novae-zelandiae	D	-	Habitat loss
Pimelea arenaria	G	SC	Gender imbalance, habitat loss
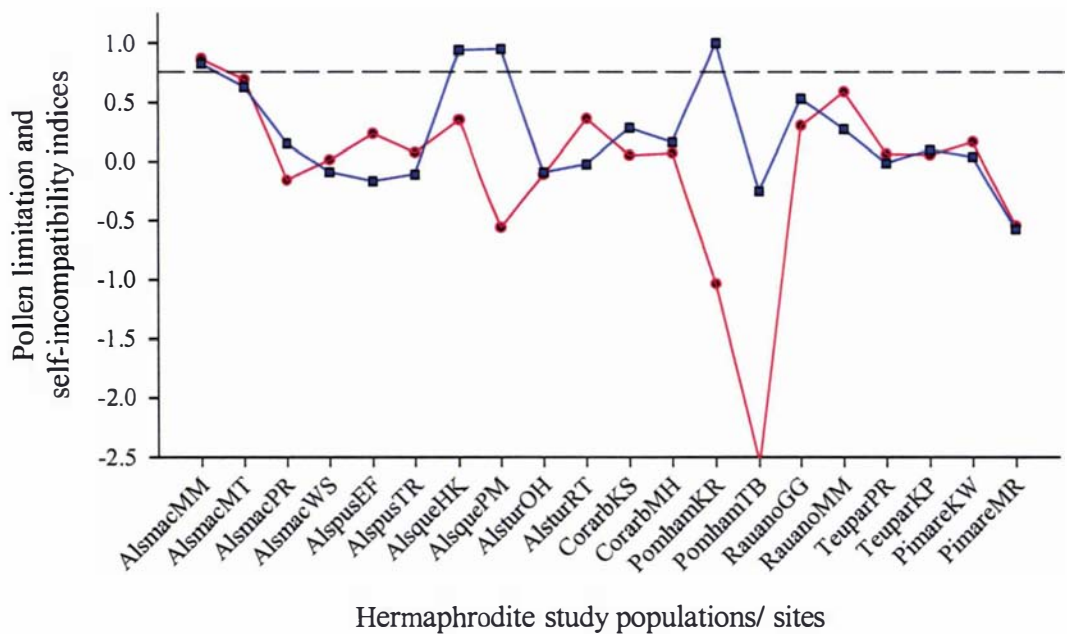
Fig. 10.1 Illustration of pollen limitation (PLI) and self-incompatibility (SII) indices of study populations with hermaphrodite flowers. The dashed line represents a value of 0.75; red = PLI; blue = SII.
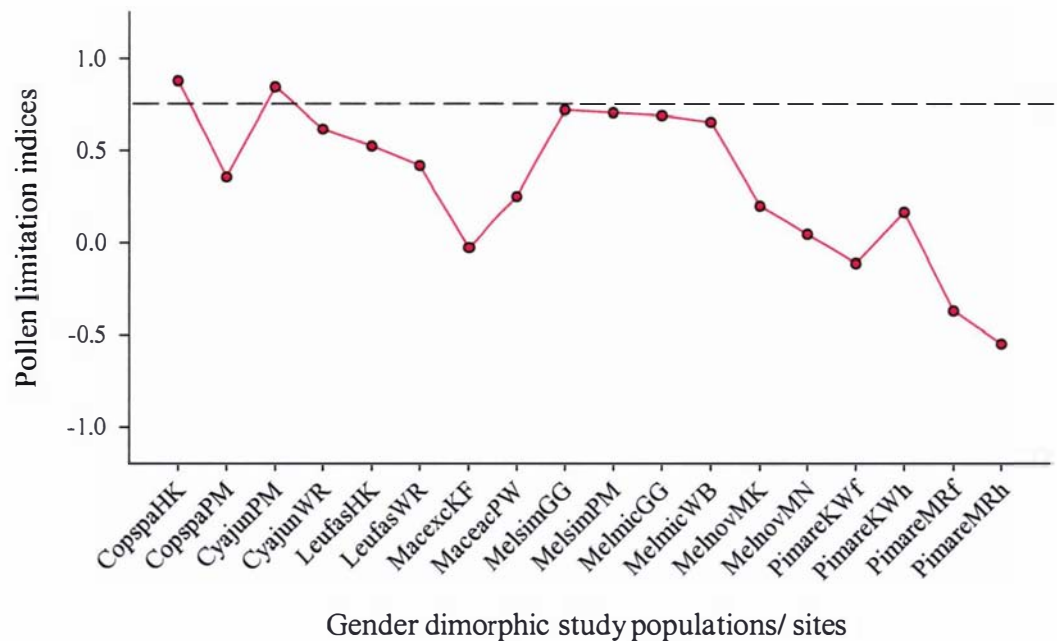

Fig. 10.2 Illustration of pollen limitation (PLI) indices of gender dimorphic study populations. The dashed line represents a value of 0.75.

10.3 Assessing fragmentation effects

Assessing the effects of fragmentation on reproductive output is retrospective at the point in time of data collection. Like most countries, all of the natural ecosystems of New Zealand have been fragmented since human occupation. Habitat loss is the most obvious threat to plant communities, and all species are susceptible. Much has been written about the negative impacts of fragmentation and the threats to biological diversity because of the magnitude of habitat alteration, degradation and destruction as a result of human activity (Laurance & Yensen 1991; Saunders et al. 1991; Primack 1993; Murcia 1995; Davies-Colley et al. 2000; Gehlhausen et al. 2000). However, natural disturbances are a normal feature of native ecosystems, and although rarely on the scale of destruction that is associated with human colonisation, the impacts are none the less similar. The oceanic climate of the New Zealand archipelago is described as unpredictable by Meurk (1995), with a wide range of episodic disturbance events that have influenced plant communities and ecosystem dynamics, including volcanic eruptions, earthquakes, fire, windstorms and cyclones, landslides, flooding, and drought (McGlone & Webb 1981; Jane & Green 1983; Shaw 1983; Jane 1986; Payton 1988; McGlone 1989; Clarkson 1990; Wardle 1991; Meurk 1995; Wells et al. 1998; Cullen et al. 2003; Smale & Smale 2003). Openings formed by natural disturbances and canopy collapse favour regeneration, particularly of early successional species.

The consequences of fragmentation on population dynamics and ecological systems is hugely variable, with multiple combinations of ecosystem and species attributes to consider (Fig. 10.3). Habitat loss and reduced species abundance are the first and obvious consequences of fragmentation. Perhaps less obvious are the secondary consequences such as edge effects. The impacts of edge effects may not be immediately apparent, apart from the abrupt boundary between the remnant and the cleared land. Edge effects can completely change the composition and frequency of plant communities that were previous occupants of that edge area (Young & Mitchell 1994), and are presumed to have deleterious consequences for the organisms that remain in the fragment (Murcia 1995).

One of the interesting outcomes from searches for suitable study populations for this research was realising that most of the populations of forest species were found on an

edge, within 50 m of an edge, or in a light well. This suggests that many of the species of this study have benefited in one way or another from habitat fragmentation, especially those species associated with native forests. For example, at the time when forest clearance was occurring after European settlement, mostly 100–150 years ago, many of the shrubs species of this study had taken advantage of newly created edge habitat and of the higher light areas within the first 30–50 m of the edge. Kelly et al. (2000) also identified reproductive benefits associated with fragmentation and edges for an endemic mistletoe. These findings have important consequences for the management of fragments, and especially the edges of fragments.

Associated with human settlement and the large-scale clearance of natural ecosystems in New Zealand has been the introduction of alien mammals and plants, many of which are having a devastating impact on the indigenous flora and fauna. Because all *Alseuosmia* species are highly palatable, one of the threats to population persistence is herbivory by introduced mammals such as goats and deer. Observation shows that *Alseuosmia* plants will produce new vegetative growth after being browsed, although at the expense of flower production. However, persistent browse over an extended period of time must inevitably cause plant death. Control of mammalian pests has been known to have a positive impact of the recovery of declining populations of native birds (James & Clout 1996; Innes et al. 1999; Dilks et al. 2003; Innes et al. 2004), but controlling animal pests alone is not enough to protect New Zealand's biodiversity. Environmental weeds are widespread in New Zealand, and are having a major and increasing impact on native plant species (Timmins 1997; Owen 1998). Approximately 20 000 plant species have been introduced into New Zealand, 2000 have become naturalised, and 247 are recognised as invasive weeds of conservation concern (Timmins 1997). Competition with invasive weeds has been reported to negatively affect seed germination and seedling growth rate of the rare herb *Pachycladon cheesemanii* (Miller & Duncan 2004), and Williams et al. (2003) reported reduced seedling richness associated with dense populations of wild ginger (*Hedychium gardnerianum*). Some of the most aggressive and damaging invasive plants initially become established at forest edges, or where there are openings in the canopy (Timmins 1997). The edge habitat is also important for native species, with many shrubs falling into the early successional category. Edges and the margins of native forest are probably important refugia for some indigenous shrub species, and maintenance of edges needs to be given more

importance, in terms of the native shrub species occupying these areas, than is currently evident (see Young & Mitchell 1994). Earlier studies of edge effects were tree-centred, focussing on the negative impacts on native tree species that die when exposed to newly created edges (Young & Mitchell 1994). The benefit of the higher diversity of native plant taxa at forest edges has not been recognised.
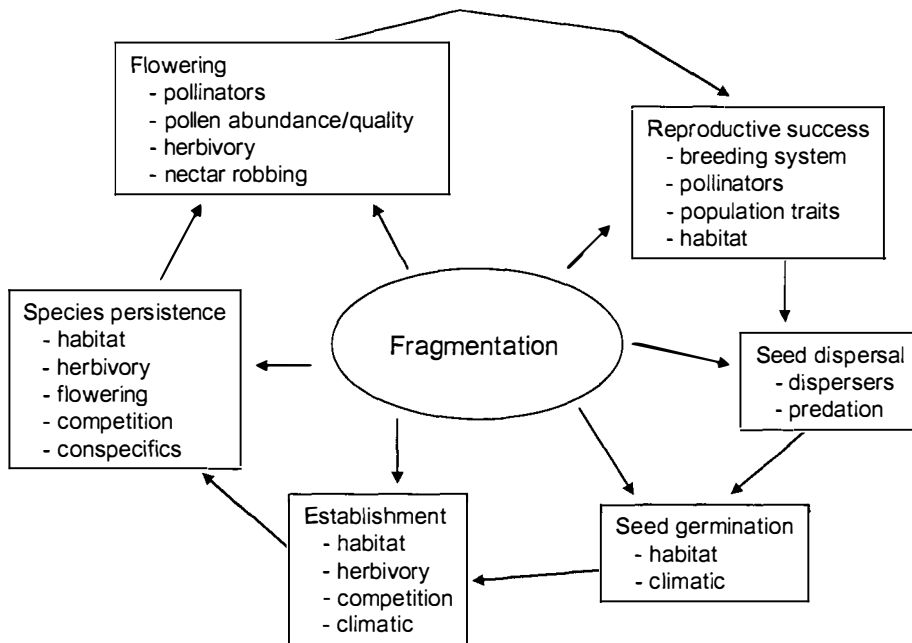

Fig. 10.3 Key processes in plant population dynamics, and some of the factors that could influence each process.

10.4   Population status

In this study, long-term population persistence was evaluated by measuring *in situ* size-class structures. The results show only seven of 34 study populations had poor or no population recruitment: *Coriaria arborea* KW, *Pomaderris hamiltonii* KR, and *R. anomalus* GG, *Cyathodes juniperina* WR, *Leucopogon fasciculatus* WR, *Melicytus novae-zelandiae* MK, and *Pimelea arenaria* MR. In each of the seven species, the results suggest lack of seedlings could be related to characteristics of the habitat that

preclude seed germination. For example, a ground covering of dense pasture grasses beneath and around adult plants of *C. arborea* KW, mown grasses under the roadside population of *P. hamiltonii* KR, low light levels under an almost-closed canopy in the *R. anomalus* GG population, and the road verge and road bank habitat of the *Cyathodes juniperina* and *Leucopogon fasciculatus* are all conditions that effectively prevent seed germination. Poor recruitment is widespread in *Pimelea arenaria* populations, despite adequate seed set, and further research is required to determine the causes. The presence of seedlings but absence of juvenile plants in the *A. quercifolia* PM site was most likely the result of seedling herbivory by introduced mammals such as hares and rabbits, or possibly rats (Best 1969; Daniel 1973; Innes 1979; Moors 1985; Miller & Miller 1995; Merrett et al. 2002; Wilson et al. 2003). Field observation suggests recruitment failure in *C. spathulata* at the HK site may be due to a lack of suitable habitat, primarily because reduced light levels associated with increasing canopy closure do not favour seedling establishment.

The remaining 27 study populations appear to be reproductively viable and provided seed set remains at the levels measured for this study, populations have the potential to persist in the longer term. Some species e.g., *Pomaderris hamiltonii*, require open, high light habitats, and when overtopped by taller species such as kanuka (*Kunzea ericoides*), flowering is reduced and plants eventually die (unpubl. data). In this event, and particularly when species have a restricted distribution, some active habitat/species management may be necessary to ensure population persistence.

10.5   Further Research

The work presented in this thesis has identified several areas for further investigation, and some of these are discussed below.

- Further investigations, particularly nationally, into the role edges play in providing habitat refugia for shrub species, and research into potential reproductive benefits in edge habitats.

- Investigations and comparisons of thresholds of male plant population density required for effective pollination in species and floras in other countries with gender dimorphic taxa.

- Further research into the reproductive biology of indigenous shrubs. The new knowledge gained of the breeding systems of two shrub species of this study suggests examination of flowers in multiple populations is needed before a breeding system can be considered completely characterised for a species. Further investigations may uncover additional discrepancies in the current knowledge of breeding systems in our shrub flora.

- Plant-pollinator mutualisms in populations of *Alseuosmia macrophylla*. Variation in the degree of self-incompatibility and polymorphism among populations of this species indicates taxonomic and reproductive biology investigations would add to our knowledge of this taxon.

- Recruitment failure in *Pimelea arenaria*. Further investigation is required to determine the cause of lack of recruitment in many North island populations.

- Investigation of the evolutionary processes of gender-distribution in the gynodioecious *Pimelea arenaria*. Clinal variation in gender ratios and floral characteristics, from north to south, presents an opportunity to investigate the evolution of gender dimorphism.

- Investigation of gender ratios in dioecious species. Male plant density has been identified as the key factor contributing to reproductive success in dioecious species. Knowledge of gender ratios and density is crucial for determining population viability, and also for restoration initiatives where self-sustaining populations are the goal.

Shrubs are an important component of New Zealand's indigenous flora, and an important component of fragmented ecosystems. This thesis research has increased our knowledge of the breeding systems and reproductive strategies of 16 lowland shrub

species, but has also highlighted the need for further comprehensive investigations into the ecology and reproductive biology of shrub species in general. The effective management of fragmented ecosystems and the success of ecological restoration initiatives depend on an understanding and knowledge of the reproductive ecology of indigenous plant species.

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