POLLINATION ECOLOGY OF NEW ZEALAND ORCHIDS

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"It will be tragic if the remaining natural areas of the world are filled with ageing plants silent as graveyards with no butterfly or sunbird pollinators working their flowers or large birds eating their fruits" (Bond 1995).

To Angélica

ABSTRACT

The New Zealand orchid flora comprises twenty-five genera and at least 100 species occurring throughout the country. Although the number of endemic species is high (69%) only four genera are endemic to New Zealand. The main physical threats to orchid survival in New Zealand are habitat destruction, modification and fragmentation. The effect of the disruption of interactions with their pollinators has never been considered. This study concentrates on this mutualistic interaction, by assessing the breeding system, pollination syndromes and pollinator-dependence of four widespread terrestrial (*Gastrodia cunninghamii*, *Thelymitra longifolia*, *Pterostylis alobula* and *P. patens*) and four widespread epiphytic orchids (*Earina autumnalis*, *E. aestivalis*, *E. mucronata* and *Winika cunninghamii*) occurring in the southern portion of the North Island.

In order to determine the breeding system and the presence of self-incompatibility, hand-pollination treatments were conducted in all eight orchid species during the flowering seasons of 2001 and 2002. Pollen grains and ovules numbers, pollen:ovule ratio and presence of floral scent glands were assessed. In those nectariferous species (*E. autumnalis*, *E. aestivalis*, *E. mucronata* and *W. cunninghamii*), the nectar standing crop was determined using the anthrone colorimetric assay for total carbohydrates. The activity of pollinator was observed both in the field and in captivity. Insects observed foraging in these orchids were identified and ranked according to their likely pollination effectiveness. Finally, measurements of pollination success and pollinia removal and deposition were used to assess whether fruit-set is pollen limited in these species and explore the effect contrasting rewarding strategies (nectar v/s deception) has on the pollination success of these orchids.

Pollination treatments in three terrestrial (*T. longifolia*, *P. alobula* and *P. patens*) and two epiphytic (*E. autumnalis* and *E. mucronata*) orchids confirmed the absence of genetic incompatibility. Despite these five orchids being self-compatible, their reproduction relies on contrasting reproductive strategies. *T. longifolia* is predominantly self-pollinated, whereas *Pterostylis* and *Earina* species are incapable of autonomous selfing and completely dependent on pollinators.

The epiphytic species *E. aestivalis* and *W. cunninghamii* are partially self-incompatible and also completely dependent on pollinators. Agamospermy is likely to occur in *G. cunninghamii* but not involved in seed-production in any of the remaining seven orchids. Both terrestrial and epiphytic species showed a positive reaction to neutral red except *E. autumnalis*. This indicated the presence of scent glands, mainly located around the column, lip and sepal tips. Pollen:ovule ratios calculated for these species ranged from 20: 1 in *E. mucronata* and *E. aestivalis* to 320: 1 in *P. alobula*.

Of the four terrestrial orchids studied, insect visitation was observed only in *P. alobula*. This orchid is pollinated by male fungus gnats of the genus *Zygomyia* (Diptera: Mycetophilidae). Pollination by sexual deception is likely to occur in species of this genus. Numerous insects were recorded visiting the nectariferous epiphytic orchids (3 orders, 13 families). Insects considered as "probable pollinator" were *Eristalis tenax* (Diptera: Syrphidae) for *Earina autumnalis*, *Dilophus nigrostigmus* (Diptera: Bibionidae) for *E. mucronata*, and *Melangyna novaezealandiae* (Diptera: Syrphidae), *Calliphora quadrimaculata* (Diptera: Calliphoridae), the Ichneumonid wasp *Aucklandella* sp. (Hymenoptera: Ichneumonidae), *Hylaeus* sp. (Hymenoptera: Colletidae) and an unidentified weevil (Coleoptera: Curculionidae) for *E. aestivalis*. In *W. cunninghamii* the species *Apis mellifera* and the native syrphid flies *Helophilus antipodus* and *M. novaezealandiae* were considered as "probable pollinators".

Levels of natural fruit-set were similarly low in rewarding and non-rewarding species fluctuating from 4.3% (*P. alobula*) to 40% (*P. patens*). Fruiting in these orchids is pollen limited, as supplementary hand-pollinations increased fruit set above 40% in all species except *P. patens*. The degree of pollen limitation varied from 0.32 (*P. patens*) and 0.94 (*P. alobula* and *E. mucronata*). Pollen limitation in these orchids may be caused by the simplicity of their flowers, the poor efficiency of their pollinators in depositing pollinia and the use of species–specific pollination systems (*e.g. Pterostylis*).

The survival capability and conservation requirements of these orchids are discussed in the light of the specific reproductive requirements revealed by this study.

RESUMEN

La familia Orchidaceae en Nueva Zealanda consta de 25 géneros y más de 100 especies distribuidas por todo el país. El número de edemismos es alto pero solo a nivel de especies (69%), solo cuatro géneros son endémicos. Aunque las principales amenazas físicas a la sobrevivencia de estas orquídeas ya han sido identificadas (destrucción, modificación y fragmentación del habitat), el efecto que la ruptura de interacciones mutualisticas pueda tener en su sobrevivencia nunca ha sido considerado. Este estudio pretende evaluar la relación mutualística planta-polinizador en cuatro especies nativas de orquídeas terrestres (*Gastrodia cunninghamii*, *Thelymitra longifolia*, *Pterostylis alobula* and *P. patens*) y cuatro epífitas (*E. automnalis*, *E. mucronata*, *E. aestivalis* and *W. cunninghamii*) creciendo en la parte sur del la Isla Norte de Nueva Zealandia, estudiando su sistema reproductivo, sindromes de polinización y la despendecia de éstas especies a su polinizador.

Durante el periodo de floración del año 2001 y 2002 se realizaron tratamientos de polinización manual en las ocho especies a fin de determinar su sistema reproductivo y la presencia de barreras de auto-incompatibilidad. También se evaluó la relación polen/ovulo, el número de granos de polen y ovulos por flor, y finalmente la presencia de glándulas productoras de aroma. En las especies nectaríferas (*E. automnalis*, *E. mucronata*, *E. aestivalis* and *W. cunninghamii*) se midió la cantidad de carbohidratos totales utilizando el método colorimétrico de antrona. La actividad de los polinizadores se observó tanto en terreno como en el laboratorio. Los insectos observados fueron identificados y clasificados de acuerdo a su eficiencia polinizadora. Finalmente, a fin de determinar si el éxito reproductivo esta limitado por la disponibilidad de polen se midió el éxito de la polinización, la remoción y deposición de polinia en las poblaciones estudiadas.

Los tratamientos de polinización demostraron que al menos tres de las orquídeas terrestres (*T. longifolia*, *P. alobula* y *P. patens*) y dos de las epifitas (*E. automnalis* y *E. mucronata*) son autocompatibles. A pesar de que estas cinco especies carecen de barreras de auto-incompatibilidad, su reproducción depende en diferentes estrategias reproductivas. *T. longifolia*, por ejemplo es autogama, mientras que las especies de los géneros *Pterostylis* and *Earina* son xenógamas obligadas y dependen completamente su polinizador para formar frutos.

Por el contrario las epífitas *E. aestivalis* y *W. cunninghamii* son parcialmente incompatibles y altamente dependiente de sus polinizadores. La agamospermia al parecer solo esta involucarada en la fomación de frutos de la especie *G. cunninghamii.* La tinción de rojo neutro presentó una reacción positiva en todas las especies, excepto en *E. automnalis*. Estas orquídeas presentan glándulas productoras de aroma ubicadas principalmente alrededor de la columna, labelo y ápice de los sépalos. Los valores de la relación polen/ovulo fluctuó entre 20: 1 y 320: 1 en *E. aestivalis, E. mucronata* y *P. alobula*, respectivamente.

De todas las especies terrestres, sólo en *P. alobula* se observó la visita de insectos. Esta orquídea es polinizada por mosquitos machos del genero *Zygomyia* (Diptera: Mycetophilidae). Es posible que la atracción de estos insectos sea mediada por un sistema de atracción por engaño sexual. Por otra parte, las orquídeas epífitas son frecuentadas por numerosos insectos (3 ordenes, 13 familias). Los insectos considerados como "probables polinizadores" fueron *Eristalis tenax* (Diptera: Syrphidae) en *Earina autumnalis*, *Dilophus nigrostigmus* (Diptera: Bibionidae) en *E. mucronata*, y las especies *Melangyna novaezealandiae* (Diptera: Syrphidae), *Calliphora quadrimaculata* (Diptera: Calliphoridae), *Aucklandella* sp. (Hymenoptera: Ichneumonidae), *Hylaeus* sp. (Hymenoptera: Colletidae) y un gorgojo sin identificar (Coleoptera: Curculionidae) en *E. aestivalis*. Para la orquidea *W. cunninghamii* la abeja común (*Apis mellifera*) y los sírfidos *Helophilus antipodus* y *M. novaezealandiae* se consideraron como sus "probables polinizadores".

Los niveles de fructificación bajo condiciones naturales de polinización fueron bajos, independiente de la presencia o ausencia de nectar. La fructificación fluctuó entre el 4.4% en *P. alobula* y el 40% en *P. patens*. El bajo éxito reproductivo de estas especies se debe a la limitación en la disponibilidad de polen, esto se confirma al observar el aumento de la fructificación tras polinizaciones manuales sobre el 40% en todas las especies, excepto *P. apatens*. La intesidad en la limitación de polen fluctuó entre 0.32 (*P. patens*) y 0.94 (*P. alobula* y *E. mucronata*).

La limitación en la disponibilidad de polen en estas especies es producto de la simpleza de sus estructuras florales, la baja eficiencia en la deposición de polinia por parte de los insectos y el uso de elaborados de sistemas de polinización (e.g. *Pterostylis*).

Finalmente, se discute la conservación y capacidad de sobrevivencia de estas especies a la luz de sus requerimientos reproductivos.

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I

The New Zealand Orchid Flora

INTRODUCTION

The study of New Zealand Orchidaceae began as early as 1769, with the first expedition of Captain Cook to New Zealand. Soon after the establishment of the first settlements, numerous collectors visited the country describing several of the current species. However, it was not until the late 1800's that the main contributions to orchid studies were made. During this period, Cheeseman (1873, 1881), Thompson (1879, 1881, 1927) and others described several species and conducted the first studies into the biology and pollination of New Zealand orchids. These studies were the basis for future researchers, and some of them represent the only source of information available today.

The major contributions to modern New Zealand orchidology have been the treatment of the family presented in the Flora of New Zealand by Moore & Edgar (1976), the field guides produced by Cooper (1981) and St. George (1999), and the books of Johns & Molloy (1983) and St. George & McCrae (1990), the last two addressing, to some extent, the origin, biology and pollination of the family in New Zealand. Despite the considerable contribution of several botanists, naturalists and amateurs to orchid knowledge, there are still aspects that require study in order to understand and successfully protect native orchid species. Orchid taxonomy and ecology (stressing plant-pollinator or plant-symbiont interactions) are in particular need for further research.

In order to evaluate the conservation status of the New Zealand orchid flora and identify those areas requiring further studies a review of the New Zealand orchid literature was conducted. This chapter includes a discussion of orchid diversity, relationships with other orchid floras, distribution and habitats, and conservation status. Also, some published data was re-worked and analysed in order to explore the diversity and distributional and phenological patterns within this group.

The New Zealand Orchid Flora

The New Zealand Orchid flora comprises twenty-five genera and over 100 species occurring throughout the country (Appendix 1). Despite the number of species and the low orchid index (0.37[1]) of New Zealand, orchids form a significant part of the native flora. The 100 species named so far (only "valid" names considered) represent almost 5% of the total native flora and 17.4% of the total number of monocots (updated from Johns & Molloy 1983). Although the number of endemic species is rather high (69% of the species, Fig. 1), most of the genera are also found in the islands of the South Pacific region and Australia. There are only four endemic genera to New Zealand (*Aporostylis* Rupp & Hatch, *Danhatchia* (Hatch) Garay & Christenson, *Waireia* Jones *et al.* and *Winika* Clements *et al.*, all of them monotypic), representing 16% of the orchid genera in the country (Fig. 2). *Pterostylis* R. Brown, *Thelymitra* J.R. Forst. & G. Forst., *Corybas* Salisbury and *Caladenia* R. Brown, are the most speciose genera with 26, 15, 13 and 9 taxa, respectively. Most of the genera are terrestrial, and only four of them are epiphytic.

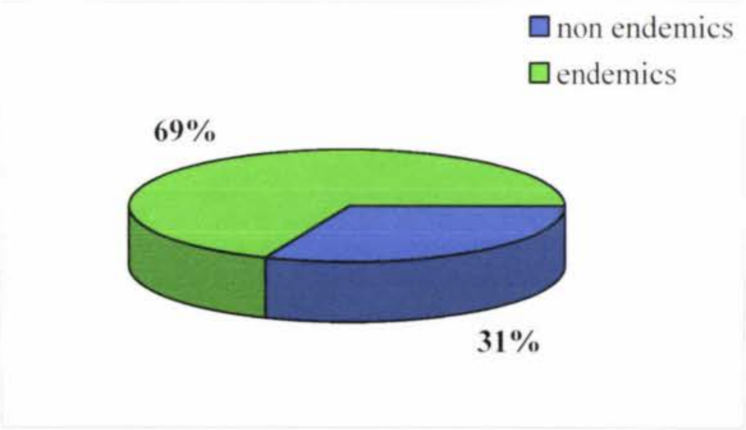

Figure 1: Percent of endemic and non-endemic orchid species in New Zealand (data from St. George 1999 and Hatch 2000).

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[1] Orchid index was calculated as the ratio of number of orchids species: country area (km^2) multiplied by 1000 (IUCN/SSC OSG 1996).

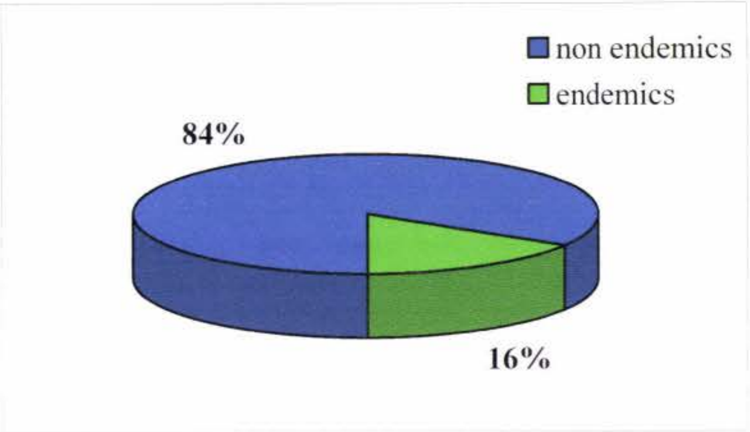

Figure 2: Percent of endemic and non-endemic orchid genera in New Zealand (data from St. George 1999 and Hatch 2000).

Distribution and Principal Habitats

Although the Orchidaceae are spread throughout the main islands as well as offshore and outlying ones, the number of genera diminishes along a latitudinal gradient. For example, the North Island contains a higher number of genera (see Fig. 3) and species (Molloy 1992) than the South and Stewart islands. The Chatham Islands, the largest of the outlying islands, and situated directly in the path of the prevailing westerly winds, support a considerable number of orchids, 26 species, including four of the mainland epiphytic orchids (Molloy 1992).

Molloy (1992) notes that the number of orchids on the subantarctic islands in the south is in direct proportion to their distance from the South Island, their size and the availability of suitable habitats. For example, the Auckland Islands have 12 species, while the smaller and more distant Campbell Island has only six.

It is well known that the habitats occupied by orchids are fairly diverse. They can be found in open grasslands, dense tropical jungles, cloud forests, hot and cold deserts or damp or dry areas, on trees or rocks that hang over the ocean and are subject to salt spray, underground, on floating vegetation "islands" in lakes and in other habitats that can support flowering plants (Arditti 1992).

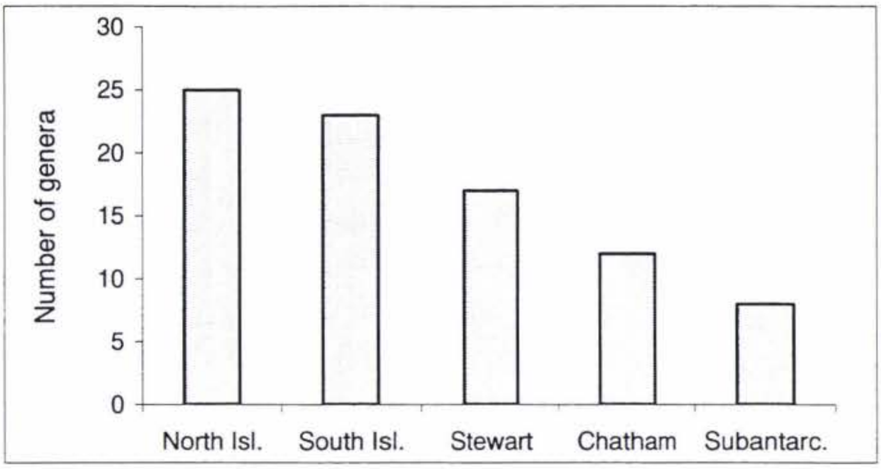

Figure 3: Number of orchid genera in the main islands of New Zealand and the offshore ones (Data compiled from St. George 1999 and Hatch 2000).

In New Zealand, orchids occur under a range of soil and climatic conditions with particular concentrations in the warmer far north, in central and southern North Island, and in the warmer northern South Island. However, a common feature of them all is the low nutrient-supplying power of the parent materials that gives rise to fungal-rich, infertile, often poorly drained soils. Disturbance of the vegetation on these sites usually leads to an abundance of terrestrial orchids (Molloy 1992).

Generally, New Zealand orchids can be found in natural forest communities, disturbed forests, natural subalpine shrubland and heath-like vegetation, natural or modified grasslands, wetlands and restiad bogs, low fertility soils and roadsides. Finally, old plantations of exotic pines, especially *Pinus nigra* and *P. ponderosa*, with their rich fungal floras, are excellent habitats for terrestrial orchids, given adequate light conditions and lack of competition from herbs, ferns and shrubs. Up to 30 or 40 species may be found in these plantations in numbers seldom if ever seen in native vegetation (Molloy 1992). This is not a unique feature of New Zealand orchids though, since it has been observed in other countries as well (Lehnebach 1999).

Currently, studies dealing with the importance of light to terrestrial native orchids in exotic plantations are being conducted (Angela Abernethy, Plant and Microbial Sciences, University of Canterbury). The aim is to establish a relationship between the understorey light quality and quantity, and the distribution of the different orchid genera under exotic timber plantations. It is believed that these exotic timber plantations offer a suitable habitat due to the lack of understorey competitors and the rich diversity of mycorrhizal fungi in the soil (*e.g.* Iwitahi, Native orchid reserve under a *Pinus nigra* stand, where the distribution of orchids in the reserve suggests that the place is a natural trap for orchid seeds) (Johns & Molloy 1983). However, some species are quite habitat-specific. For instance, two of the terrestrial saprophytic orchids, *Danhatchia australis* and *Corybas cryptanthus*, have fairly precise habitat requirements. The former is unknown outside stands of Taraire trees (*Beilschmiedia tarairi*), while the latter is known mainly from a few sites in Beech forest (Johns & Molloy 1983).

The epiphytic species seem less selective in their habitats than the ground orchids. They grow mainly in low altitude mixed forests of native conifers or angiosperms, perhaps due to their common subtropical origin (Johns & Molloy 1983). Occasionally though they are found perched on introduced trees such as *Salix* sp. and *Acacia* sp.; and may additionally be found on rocks and stony forest floors, exposed coastal rocks and rocky ridges and gorges inland (Johns & Molloy 1983).

Floral Structures in the Orchidaceae

The typical orchid flowers are zygomorphic and resupinated, *i.e.* the ovary or the pedicel twists or bends during development by 180° and in so doing, positions the lip on the lower side of the flower (Dressler 1993). The perianth of these flowers is formed by two whorls of segments usually similar in colour and texture, commonly referred as "sepals" for the outer three segments (1, Fig. 4) and "petals" for the inner two segments (3, Fig. 4) (Dressler 1993). The dorsal sepal is often wider than the other pair and may form a hood over the flower (*e.g. Pterostylis*), while the central petal is almost always greatly modified to form a special structure known as the labellum or lip (5, Fig. 4). The function of the labellum is to attract insects and to provide a landing stage for them (Hoffman & Brown 1992).

The labellum may be much larger, variously lobed or fringed, ornamented with ridges or calli, and distinctively coloured (4, Fig. 4). Less often the labellum is much smaller than the other two petals (*e.g. Pterostylis*) and very rarely may be almost indistinguishable from them (*e.g. Thelymitra*) (St. George & McCrae 1990).

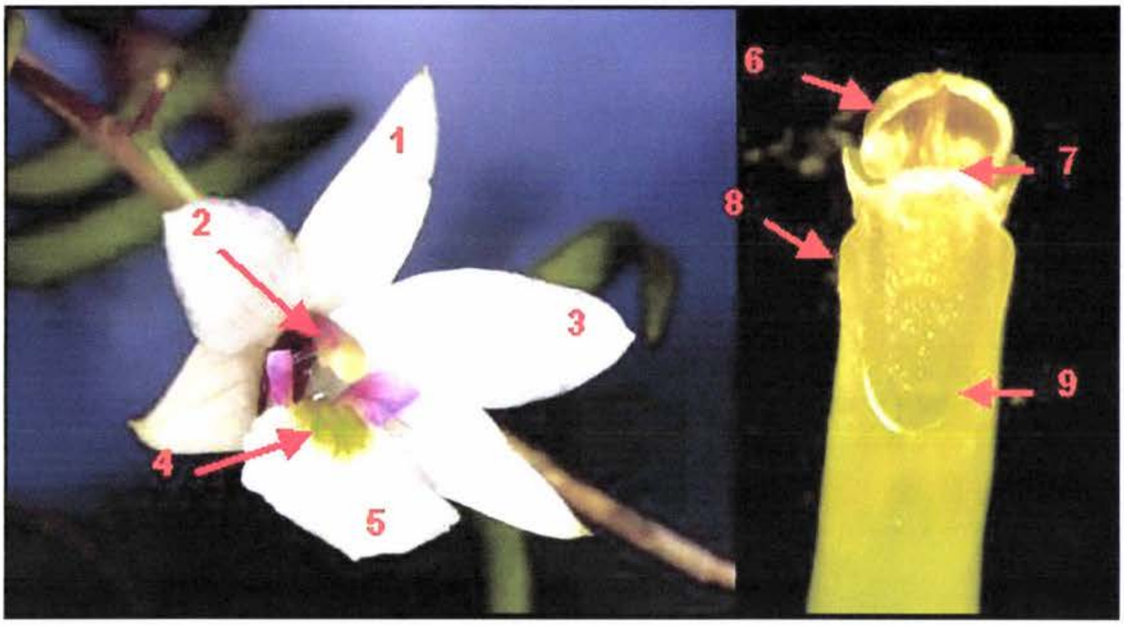

Figure 4: Floral structures of an orchid flower (**1**: dorsal sepal; **2**: column; **3**: petal; **4**: ridges; **5**: labellum; **6**: anther; **7**: rostellum; **8**: column wing; **9**: stigma).

The reproductive parts of the flower, *i.e.* style and stamens, are united to form a structure known as gynostemium or column (2, Fig. 4). All New Zealand orchids have a single anther atop the column (6, Fig. 4) (St. George & McCrae 1990) where the pollinia are located. The column often has lateral wings (8, Fig. 4). Below the anther, is the stigma (9, Fig. 4). Between the anther and the stigma, is located the rostellum (7, Fig. 4). This organ aids in gluing the pollinia to the pollinator (Dressler 1993). The pollinia within the anther are often attached to each other either directly, or by stalk or stipe, to a viscid disc on the rostellum (e.g. *Earina*). The viscidium disc sticks to the pollinator and it is removed with the pollinia as a unit (Dressler 1993). In this family, pollen grains are joined together by elastoviscin forming a compact and coherent structure called the pollinium (*pl.* pollinia). The cohesion of the pollen grains is highly variable within the family but hard pollinia are the most common among orchids (Dressler 1993).

Unlike the flowers, the fruit (capsules) of orchids are remarkably uniform (St. George & McCrae 1990, Arditti 1992, Dressler 1993). The ovary is made up of three carpels that split and separate into valves, thereby releasing the seeds (Arditti 1992).

Phenological Patterns in the New Zealand Orchid Flora

Although the main flowering peak of the Orchidaceae in New Zealand occurs during mid November, December to early January (with 55, 60 and 36 species, respectively), there are always several species flowering all through the year (Fig. 5). This flowering trend is comparable with the general angiosperm flowering pattern reported by Godley (1979). A second flowering peak occurs during May to July. This "winter flowering" may be presented in three patterns: some species extend their flowering from autumn into winter; some species extend their flowering from autumn through winter into spring or summer; and others begin flowering in winter and may continue long after winter is over.

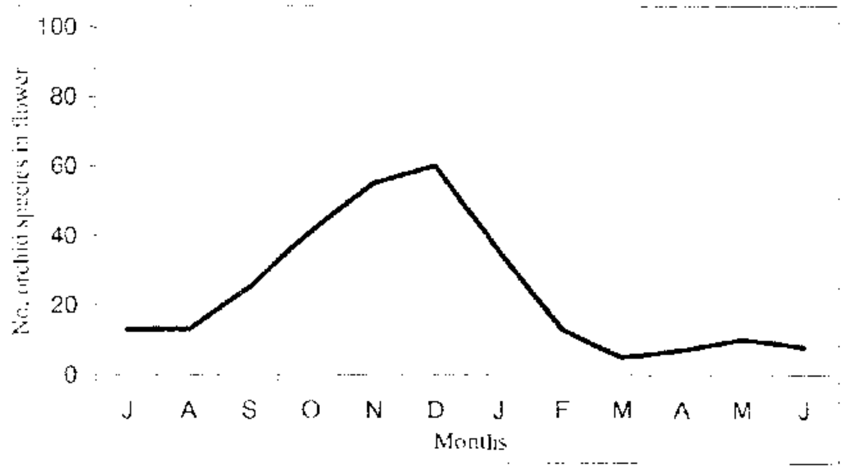

Figure 5: Flowering curve of the species of Orchidaceae along the year (June to July) in New Zealand (Data compiled from St. George 1999).

The first and second groups are predominantly in the North Island. The mid autumn-winter flowering is mainly formed by the terrestrial species of the genus *Pterostylis*. It is interesting to point out that St George (1999) has reported most of the autumn-winter flowering species as out-crossing species.

Relationships and Origin of the New Zealand Orchids

Following Dressler (1993), most of the New Zealand genera belong to the subfamilies Orchidoideae (18 genera), Epidendroideae (6 genera), Spiranthoideae (1 genus), and all are considered relatively derived. The outline of the classification is presented in the Table 1.

Table 1: Classification of the New Zealand orchid genera *sensu* Dressler (1993).

Subfamily	Tribe	Subtribe	Genera
Spiranthoideae	Spiranthideae	Spiranthinae	*Spiranthes*
Epidendroideae	Gastrodieae	Gastrodiinae	*Gastrodia*
	Dendrobieae	Bulbophyllinae	*Bulbophyllum*
		Dendrobiinae	*Winika*
		Aeridinae	*Drymoanthus*
	Calypsoeae		*Danhatchia*
	Epidendreae	Glomerinae	*Earina*
Orchidoideae	Diurideae	Acianthinae	*Acianthus, Cyrtostylis, Corybas, Townsonia*
		Caladeniinae	*Aporostylis, Caladenia, Waireia Adenochilus*
		Cryptostylidinae	*Cryptostylis*
		Diuridinae	*Orthoceras*
		Drakaeinae	*Chiloglottis, Caleana*
		Prasophyllinae	*Genoplesium, Prasophyllum, Microtis*
		Pterostylidinae	*Pterostylis*
		Thelymitrinae	*Calochilus, Thelymitra*

The Orchidoideae as a group may be basically southern, though one of its subtribes, Orchideae, is now widespread and is the principal orchid group of north temperate areas (Dressler 1993). All species in this subfamily have soft herbaceous leaves. Most of the genera have root-stem tuberoids, which become dormant during the dry season or winter. During the growing season a new tuberoid is paired with an older one. The Orchidoideae may be closely related to the Spiranthoideae, but the evidence for such a relationship is weak (Dressler 1993).

The second subfamily well represented by native orchids is the Epidendroideae, in which the primary derived feature is the possession of eight pollinia. This may have evolved in the Epidendroideae only once, and this phylad shows a reduction series to six, four, and two in most subtribes (Dressler 1993).

Finally, the Spiranthoideae is represented in New Zealand by *Spiranthes sinensis* (Pers.) Ames. This subfamily as a whole is a tropical group, with some representatives in cooler areas both to the north and the south (Dressler 1993).

The origin of the New Zealand Flora has been largely discussed and several hypotheses have been proposed through the years (Raven 1973, Wardle 1978, Pole 1994, Macphail 1997, Lockhart *et al.* 2000, Winkworth *et al.* 2000). Recently, molecular and pollen record evidence, suggest multiple origins of the components of NZ flora, with elements of an ancient Gondwanic ancestry, as well as recent long-distance dispersal from the South Pacific region and Australia and *in situ* origin and radiation (Lockhart *et al.* 2000, Winkworth *et al.* 2000). However, the origin of New Zealand orchids is not totally understood. At the present there are considered to be three main distributional patterns (Hatch 2000)

- A presumably ancient, overland trend, that began in Asia and brought us the New Zealand representatives of *Bulbophyllum*, *Corybas*, *Danhatchia*, *Drymoanthus*, *Earina*, the short-columned *Gastrodia*, *Microtis*, *Spiranthes* and *Winika*.

- A distinctly powerful west-to-east trend[2], probably windborne, that still brings East African orchids to Western Australia, Eastern Australian orchids to New Zealand, and from thence to the Chatham's. This gives us the Australia-originating genera *Acianthus*, *Adenochilus*, *Caladenia*, *Calochilus*, *Chiloglottis*, *Cryptostylis*, *Cyrtostylis*, *Genoplesium*, *Orthoceras*, *Prasophyllum*, *Pterostylis*, *Thelymitra* and *Townsonia*.

- And lastly, the tendency to local differentiation (*e.g. Waireia*).

It appears that roughly half of the New Zealand orchids species are immigrants, while the other half have evolved as endemics in this country. Johns & Molloy (1983) assume that the endemic species evolved from ancestral orchids already in New Zealand. The moment when this happened can only be guessed, as the fossil record of the family in New Zealand is almost non-existent. The only fossil orchid pollen known with certainty comes from ice age sediments about 1.5 million years old (Macphail 1997).

Johns & Molloy (1983) propose that plant extinction rather than plant evolution took place during the two million years of the great ice age, suggesting that the endemic orchids survived the glaciations and probably evolved when the present landforms began to take shape some 12-15 million years ago. Furthermore, it is believed that the orchid family had already completed a major part of its evolution by the Cretaceous (65-135 mya), when most of the flowering plant families were differentiated (Johns & Molloy 1983). At that time, the Gondwana super-continent was only just starting to break up and drift apart. The pieces were still close enough, though, for migration of orchids and other plants to continue. The northern orchids, which probably originated in tropical Malaysia, were able to migrate south, just as some of the ground orchids no doubt migrated north from their southern point of origin. New Zealand, Australia and New Caledonia undoubtedly received contributions from both sources and from each other, but as the pieces drifted further apart some 50 mya, New Zealand gradually become isolated and more dependent on long-distance dispersal.

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[2] However, Johns & Molloy (1983) point out that *Corybas macranthus* spreading south to Macquarie Island could be seen as evidence for an east-west migration route as well.

By 30 mya much of the young landscape was worn down by erosion, which may have reduced the ancestral orchid stocks. However, when mountain building was renewed (12-15 mya) evolution of the present New Zealand orchid flora probably reached its maximum (Johns & Molloy 1983). It is necessary to keep in mind though that this is just a hypothesis of the origin of New Zealand orchids, and that further phylogenetic studies must be conducted before a definitive explanation may be reached.

New Zealand Orchids Survival and Conservation

In recent years, the conservation status of native plants in New Zealand and Australia has been examined independently and the orchids of both countries have been considered along with other groups. Orchids have occupied a prominent place in the recent lists of New Zealand threatened plants. For example, Cameron *et al.* (1993) recognise 12 orchid species and four unnamed taxa as threatened, using IUCN Red Data Books categories. In addition, four species and two unnamed taxa are ranked as "local", giving a current total of 16 orchid species and six unnamed taxa considered to be under some level of threat. Out of this total, four species and all six unnamed taxa are currently regarded as endemic to New Zealand and should be given first priority in the Crown's conservation strategy (de Lange 1995). de Lange (1995) points out that more than half of the orchids recognised as threatened in New Zealand, specially those ranked in the higher categories of threat, are vagrant immigrants from Australia where, with possibly one exception, they are not known to be threatened, and that they should only be considered as important elements to understand dispersal of the family and colonisation mechanisms. However, Ecroyd (1995) states that neophytes, such as *Caleana minor*, a mainly Australian species occurring in Rotorua, should be considered in the conservation efforts of the flora, otherwise, New Zealand risks losing more of its native species and hence its natural richness in biological diversity. This site in Rotorua is currently considered as the eastern limit for the distribution of the genus *Caleana*.

de Lange *et al.* (1999) have recently reappraised the conservation status of New Zealand's threatened and uncommon vascular plants. In this report, herbaceous and short woody vegetation are the most common life forms amongst the threatened taxa; in this list the Orchidaceae are represented by four threatened species, 20 uncommon species and four taxonomically undetermined. These authors also showed that open sites, including rocky areas, riverbeds and coastal sites, are the most common habitat type for the threatened and uncommon flora, habitats where most of the orchids are usually found.

The main threats to orchid survival are summarised in the Orchid Status Survey and Conservation Action Plan by the Orchid Specialist Group of the IUCN (IUCN/SSC OSG 1996). The following are considered the main threats:

1. Habitat destruction, modification and fragmentation: logging, agriculture activities and plantations, habitat fragmentation, urban development and mining.

2. Collecting: horticultural trade, amateur collection, and collection of consumable orchids.

Although the main threat to orchids is habitat destruction, the factors limiting terrestrial and epiphytic orchids survival are rather different. For terrestrial orchids several activities such as woodcutting and opening of forests, grazing, mowing, hydrological changes, competition with invasive alien species, herbicides used along roads strikingly change population dynamics (*e.g.* flowering individuals, seedling recruitment). On the other hand, although epiphytic orchids show noticeable resilience to land conversion, many are more or less host-specific, and exposure effects and also the interactions with their pollinators constrained their distribution (IUCN/SSC OSG 1996). Displacement of their native hosts by invasive species may constrain the occurrence and distribution of epiphytic orchids as well (Tremblay *et al.* 1998).

In New Zealand, the main threats to orchid survival also appear to be related to habitat destruction and over-collection. Drainage of swamps, and the destruction of habitats due to the spread of farms and towns have been reported as the main threats (St. George 1999). An example of a species affected by these activities is *Pterostylis cernua*, a recently described species, in which the type locality (a roadside in the South Island), has been already destroyed by roadworks.

Over-collection has caused a substantial impact on some uncommon taxa (Norton *et al.* 1994). Some examples are, *Corybas carsei* (Cheeseman) Hatch., where plants were collected for their flowers at all previously known sites, and now is just restricted to one site in the North Island (Norton *et al.* 1994); and *Thelymitra matthewsii* Cheeseman, where virtually all extant plants were collected by R.H. Matthews prior to its description in 1911, and only recently has it been rediscovered in northern North Island (Wilson & Given 1989).

Despite the well known effect of these menaces and the reduction of orchid populations in New Zealand, studies dealing with basic biology of the species and recovery and conservation strategies for those endangered are scarce. For example, there have only been two studies involving the long-term monitoring of a population, *Thelymitra* "Ahipara" which was relocated by Department of Conservation (de Lange *et al.* 1991) and the effect of fire on *Corybas carsei* by Clarkson *et al.* (1993).

The successful management of orchid populations is not necessarily a matter of maintaining pristine conditions or very old forests growth but often encouraging certain kinds of disturbance so as to maintain a successional or at least a patchy habitat (IUCN/SSC OSG 1996). Johns & Molloy (1983) have proposed this idea in New Zealand as well. These authors suggest that conservation of native second growth on hill country should be the first step to orchid conservation. In New Zealand these areas form some of the best orchid habitats with as many as 20 species on any one site. However, they are all subject to continuous change and if we wish to conserve the orchids in them the vegetation might have to be manipulated in such a way that successional stages and habitats are maintained. Management of these areas may include burning, cutting and grazing. A useful example cited by Johns & Molloy (1983) is the Bankside Scientific Reserve of short-tussock grassland and kanuka shrubland set up on the Canterbury Plains in 1969. The orchid flora then consisted of *Prasophyllum colensoi*, *Microtis unifolia*, *Pterostylis mutica*, *Thelymitra longifolia* and *T. pauciflora*. In the first few years, following the exclusion of animals, the orchid population rose dramatically. Gradually, however, these orchids have succumbed to increasing competition from grasses and shrubs.

Notwithstanding the accumulated knowledge about New Zealand orchids, understanding of their ground-biology is still far from complete. This information is crucial to successfully developing conservations policies and/or restoring populations of threatened species (Falk & Holsinger 1991, Given 1994, Palmer 1995). For New Zealand orchids, studies including taxonomic revision of species and genera to basic biology and species autecology are required.

Despite the fact that the knowledge of orchid pollination has been considered a priority for their effective conservation (Sipes & Tepedino 1995), pollination studies in New Zealand orchids are still far from complete. Pollination mechanisms in orchids are generally highly specialised, and species-specific pollination systems occur in many species (van de Pijl & Dodson 1966). However, specialisation makes species more dependent and vulnerable to loss of the mutualistic partner (Bond 1994, 1995). Disruption of mutualistic pollination relationships may pose a serious threat to species survival; and the effect on species reproductive success has already been observed in several bird-pollinated New Zealand plants (Anderson 1997, McNutt 1998, Robertson *et al.* 1999, Montgomery *et al.* 2001).

In order to assess the probability of extinction due to pollinator failure, Bond (1994, 1995) suggests considering three main aspects of this ecological interaction; the chances of mutualisms failing, the degree of reproductive dependence on the mutualism and the importance of seeds in the demography of the plant studied. The main goal of this thesis is to assess the survival capability of a eight widespread New Zealand orchids (four terrestrial and four epiphytic) by studying their breeding system, pollination success and identifying and evaluating the role of pollinators in sexual reproduction and their impact on these species survival.

II

Pollination of Some New Zealand Terrestrial Orchids

INTRODUCTION

For isolated islands such as New Zealand, a relict or dispersed flora must survive with a similarly restricted suite of pollinators and dispersers (Webb & Kelly 1993). In order to avoid extinction in these environments, plants may adjust their mating-system, evolving from primarily out-crossing ancestors to selfing races or species (Takebayashi & Morrell 2001). Flowering strategies and morphological features of New Zealand terrestrial orchids suggest that self-pollination is common within the group. Molloy (1990) estimated that at least 60% or more of the orchids in the country are predominantly self-pollinated and self-fertilised, features that make New Zealand orchid flora unique.

Species of the genus *Thelymitra* and *Pterostylis* are good examples of self-pollinating taxa (van de Pijl & Dodson 1966, Catling 1990). The first description of mechanical self-pollination in the New Zealand *Thelymitra* dates from more than a century ago (Cheeseman 1881). Since then, obligate autogamy has been considered a rule in the genus. This assumption is based on features that do not necessarily suggest autogamy, such as the unmodified labellum, the perianth extension being sun-induced, but a complete fruit-set of all flowering individuals in the population (Darwin 1877, Cheeseman 1881, Thompson 1881, Molloy 1990, see review in Burns-Balogh & Bernhardt 1988).

Despite the prevalence of self-pollination in the terrestrial orchids and the relative scarcity of insects in New Zealand, the occurrence of insect-dependent species has also been reported (Cheeseman 1873, Darwin 1877, Thompson 1927, Molloy 1990, St. George 1999 and others). The pollination mechanisms evolved in out-crossing species of *Pterostylis* are clear examples. In this genus the labellum is attached to the rest of the flower by a hinge that flicks back into the hood when touched. This action traps the visitor with its back against the column, and it is thrown into contact with the stigma and may pollinate the flower if it carries pollinia from a previous visit (Johns & Molloy 1983).

Frequently, pollination mechanisms in insect-pollinated orchids are highly specialised, and species-specific attraction of pollinators is characteristic of many of the more evolved species of orchids (van de Pijl & Dodson 1966). This kind of limitation of pollinator diversity and specialisation on specific pollinators may enhance fitness by reducing the cost of successfully transferring male gametes between individuals (Tremblay 1992). However, specialisation also makes species more dependent and vulnerable to loss of the mutualistic partner.

Terrestrial orchids are an important component of the orchid flora in New Zealand (over 90 species). Unfortunately, they are also the most threatened within the family. Currently 19 uncommon ground orchid species and four taxonomically undetermined taxa are included in the list of threatened and uncommon New Zealand plants (de Lange *et al.* 1999). Although the main threats to these orchids have been identified (habitat destruction, modification and fragmentation), disruption of ecological interactions with their pollinators and/or associated fungi has not been considered.

Disruption of mutualistic pollination relationships may pose a serious threat to species survival; and the effect on species reproductive success has already been observed in some bird-pollinated New Zealand plants (Anderson 1997, McNutt 1998, Robertson *et al.* 1999, Montgomery *et al.* 2001). There have been few pollination studies on terrestrial orchids in New Zealand. Most of the information available is based on morphological and phenological observations, none of them in great detail and a few involving isolation of plants or hand pollinations (Godley 1979). In order to assess the probability of extinction due to pollinator and disperser failure, Bond (1994, 1995) suggests considering three main aspects of the interaction; the chances of mutualisms failing, the degree of reproductive dependence on the mutualism and the importance of seeds in the demography of the plant studied. This chapter concentrates on the first two aspects by studying the breeding system, pollination syndromes and pollinator-dependence of a select group of terrestrial orchids.

METHODS

Species description

Gastrodia cunninghamii Hook f. (Fig. 1) belongs to a genus of about 15 species distributed throughout the Australasian region (New Zealand, Australia, Malaysia, India and Japan) (Johns & Molloy 1983). It is a terrestrial saprophytic orchid that relies on a mycorrhizal association for its carbohydrate nourishment (Campbell 1962). The plant sometimes reaches a meter in height and bears up 70 flowers on its single stem. The flowers are knobbly and tubular and formed by the fusion of the sepals and petals, which are separated only at their tips. The labellum is attached at the inside of the tube so that only the black-tipped yellow end shows (St. George 1999). *G. cunninghamii* occurs both in the North and South Island, also in Stewart and Chatham Islands (Moore & Edgar 1976, St. George 1999). Although the species is widely distributed, it has a local occurrence (Johns & Molloy 1983). The species is generally associated with *Nothofagus* forests, however sometimes appears in association with exotic species (Moore & Edgar 1976, St. George 1999).

The genus *Thelymitra* is distributed throughout Australasia and Malaysia and is regarded as being primitive in the Orchidaceae due to its reversion to an actinomorphic state (van der Pijl & Dodson 1966). The labellum is not or hardly differentiated from the other perianth segments and the flowers are of bright colours, generally blue or pink (Jones 1981). The New Zealand species are all either Australian, or derived from Australian species, and were probably windborne originally across the Tasman Sea (Hatch 1949). One of the most widespread species in New Zealand is *Thelymitra longifolia* J. R. et Forst. f. (Fig. 1). *T. longifolia* usually found in clay banks, grasslands and under scrub. The plant can be quite variable in shape and size, the flowers are white or tinged pinkish. The inflorescence bears 1 to 20 flowers of *ca.* 1.5cm across. The petals and labellum are alike. The column-arms are round in cross section each with numerous trichomes. *T. longifolia* is widely distributed in the North and South Island and also occurs in Stewart Island, Chatham and Auckland Island (Moore & Edgar 1976, Johns & Molloy 1983).

With over 150 species and several natural hybrids, *Pterostylis* is found in the Australasian region, with species in Papua New Guinea, New Caledonia, Australia and New Zealand (St. George 1999). Species included in this study are *P. alobula* (Hatch) L.B. Moore and *P. patens* R. Br (Fig. 1). When flowering, *P. alobula* is *ca.* 15cm height with sessile, bracteated leaves. The plant usually bears a solitary and erect flower. The labellum is narrowed in its upper half, with slightly swollen, truncate or crenulated tip. *P. patens*, in contrast, is a robust grass-leafed plant of montane and subalpine forests. When flowering the plant may be 15 to 100cm tall, topped by a single flower considerably bigger than *P. alobula*, 5 X 3cm. The most distinctive character of this species is the curvature of the dorsal and lateral sepals. The tip of the dorsal sepal is curved downward, while lateral ones are strongly recurved, often meeting behind the base of the dorsal sepal and below the ovary. Both species are endemic to New Zealand.

Figure 1: *Pterostylis alobula* (top left), *Gastrodia cunninghamii* (bottom left), *P. patens* (centre) and *Thelymitra longifolia* (right).

Study sites

Paengaroa Scenic Reserve (39°38'S / 175°43'E) (Fig. 2): The reserve occupies an area of 101 ha. It is a forest remnant situated on the banks of the Hautapu River in the Rangitikei Ecological Region. The reserve is entirely surrounded by farmland although other small forest remnants occur nearby. The river terrace forest is a typical podocarp/broad-leaved forest dominated by *Dacrycarpus dacrydioides*, *Prumnopitys taxifolia*, *Podocarpus totara*, *Sophora microphylla*, *Pittosporum eugenioides* and *Plagianthus regius* while the understorey vegetation comprises mainly divaricating shrubs. The orchid *G. cunninghamii* was studied in this site.

Takapari Road, Ruahine Forest Park (40°4'S / 175°58'E) (Fig. 2): This Park is part of the Hawke's Bay and the Manawatu - Wanganui regions. Takapari Rd. is located in the southern Ruahine Range. This area is well defined by natural topographical features that separate it from the rest of the Ruahine Range. Vegetationally the southern Ruahine can be defined in three main altitudinal vegetational belts; podocarp-hardwood forest, *Weinmannia* forest or its scrub replacement, and *Plagianthus regius* scrub (van Essen 1992). Populations of *Pterostylis patens* occur all along the first two belts. Some of the species occurring in the area are *Dacrydium cupressinum*, *Ripogonum scandens*, *Weinmannia racemosa* and *Pseudowintera colorata* (for an extended list of species in the area refer to van Essen 1992).

Forest Hill Road (40°21'S / 175°32'E) (Fig. 2): Study sites are beside a public road, located *ca.* 10km from Palmerston North city. There are several populations of *Pterostylis alobula* along the roadside, some of them among remnants of native shrubs of *Kunzea ericoides* and the exotic *Cytissus scoparius* and under a pine tree plantation.

Pahiatua Track (40°24'S / 175°42'E) (Fig. 2): The study site is located *ca.* 15km from Palmerston North city, on the roadside. This site supports populations of three orchids species, *Orthoceras novae-zelandiae*, *Thelymitra longifolia* and *Microtis* sp., as well as a secondary growth scrub dominated by *Cytissus scoparius*. A *Pinus radiata* plantation tops the bank. Only *T. longifolia* was studied in this site.

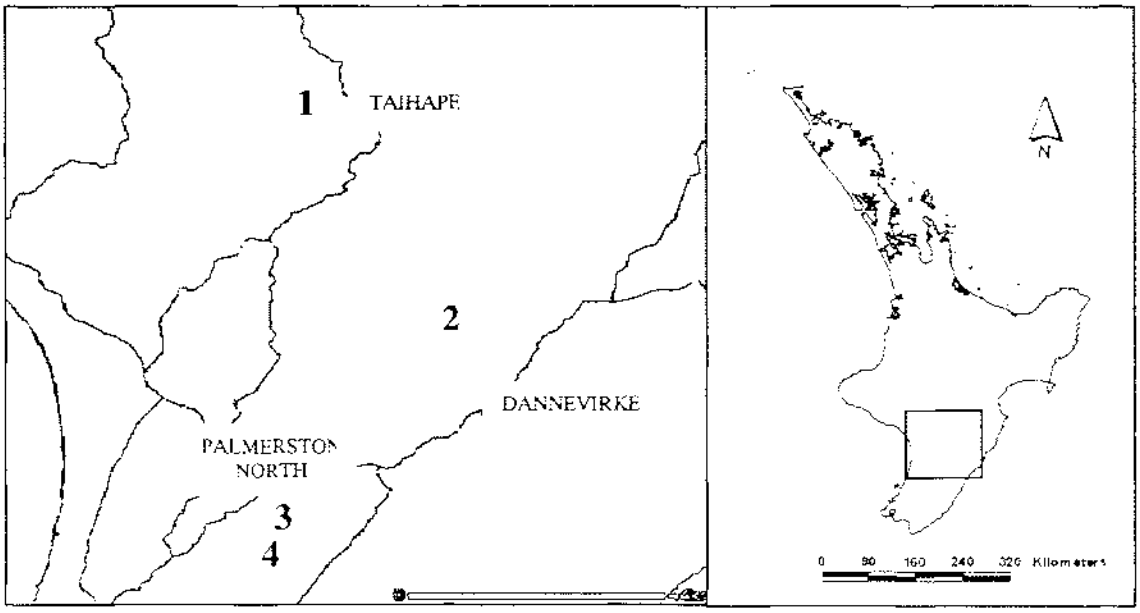

Figure 2: Locations of the populations of *Gastrodia cunninghamii*, *Pterostylis patens*, *P. alobula* and *Thelymitra longifolia* where pollination experiments were conducted (1: Paengaroa Scenic Reserve; 2: Ruahine Forest Park, 3: Forest Hill Road and 4: Pahiatua track).

Breeding system, fruit-set and self-compatibility

In order to determine the breeding system and presence of self-incompatibility in these orchids, the following hand-pollination treatments were performed during the flowering season of the year 2001 and part of the 2002:

Direct autogamy: Flowers were bagged before anthesis until senescence, excluding pollinators. This treatment tests whether fruit-set occurs by automatic self-pollination.

Apomixis: Before anthesis, the entire pollinarium was removed with forceps, and the flowers were then bagged until senescence. The treatment allowed determination of agamospermy.

Self-compatibility: Pollinarium was removed and flowers bagged until stigma receptivity. Flowers were considered receptive when the stigmatic surface was sticky and moist. Flowers were then pollinated with the whole pollinarium from another flower on the same inflorescence and bagged again until wilting. Pollinia from the same flowers were used in hand-self pollination of *Pterostylis* species.

Cross-pollination: Same as above, but flowers were pollinated with pollen from another individual.

Natural pollination: Floral buds were tagged and allowed to develop to fruit under natural condition of pollination.

In *G. cunninghamii* the entire perianth was removed before anthesis using a scalpel. Flowers were then emasculated using forceps and bagged until stigmas were ready to be pollinated. This was done for all treatments but direct autogamy and natural pollination.

Before dehiscence, capsules were collected and stored in paper bags. The presence of self-incompatibility was scored using the self-compatibility index (S.C.I.: number of fruits produced by hand self-pollination/number of fruits produced by hand cross-pollination). Fruit-set was analysed by means of generalised linear model with a binomial error distribution and logit link function using SAS (SAS Institute 2001).

Pollen and ovule numbers and pollen/ovules ratio (P/O)

P/O ratios have been considered as a good indicator of plant's reproductive strategies (Cruden 1977). To determine the P/O ratio, the entire pollinia from ten flowers, each from a different individual, were collected. Pollinia were macerated and stained with the Alexander's differential dye (Alexander 1980) for counting. Pollen grains were counted using a haemocytometer. Pollinia from *G. cunninghamii* were soaked in KOH 10% for 5 hours before macerating them. Ovules in the Orchidaceae do not finish their development until pollination has taken place (Neiland & Wilcock 1995), making their management and observation difficult. For this reason capsules in early ripening stage were used to determine ovule numbers (Cruden, personal communication). Ovules from one carpel were sub-sampled in 500μL of water and counted under the stereomicroscope. P/O ratios were obtained following Cruden (1977).

Osmophores

Floral fragrances are important attractants for pollinators and, in some species, important sexual isolating mechanisms. Floral fragrances are produced by scent glands called osmophores. Since osmophores may or may not be morphologically and anatomically distinguished from adjacent tissues in the perianth, at least 10 fresh flowers of each species were submerged in a stain bath of 1:1,000 neutral red: tap water for two to 12 hours following Stern *et al.* (1986) and Vogel (1990). According to these authors, those tissues with presumptive osmophoric function stain deep red while other tissues remain unstained. Neutral red is a vital stain that is absorbed by, or reacts with, substances in the cell sap that are involved in the scent production. Staining was assessed after one, two, four, six and 12 hours. After recording the areas with a positive reaction to the stain, they were photographed using a stereomicroscope (Zeiss MC80).

Phenology and pollinators observations

Phenological phases, growth period, flowering and fruiting were observed in tagged plants both in the field and shade-house at the Institute of Natural Resources, Ecology, Massey University. Floral longevity of *P. alobula* was observed in flowers tagged before anthesis and visited daily until senescence. In the remaining species, phenological stages were assessed by weekly visits to the study sites.

Pollinator activity was surveyed while fieldwork was conducted. Insect observations were conducted only during the day from 10:00 am to ~ 18:00 pm and under different weather conditions and throughout the flowering season. Special attention was paid to the insect's behaviour while visiting the flowers. Some of the insects were collected and identified. Pollinators were ranked according to their pollinator efficiency following Adams & Lawson (1993).

RESULTS

Phenology and pollinators

Figure 1 is based on observations conducted during part of the year 2000 and mainly 2001. In general, flowering periods last for at least 2 months in *G. cunninghamii* and *T. longifolia*. In the winter-flowering species *P. alobula* the flowering period extends for almost four months while in the summer flowering species *P. patens* only one month (Fig. 3).

The saprophytic *G. cunninghamii* starts sprouting through the ground during mid November like an "asparagus shoot". Flowering spikes can be observed by early January. Flowering is acropetal and, unlike most of the Orchidaceae, flowers are non-resupinated (*i.e.* the labellum is in the upper side the flower). Flowers change their orientation during flowering and fruiting stages. Before anthesis floral buds are pointing downwards, during late anthesis and after pollination takes place they are pointing upwards. Once ripe, capsules are pointing downwards again. Capsules take almost two months until dehiscence occurs.

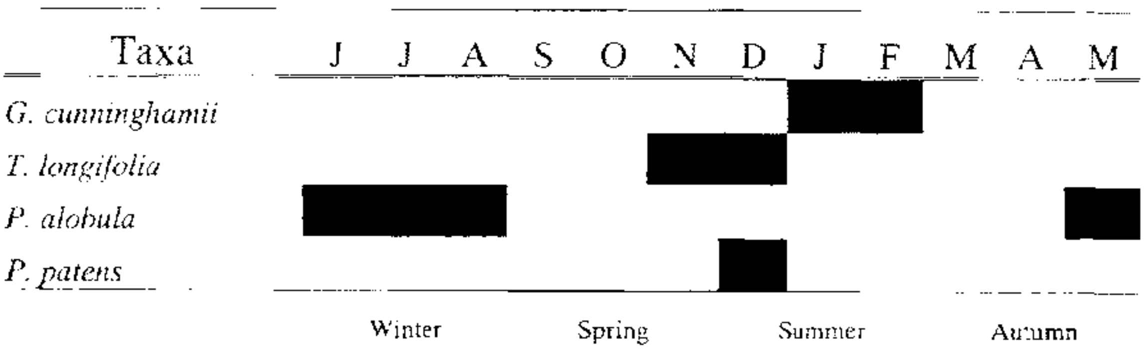

Figure 3: Flowering times of some New Zealand terrestrial orchids *Gastrodia cunninghamii, Thelymitra longifolia, Pterostylis alobula* and *P. patens* in the North Island.

T. longifolia flowers from early November to early January, forming a flowering spike with 3 to 20 flowers, flowering is acropetal. Flower life span is rather short, ± 5 days. Although floral senescence may be triggered to some extent by the occurrence of self-pollination, flowers with pollinia deposited on the stigma and still in total anthesis were observed in numerous occasions. As reported before in the literature (e.g. Catling 1990), the anthesis of the perianth is subject to environmental conditions, *i.e.* the flowers only open in sunny and warm days.

The winter flowering "green hood orchid" *P. alobula* flowers from mid May to early August. Flowering within the population is gradual, so individuals at different developmental stages can be observed and out-crossing is promoted. Floral life span in this species is long - marked flowers often lasted over a month. Pollination (*i.e.* pollen deposition) does not seem to trigger floral senescence. Flowers that were hand cross- and hand self-pollinated did not wilt until after a month (26/06/2001 to 27/07/01). Further studies should be conducted in order to understand the physiological events triggering floral senescence in this species. Capsules complete their development after three months, September-October, and seeds are dispersed through three splits along the ovary. Finally, *P. patens* flowers only during December. Contrary to *P. alobula*, floral life span is strongly affected by pollination. Fifteen days after hand-pollinations were conducted, flowers had wilted and the stigma and ovary had already swelled. About two months later capsules were fully developed and seed dispersal was already occurring (mid February).

Although the activity of pollinators was surveyed in all four taxa, insect visitation was observed only in *P. alobula*. Male fungus gnats of the genus *Zygomyia* sp. (Mycetophilidae) were found both alive and dead inside the flowers on several occasions, and some of them were found imprisoned by the column wings and in a position ready to remove the pollinia (Fig. 4). Pollination in the genus *Pterostylis* has been previously described (Johns & Molloy 1983 and Proctor *et al.* 1996). In this genus the labellum is attached to the rest of the flower by a hinge that flicks back into the hood when touched. This touch-sensitive movement is produced by rapid turgor changes in the hinge. This action traps the visitor with its back against the column, and if the insect is carrying pollinia these are thrown into contact with the stigma. The column of these species has two wings near the tip that project towards the lip. The only way the insect can escape is by pushing between these wings with its back towards the column, picking up pollinia on its thorax as it does so (Fig. 4). Despite no insects were observed visiting

P. patens, pollination by fungus gnats might be likely here too (two other fungus gnats were collected in a third species of *Pterostylis* flowering in the area).

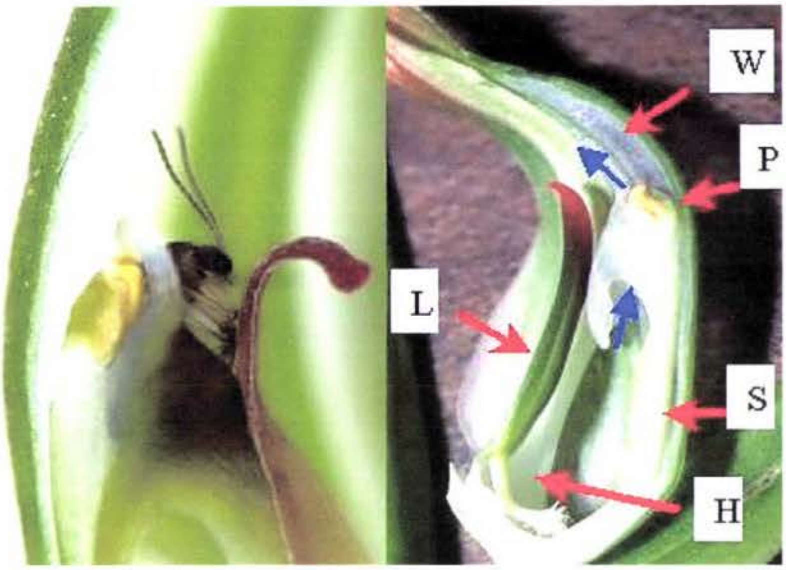

Figure 4: *Pterostylis alobula* with *Zygomyia* sp. (Mycetophilidae) imprisoned by the column wings and the tip of the labellum and detailed of the internal floral structures of a "green-hood" orchid (**L**: labellum; **H**: hinge; **S**: stigma; **P**: pollinia; **W**: window-panes. Blue arrows indicate the path used by the fungus gnat when escaping from the trap).

Finally, aphids were usually found inside the flowers of *G. cunninghamii*, perhaps attracted to the "pseudopollen" this species forms at the base of the labellum. The function aphids may have in the pollination of this orchid is unknown.

Osmophores

Despite the fact that these four species are scentless to the human perception, neutral red positively stained several areas of the perianth (Table 1). Evidence of positive staining was observed after two hours of soaking. The presumptive osmophoric areas of these orchids were mainly found around the column, lip and sepal tips. The latter were particularly noticeable in both species of *Pterostylis*. In these species scent glands seem to be located in the same areas of the perianth; column wings and the tip of lateral sepals and labellum. Positive staining of structures such as the stigmatic surface and pollinia were not considered as osmophoric due to their absorbent nature (Lehnebach 1998).

Table 1: Absorbance of neutral red by the floral structures of four New Zealand terrestrial orchids.

Species	Column	Labellum	Petals	Lateral sepals	Dorsal sepal
G. cunninghamii	No reaction	Ridges & warts on the lip	Positive reaction was observed in the points were sepals and petals join to form the tubular perianth		
T. longifolia	Column wings, cilia and around the pollinaria	No reaction	No reaction	No reaction	No reaction
P. alobula	Column wings	Tip of the lip	No reaction	Sepal tips	No reaction
P. patens	Column wings	Tip of the lip	No reaction	Sepal tips	No reaction

Pollen and ovule numbers and P/O ratios

All species studied are monandrous orchids (*i.e.* one anther per flower), therefore the number of pollen presented in the Table 2 correspond to the number of pollen grains per flower. The type of pollinia observed in these orchids varies in cohesion and aggregation. In *G. cunninghamii* pollinia are formed by several massulae weakly held together by viscin threads. Tetrads within the massulae are strongly bond together; therefore soaking in KOH (10%) was essential to count them. Pollinia in *T. longifolia* are rather soft and powdery, and weakly joined to the viscidial disc. In this species pollen grains are found as monads rather than tetrads. Pollen grains of the species of *Pterostylis* are presented as tetrads (*P. alobula*) and monads (*P. patens*). This genus has soft pollinia and they may be removed either as a coherent unit or as single pollinium.

Among the terrestrial orchids, *P. alobula* had the highest number of pollen grains per flower (366 857± 3831.14), whilst the lowest was observed in the self-pollinating species *T. longifolia*. *G. cunninghamii* had the highest number of ovules per flower *ca.* 11 400. Pollen/ovule ratios (P/O) calculated fluctuated from 320: 1 in *P. alobula* to 24:1 in *T. longifolia* (Table 2).

Table 2: Pollen and ovule numbers (mean ± standard error, n=10), P/O ratios and tetrad/monad sizes of four terrestrial New Zealand orchids.

Species	Pollen # per flower	Ovule # per flower	P/O Ratio	Tetrad & Monad size (μm)
G. cunninghamii	283 821 ± 3721.39	11 400 ± 191.25	25: 1	44.6 X 25.4
T. longifolia	114 165 ± 9099.94	4 842 ± 213.00	24: 1	26.6
P. alobula	366 857 ± 3831.14	1 147 ± 7.70	320: 1	42.3 X 27.8
P. patens	262 837 ± 3419.66	6 333 ± 801.30	42: 1	24.6

Breeding system and genetic incompatibility barriers

Detailed figures regarding treatments, number of flowers pollinated and fruits yielded are presented in the Appendix 2. Overall, *T. longifolia*, had the highest percent of fruit-set after hand self-pollination (70%), whereas *P. alobula* the lowest (34%). Fruit-set after hand cross-pollination across species ranged from 44 to 83% (*P. alobula* and *T. longifolia*, respectively). Fruit-set percentages obtained in this treatment were higher than those obtained after hand self-pollination in all species but *P. patens* but these differences are not statistically different (Appendix 4). *G. cunninghamii* showed the lowest fruit-set after cross-pollination, only 20% of the flowers produced fruits (Fig. 5).

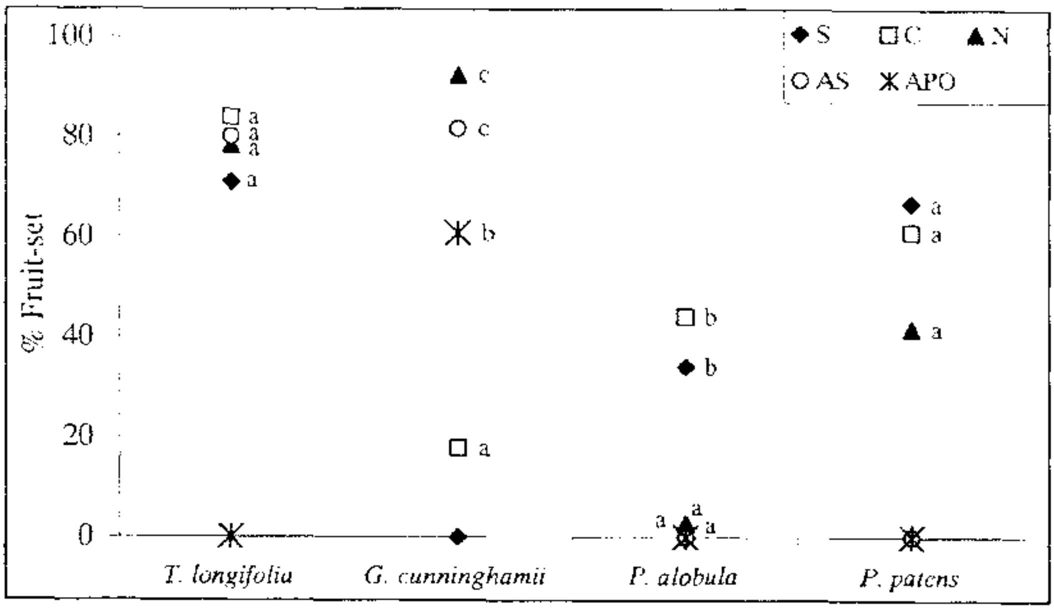

Figure 5: Percentage of fruit-set obtained in the four species studied (**S**: hand self-pollination; **C**: hand-cross pollination; **N**: natural pollination; **AS**: automatic self-pollination; **APO**: apomixis). Shared letters within species indicate means that are not significantly different.

Automatic self-pollination occurs in *G. cunninghamii* and *T. longifolia*- 80% of un-manipulated flowers, bagged until senescence produced capsules (Fig. 5).

Natural fruit-set was extremely high in *T. longifolia* and *G. cunninghamii*, reaching 78 to 90% respectively. Conversely, natural fruit-set was low in both species of *Pterostylis*. The winter flowering orchid *P. alobula* showed a remarkably low fruit-set; only two out of 73 flowers (2.7%) tagged in the wild during the years 2000 and 2001 produced capsules (Fig. 5).

Response of *G. cunninghamii* to the pollination treatments is rather unclear. This orchid did not produce capsules through hand self-pollination treatments but through automatic self-pollination, cross-pollination and agamospermy.

The species *T. longifolia*, *P. alobula* and *P. patens* are self-compatible. The self-compatibility index (S.C.I.), a ratio between fruits set after self-hand pollination and number of fruits yielded after hand-cross pollination, obtained for all taxa are shown in the Table 3. *G. cunninghamii* was not included in this analysis for the reasons explained above.

Table 3: Self-compatibility index (S.C.I.) obtained in the three species of terrestrial orchids (a score of 1.0 would indicate equal self fruit-set to outcross, a lower score a trend to self-incompatibility).

Taxa	S.C.I.
T. longifolia	0.68
P. alobula	0.77
P. patens	1.17

DISCUSSION

Reproductive biology

Pollination treatments in this group of terrestrial orchids confirmed the absence of genetic incompatibility in three of the species studied, *T. longifolia*, *P. alobula* and *P. patens*. Self-compatibility seems to be a generalised condition in the flora of New Zealand (Godley 1979), and is likely to be related to the unspecialised pollinating fauna and the long isolation of the country, both of which could impose constraints on dispersal and establishment of self-incompatible taxa (Webb & Kelly 1993). Additionally, self-compatibility in terrestrial orchids has been frequently demonstrated (Thien & Marcks 1972, Mehrhoff 1983, Beardsell *et al* 1986, Sydes & Calder 1993, Sipes & Tepedino 1995; Clayton & Aizen 1996, Galetto *et al.* 1997) and although this may suggest it is a common condition in the Orchidaceae there are no firm statistics (Dressler 1993).

Despite these three orchids being self-compatible, their reproduction relies on contrasting reproductive strategies. *T. longifolia* is predominantly self-pollinated, whereas *Pterostylis* species are cross-pollinated and have an absolute dependence on insects to achieve pollination. The demonstration of self-pollination in the genus *Thelymitra* is not new, in fact autogamous species in this genus probably number well over half (Jones 1981). However, despite the high fruit-set produced through automatic self-pollination in *T. longifolia* (nearly 80%), cross-pollination may be involved in fruit-set as well. In this study, fruit-set was higher after hand cross-pollination than through hand self-pollination (83.87 and 70.83%, however non statistically significant), suggesting that *T. longifolia* might favour to some extent fertilisation by pollen from a different individual than self.

It is likely that self-pollination has recently evolved in *T. longifolia* as a "fail-safe mechanism" due to the lack of suitable pollinators, climatic conditions during flowering, or high competition for them at certain times of the year (*e.g.* Hogan 1983). If cross-pollination does not occur, flowers will self-pollinate. This may be considered as bet-hedging strategy in which the reproductive output of *T. longifolia* is optimised when environmental conditions are rather fluctuating (Wells & Lloyd 1991). Lloyd

(1992) and Newstrom-Lloyd & Ward (2001) have described this "delayed self-pollination" as mechanism to assure reproduction in those species arriving by long distance dispersal, predicting that it will be common in the New Zealand flora.

The presence of functional floral attractants to promote allogamy and records of burrowing bees (*Leioproctus fulvescens*) visiting and removing pollinia of *T. longifolia* in northern New Zealand populations (Jones 1981) reinforce this idea. Neutral red positively stains the trichoma brushes of the column, suggesting the presence of active scent emission in the area. Bernhardt & Burns-Balogh (1986) and Copper & Calder (1990) propose that these structures attract pollinators by resembling rewarding stamens (pseudanthery) of other rewarding co-flowering species (for more about food deception syndromes and pseudanthery refer to Dafni (1984) and Dafni & Bernhardt (1990)). It is likely that the trichomes observed in *T. longifolia* function as olfactory and visual cues to attract pollinators promoting cross-pollination. The presence of osmophores in the column of *Thelymitra* species has been observed in the Australian partially autogamous species *T. nuda* (Bernhardt & Burns-Balogh 1986) and in the out-crossing *T. epipactoides* (Copper & Calder 1990).

Pollination studies in the Australasian genus *Pterostylis* have been scanty and as a result generalisations are plentiful, most of them incorrect (Jones 1981). The genus appears to belong to a guild of terrestrial, winter early-spring flowering orchids (*Acianthus* and *Corybas*) that are pollinated exclusively by microdipterans in the families Culicidae, Phoridae and Mycetophilidae (Bernhardt 1995a). In the autumn-winter flowering *P. alobula* pollination is achieved by male fungus gnats of the genus *Zygomyia* sp. (Mycetophilidae) and although no insects were observed in this study visiting or pollinating the summer-flowering *P. patens*, pollination by male fungus gnats is strongly suspected. This supported by observations on a third co-flowering species of *Pterostylis* in the same area, as two male fungus gnats, *Aneura longipalpis* (Mycetophilidae) and *Cerotelion* sp. (Keroplatidae) were discovered inside the flowers of *P. australis*.

The flowers of *Pterostylis* in New Zealand are trap-flowers, similar in construction to "kettle trap" flowers studied by Vogel & Martens (2000) in the genus *Arisaema* (Araceae) also pollinated by fungus gnats and to other flytraps such as *Sarracenia* and *Nepenthes* (van der Pijl & Dodson 1966). However, the kettle trap in *Pterostylis* is not necessarily lethal. In *Pterostylis* the prospective pollinator is trapped between the labellum lamina and the column wings and rostellum for 30-90 seconds until the labellum hinge begins to reset spontaneously (Jones 1981).

The attractant mechanism used by these deceptive nectarless orchids is still unclear, and several hypotheses have been proposed (see van der Pijl & Dodson 1966, Jones 1981, Bernhardt 1995a, b). Vogel (1990) suggests that species pollinated by fungus gnats tend to produce greenish to rusty to deep-iodine-coloured flowers that smell like carrion or ripe fungi. Hyett cited in van der Pijl & Dodson (1966), suggests that the genus *Pterostylis* is almost entirely sapromyophilous, pointing out the strongly unpleasant odour of some Australian species such as *P. mutica*. However, emission of carrion-like or fungi scent has never been reported in any of the New Zealand *Pterostylis* and was never perceived in *P. alobula* or *P. patens*.

Alternatively, Jones (1981) suggests light attraction. Light has a particular attraction for dipterans and it has been suggested that these orchids may be "window flowers" with the clear crystalline panels in the hoods concentrating the light on the inside of the flower and thus attracting the fungus gnats (Jones 1981). However, presence of these structures does not explain the attraction of the fungus gnats into the flower, but their movements inside of the trap. The presence of colourless translucent areas in the perianth such as those in *P. alobula* and *P. patens* has been reported in other kettle trap flowers (Dafni 1984, Vogel & Martens 2000) (Fig. 4). Dafni (1984) and Vogel & Martens (2000) explain that since flies are positively phototropic once inside the flower trap, they will try to escape through the trap's entrance before reaching the reproductive organs. The light that comes through these "window-panes", usually located at the bottom of the trap and close to the reproductive organs, will lure the insects deep into the trap assuring insect visitation.

Pseudocopulation has been considered the most likely pollination system of this genus. Visits by male fungus gnats to the flowers of the Australian species *P. boormanii* (Bates 1977), *P. rufa* (Beardsell & Bernhardt 1983) and *P. curta* (Bernhardt 1995a) support this idea. Furthermore, Bernhardt (1995a, b) proposes that the labellum lamina in *Pterostylis* is ornamented and coloured (and also scented) to resemble a female gnat. Unfortunately, this is not likely to explain the attraction of fungus gnats in *P. alobula* and *P. patens*, since these plants belong to a different section of the genus, in which the labellum is neither exposed or insectile as those observed by Bates (1977) and Beardsell & Bernhardt (1983) (*c.f. P. tristis* or *P. tanypoda* in New Zealand).

Considering the ideas presented above and the observations in this study of *P. alobula* and *P. patens* and that all fungus gnats collected here were male, I propose the attraction of fungus gnats by sexual deceit seems the most plausible. Sexual deception exploits the reproductive stimuli of pollinators by the emission of odours that probably act as a species-specific sexual attractant. This would explain the presence of osmophores observed in the sepal tips, labellum and column wings of *P. alobula* and *P. patens*. The events leading to the pollination of these species would include: attraction of fungus gnats by volatile sexually stimulating signals produced in the flower (osmophores), the insect lands in the flower and triggers the labellum, this action imprisons the insect in the floral chamber, the presence of colourless translucent areas in the perianth guides the insect deep in the trap where the stigma is located. From here the events are the same as described by Johns & Molloy (1983) and Proctor *et al.* (1996). Pollination by sexual deception seems to be limited to the Orchidaceae and rather common in the family (Dafni 1984). It is believed that this system may represent an intermediate stage in pseudocopulatory systems toward true pseudocopulation (Dafni & Bernhardt 1990).

Despite this apparently specific and efficient system, natural fruit-set was low in both species and particularly in *P. alobula* (2.7%). Sabat & Ackerman (1996) suggest that xenogamous orchids usually have a low fruit-set, less than 10%. Fruit-set in xenogamous plants may be limited by pollinator availability or the amount of resources allocated to reproduction (Primack & Hall 1990, Kearns & Inouye 1993). But hand pollination increased fruit set considerably, so resources are not limiting fruit production.

Low fruit set in *P. alobula* may be explained by the low availability of pollinators, damage by herbivores and effect of the accumulation of heterospecific pollen on the stigmas. Flowering in *P. alobula* peaks during the New Zealand winter months of June and July. During this period climatic conditions are harsh, and insect activity is dramatically reduced. Meteorological factors may have a detrimental effect in the reproductive success of orchids (Hogan 1983). Most of the populations of *P. alobula* occur in open areas where they are usually subject to damage by frosts. Low pollinator availability may also explain the floral persistence observed in this orchid, *ca.* a month. Long-lived flowers in the Orchidaceae have been interpreted as an adaptation for highly specialised pollination systems where the reproduction depends upon specific pollinators, that may be relatively uncommon (Primack 1985). The effect of pollinator availability and their pollinating efficiency will be explored further in chapter IV.

Floral herbivory can affect reproductive success of plants directly by the consumption of reproductive organs and indirectly through the reduction of attractiveness to pollinators of flowers with damaged petals (Malo *et al.* 2001), in the particular case of *P. alobula* by damaging the trap system. Floral and capsule predation by the leaf-roller caterpillar of the native Lepidotera *Cnephasia jactatana* had a considerable effect in the final natural fruit-set and also damaged some of the hand-pollination treatments. This species is an important orchard pest in New Zealand, dramatically damaging kiwifruit soon after fruit-set (Steven 1990).

Pollen of the exotic species *Pinus nigra* was observed on stigmas of *P. alobula* in several populations. Although the effect in the reproductive success was not quantified, contamination of the stigmas by heterospecific pollen may be detrimental for fruit set by reducing the stigmatic area available to receive conspecific pollen (Neiland & Wilcock 1999). Lastly, low fruit-set in these species, both under natural condition and through hand-pollination treatments (none of the treatments reached 100% of fruit-set) could be explained by limitation of resources. But this is more likely to apply for long-lived orchids (*Pterostylis* forms a new tuber every season).

The breeding system of the saprophytic *G. cunninghamii* is difficult to assess due to the nature of the data obtained in this study. In general this species has been considered as autonomously autogamous. Morphological adaptations such as an extremely short column that places the pollinia immediately above the stigma reinforce this idea. Fruit set was observed after flowers were bagged but not after hand self-pollination. The failure of the treatments conducted in this species may be attributed to the inappropriate handling of the pollen or the stress caused by removing the entire perianth. Loss of viability of the pollen seems to be the most likely explanation, removal of floral structures in other orchid species have been performed before by the author in order to pollinate the flowers and fruit-set has never been dramatically affected (and this is also rather common procedure among orchid horticulturists).

Another indicator of the reproductive strategy used in flowering plants are P/O ratios. Cruden (1977) predicts that P/O ratios in autogamous and cleistogamous species are lower than in xenogamous species. This based on the idea that in autogamous species the likelihood of arrival of a considerable amount of pollen to the stigmas is more certain. However in orchids, and other plants with pollen clumped in pollinia, P/O ratios do not follow Cruden's principle, and they are usually lower than those observed in cleistogamous plants (Cruden 1977). Except for *P. alobula*, P/O ratios observed in this study fluctuated between 24 to 42: 1. A similar range of P/O values (1.2:1 to 36: 1) have been observed in a range of xenogamous and autogamous European orchids (Neiland & Wilcock 1995, Nazarov & Gerlach 1997).

Neiland & Wilcock (1995) proposed that P/O ratios in orchids are more likely to be related to pollinator efficiency and the mode in which the pollinia are removed than to the breeding system. Thus lower P/O ratios are expected to be associated with orchids that are visited by Hymenoptera rather than Diptera, which forage in a more random way (Neiland & Wilcock 1995). This might explain the extremely high P/O ratio observed in *P. alobula*. In this orchid fungus gnats removed only part of the pollinia. Van der Pijl & Dodson (1966) and Bernhardt (1995a) suggest this is an adaptive feature since such a small dipteran could not support the weight of four pollinia in flight. Consequently if only a portion of the pollinia is removed, a high count of pollen grains in this species is highly advantageous as it may allow pollinia to father seeds on several subsequent flowers and still have a prospect of fertilising all the ovules.

Conservation implications

In general, the survival of plants will vary on how much they depend on biotic and abiotic environmental elements. Although habitat destruction, modification and fragmentation have been considered as the main threats to orchids survival (IUCN/SSC OSG 1996), disruption of ecological interactions such as pollination, seed dispersion and mycorrhizal associations may be just as threatening.

The species more likely to be affected by pollinator failure are *Pterostylis alobula* and *P. patens.* These species (especially *P. alobula*) have a species-specific pollination syndrome by male fungus gnats. Moreover, both species are obligate xenogamous and depend absolutely on their pollinator to set-fruit. Therefore, habitat transformations may have a considerable effect in the reproductive success of these species, and it is likely that the lower fruit-set observed in *P. alobula* in contrast to *P. patens* is the result of the habitat clearance and fragmentation; *P. alobula* was studied in highly disturbed area whereas *P. patens* in a protected area. These species have some compensatory mechanisms to increase their survival capability such as clonal propagation and production of considerable amount of seeds per capsule. Arditti (1967) reports that orchids may produce 1,000 to 6,000,000 seeds per capsule. Therefore, the low fruit-set observed in *Pterostylis* species may not drastically jeopardise their survival.

In contrast, *Thelymitra longifolia* is the least vulnerable to mutualism disruption, except for the mycorrhizal association. This orchid is a facultative xenogamous and fruit-set is predominantly produced by self-pollination; therefore its dependence to pollinators is nil. Although self-pollination may assure fruit-set in *T. longifolia* and maintain genotypes already adapted to the local environment, it might be a disadvantageous strategy when progeny face changes in the environmental conditions. Then, the protection of *T. longifolia* will dependent on the maintenance of a stable habitat or successional stage. Fire, controlled grazing and winter-spring waterlogging have been proposed before as management actions for other New Zealand and Australian orchid species (Clarkson *et al.* 1993, Sydes & Calder 1993).

Finally, despite mechanisms such as clonal propagation and large seed production promote recruitment and the establishment of new populations; compensating for the risk of extinction, orchid colonisation is significantly site-limited (Calvo 1993). Orchid seeds are heterotrophic, and will germinate only if a precise mycorrhizal infection takes place, establishment will be restricted only to sites where the appropriate mycorrhiza is present. Consequently, survival of these species will depend on the appropriate functioning of another ecological interaction, *i.e.* mycorrhizal dependency and nutrition - a field largely unstudied in New Zealand.

III

Pollination of Some New Zealand Epiphytic Orchids

INTRODUCTION

Because of their unique combinations of vegetative and reproductive features and the extraordinary diversity promoted by specialised pollination syndromes, orchids are one of the commonest epiphytes in the tropics (Benzing 1989, 1990). Evolution of pollination syndromes in epiphytic plants has been profoundly influenced by constraints imposed on plant size and distribution by the epiphytic habitat (Ackerman 1986). In epiphytes, plant size is usually small and this affects the floral display and reward production. Epiphytes are often hyperdispersed and this influences pollinator foraging behaviour. Consequently, it may be expected that the pollination of epiphytic flowering plants is more specialised than densely distributed terrestrial species. This is known as the dispersion-specialisation hypothesis (Ackerman 1986).

Ackerman (1986) and Benzing (1990) proposed the evolution of three pollination strategies in epiphytic flowering plants, trap-lining pollination, and, in many Neotropical orchids, deceptive pollination and pollination by male scent-collecting euglossine bees. In the first system, plants produce few flowers per day for long periods and the flowers contain a high quality reward that is exclusively available to large trap-lining pollinators. The second is based on deceit; usually the deception system exploits sexual, feeding or egg-laying behaviours of their pollinators. The third strategy is pollination by male euglossine bees. In this system flowers produce fragrances that serve as specific attractants and perhaps precursors to sex pheromones of the bees, pollination occurs when male bees visit the flowers to collect these fragrances (Ackerman 1986). Benzing (1990) suggests that if these plants were utilising generalised pollinators instead, or were less attractive to them, reproductive output would be dramatically reduced.

In the temperate forest of New Zealand epiphytic orchids are represented by four genera (*Bulbophyllum*, *Drymoanthus*, *Earina* and *Winika*), all of them basically tropical in origin (Hatch 2000). Among these, *Earina* and *Winika* are the most conspicuous. As in the New Zealand terrestrial orchids, pollination syndromes of epiphytic orchids are still largely unknown and speculation about their pollinators and breeding system is abundant in the literature. Insect pollination has been generally suggested for New

Zealand *Earina* (Thompson 1881, Johns & Molloy 1983, Molloy & St George 1994, St. George 1999), but never confirmed. The breeding system of *Earina* species is also unclear. Although Thompson (1927) reported the lack of fruit-set after hand cross- and hand self-pollination in *E. autumnalis*, Molloy (1990) suggested self-pollination is the main source of fruit-set. Similarly, the reproductive biology of *Winika cunninghamii* is far from understood, and the inconsistency in the literature is remarkable. Johns & Molloy (1983) suggested that *W. cunninghamii* relies on pollinators, as plants in insect-proof cages do not set seeds, nevertheless Clements *et al.* (1997) indicate that automatic self-pollination is likely to be responsible for fruit-set. Unfortunately, none of these authors explain the nature of the observations conducted and/or the data collected to support these statements.

In order to determine the pollination syndromes that have evolved in some New Zealand epiphytic orchids, this study will examine the reproductive biology and pollinators associated to *Earina* and *Winika*. Also, this chapter will attempt to explore the dispersion-specialisation hypothesis proposed by Ackerman (1986) with regard to the pollinations systems New Zealand epiphytic orchids display. Following Bond (1994, 1995), the risk of extinction of these orchids will be evaluated by addressing the probability of pollinator failure and their reproductive dependence on pollinators.

METHODS

The species included in this study belongs to the genera *Earina* and *Winika*. These genera are commonly found throughout New Zealand, Stewart Island and the Chatham islands. In the North Island they mainly occur in fragments of low altitude mixed forests of native conifers and broad-leaved species, and occasionally on exotic trees such as willows (*Salix* sp.) and wattles (*Acacia* sp.) (Johns & Molloy 1983).

Species description

The genus *Earina* is considered to be part of the tribe Epidendreae, subtribe Glomerinae (Dressler 1993). It comprises about 10 species occurring in Fiji, Vanuatu, Samoa and New Caledonia and New Zealand. In New Zealand it is represented by three taxa; *E. autumnalis, E. mucronata* and *E. aestivalis*. The taxonomic status of the *E. aestivalis* is still unclear, and it has been variously considered as a synonymous, but late flowering form of *E. mucronata,* or as a different species (Cheeseman 1919, Moore & Edgar 1976, Parson *et al* 1995, St. George 1999).

The species *E. autumnalis* (Fig. 1) has either short erect stems or larger drooping stems. When flowering it forms a panicle *ca.* 5-10cm long. The flowers are small (*ca.* 13mm across) white and waxy. The labellum is broad and obtuse, except for the yellow base, which stands erect and half-encloses the column. The diagnostic feature of this species is the two short crescent-shaped ridges near the base of the labellum; these leading down to a small, brightly coloured pit, the nectary (Moore & Edgar 1976).

The species complex *E. mucronata*/*E. aestivalis* (Fig. 1) usually has pendulous stems. When flowering, it forms a panicle of 10cm. The flowers are yellow to orange and 1cm diameter. The labellum is much broader and showier than the rest of the floral elements. Flowers are widely open when anthesis is complete; near the base two inconspicuous ridges lead down to a small pit where the nectary is located (Moore & Edgar 1976, St. George 1999). For convenience, the two taxa will here be treated as separate species.

Taxonomically and geographically isolated in New Zealand, the genus *Winika* (formerly *Dendrobium*), in the section Macrocladium of the tribe Denbrobieae, has always been considered marginal to *Dendrobium* and is now treated as a monotypic endemic genus (Clements *et al.* 1997, Hatch 1998). *W. cunninghamii* bears flowers of almost 3 cm across (Fig. 1). The sepals and petals range from white to green in colour. The 3-lobed labellum is attached to the column by a column foot, where the nectary is located. Lateral lobes of the labellum are usually pink or purple, although entirely white forms are sometime found. This species is found throughout New Zealand. It grows as epiphytic on a wide range of native conifers and broad-leaved trees and is also rupestral on rocks of various lithologies, and sometimes on exposed buttressed tree roots (Clements *et al.* 1997). It is rarely found on the open forest floor (Johns & Molloy 1983). Although *W. cunninghamii* is fairly common, it has apparently been over collected near population centres (St. George 1999).

Figure 1: *Earina autumnalis*, *E. mucronata*, *Winika cunninghamii* and *E. aestivalis* (Clockwise, from left top).

Study Sites

Putara, Tararua Forest Park (40°41'S / 175°32'E) (Fig. 2): The Tararua Forest Park protects mountain landscapes, considerable tracts of indigenous forests and the upper catchments of many rivers. It is important for soil conservation and provides habitat for indigenous birds, fish and invertebrates. Management issues in the area include control of possums, deer and goats. At low altitudes species such as *Nothofagus fusca* and *Dicksonia squarrosa* dominate the forests. Several populations of *Earina mucronata*, *E. autumnalis* and *Winika cunninghamii* can be found growing on trees or rocks along the bank of the Mangamaire stream. All three species were studied at this site.

Marima Domain (40°30'S / 175°42' E) (Fig. 2): This Park, surrounded by farmland, is located at 30km from Palmerston North and supports a forest dominated by *Podocarpus totara* and *Melicytus ramiflorus*. Most of the trees in the park are rich in epiphytes such as *Astelia* sp, *Lycopodium* sp., *Asplenium flaccidum*, *Pyrrosia eleagnifolia*, *E. mucronata* and *E. aestivalis*. The latter were studied in this area.

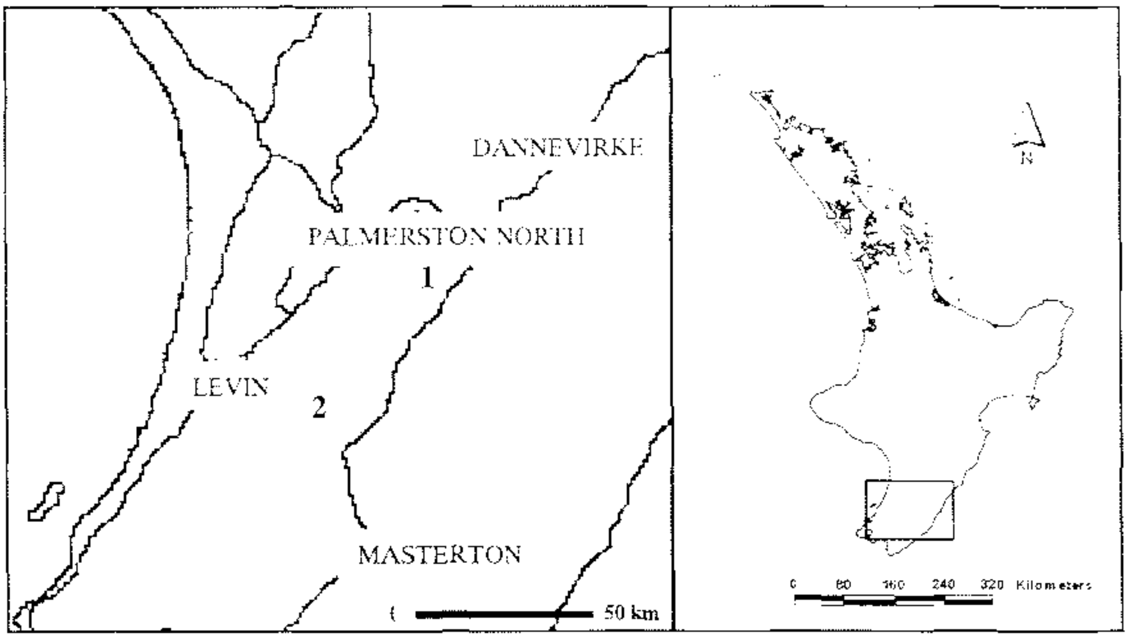

Figure 2: Locations of the populations of *Earina aestivalis* (1 and 2), *E. mucronata* (1 and 2), *E. autumnalis* (2) and *Winika cunninghamii* (2) were pollination experiments were conducted (1: Marima Domain; 2:Putara).

Reproductive Biology

In order to determine the breeding system and presence of self-incompatibility barriers in these orchids, hand-pollination treatments were performed during the flowering season of the year 2001 and part of the 2002 as described in chapter II. Fruit-set was analysed by means of generalised linear model with a binomial error distribution and logit link function using SAS, SAS Institute (2001). Pollen and ovule numbers, P/O ratios and osmophores where studied as described in Methods, chapter II.

Phenology and pollinators observations

Phenological phases, growth period, flowering and fruiting were assessed as mentioned in chapter II. Pollinator activity was surveyed while the other fieldwork was conducted, with special attention to their behaviour while visiting the flowers. Later, some of the insects were collected and identified. Pollinators were ranked according to their likely pollination efficiency following Adams & Lawson (1993).

When the insects' activity or the function they have in pollination was dubious, flowering branches were collected and brought back to the laboratory. Here, branches of *E. aestivalis* and *W. cunninghamii* were placed in a glass flask (covered with a mesh) together with the collected insects and left aside as showed below in Fig 3. Insect behaviour was the observed during the day for two days. At the end of the second day, the number of pollinia depositions and removals in virgin flowers was evaluated.

Figure 3: Glass flask used in the lab to observed insects behaviour and pollination efficiency.

Nectar Composition

Using filter-paper wicks (McKenna & Thomson 1988), nectar was collected from at least 10 flowers of each of the species under study. Wicks were air-dried at room temperature and then used to determine nectar sugar contents using the anthrone colorimetric assay for total carbohydrate technique as described in Appendix 6. This assay uses a strong acid (H_2SO_4 conc.) to hydrolyse all sugars present in a sample to monosaccharides. These in turn are dehydrated to furfural that forms a blue compound with anthrone. The intensity of the blue is proportional to the amount of carbohydrate present in the original sample. The intensity of the blue colour was read in the spectrophotometer and compared to a series of standards made from sucrose (Appendix 6, 7).

RESULTS

Phenology and Pollinators

Flowering periods of all four species extended for almost 3 months during 2000 (Fig. 4). However, unlike the previous season *E. mucronata* presented a mass flowering during 2001 (after *ca.* 20 days all flowering plants in the study sites had already finished blooming). Flower life span observed was of 5 ± 0.23 days in *E. aestivalis*, 6.20 ± 0.2 in *E. autumnalis*, and 6.3 ± 0.27 in *E. mucronata* and 9.57 ± 0.6 days in *W. cunninghamii* (mean ± standard error, n=10).

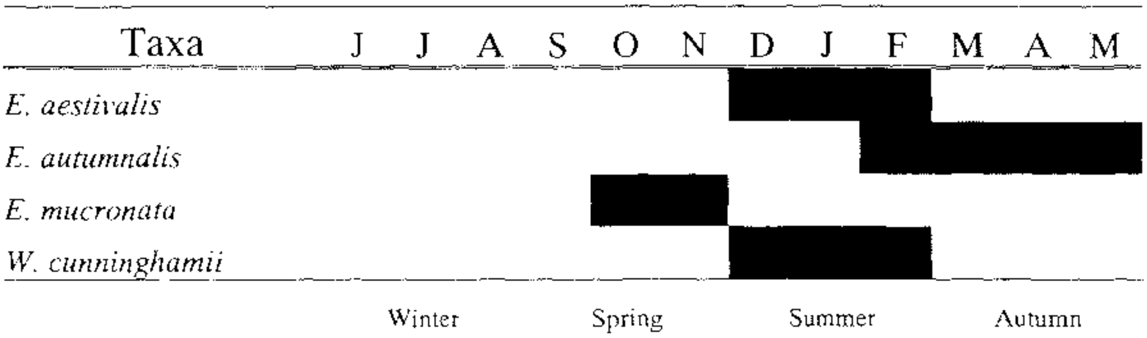

Figure 4: Flowering times of the New Zealand epiphytic orchids *Earina aestivalis*, *E. autumnalis*, *E. mucronata* and *Winika cunninghamii* in the North Island.

The genus *Earina* showed two flowering peaks. The first peak occurs during late spring and summer (*E. mucronata* and *E. aestivalis*) and the second in autumn and early winter (*E. autumnalis*). In these taxa, the leafy shoot takes almost three months to complete development and initiate flowering. Capsule ripening takes over three months. Seed dispersal occurs during good weather conditions. Dispersal is assisted by the presence of elaters in the capsules, that by hygroscopic movements expulse the seeds from the placenta (Halle 1986).

In *W. cunninghamii*, the floral buds begin their development during early October, and flowering occurs about two months later (December–January). Blooming in this species is not as synchronised as in *Earina*, and it is usual to find flowers at different stages of development or anthesis during the flowering period. Also, unlike *Earina*, the capsules are ripe after one month since the pollination occurred (March). When ripe, the capsules of *W. cunninghamii* do not split longitudinally as is usually observed in most of the Orchidaceae, but develop three openings at the apical end, where the column base was situated. Seeds are shaken out and wind-dispersed.

The insects observed visiting *Earina* species belong to 13 families: Diptera- Calliphoridae, Tipulidae, Bibionidae, Muscidae, Empidae, Syrphidae, Tachinidae; Coleoptera- Curculionidae and Carabidae and Hymenoptera- Ichneumonidae, Formicidae, Colletidae and Apidae. Nectar seems to be the unique reward since pollinia are strongly packed and waxy, and impossible to break apart mechanically. The insects collected during 2000, 2001 and 2002 are listed in the Appendix 9.

In *E. autumnalis*, the only insect species considered capable of achieving pollination and considered a "probable pollinator" was the cosmopolitan syrphid fly *Eristalis tenax* (Syrphidae). Individuals of this species were captured on two occasions carrying the four pollinia attached to the mouthparts (Fig. 5). Other visitors, on the other hand, were diverse and numerous, 6 taxa belonging to 5 families were recorded. Insects in this group were unlikely to achieve pollination because of their size or their behaviour while visiting the flowers, which was usually from the side and away from the reproductive structures (nectar thieves).

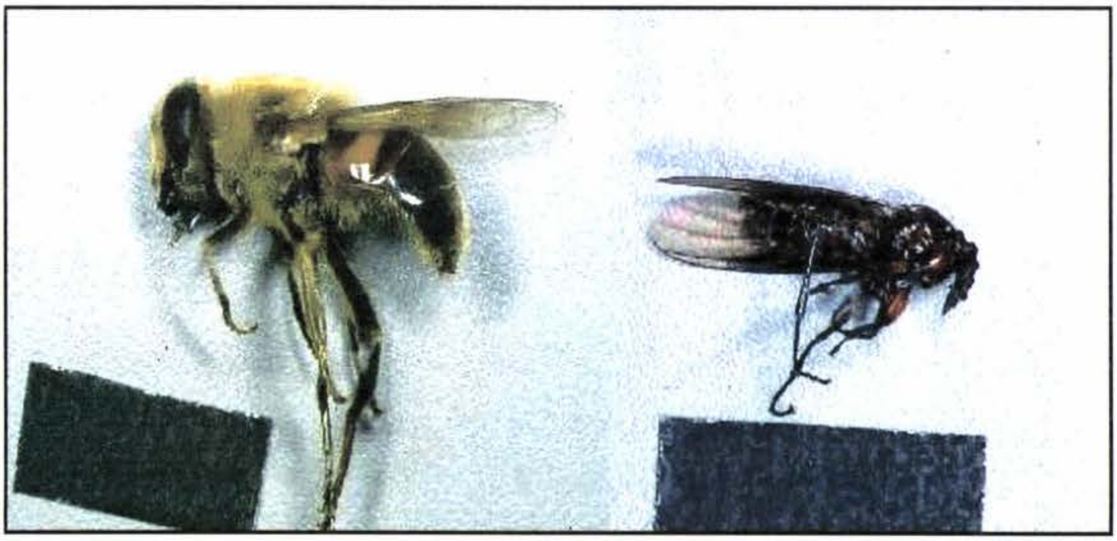

Figure 5: *Eristalis tenax* (Diptera: Syrphidae) (left) and *Dilophus nigrostigmus* (Diptera: Bibionidae) (right) collected after visiting flowers of *Earina autumnalis* and *E. mucronata*, respectively. (Black bar represents 1cm).

In *E. mucronata*, only one species may be considered as a pollinator, *Dilophus nigrostigmus* (Bibionidae) (Fig. 5). Unfortunately, this species was never observed carrying pollinia. The remaining two insect taxa (*Ceratochilus* sp. and *Hilara* sp.) were considered as visitors only because of their small size. A small beetle was also observed feeding on nectar from the underneath of the mesochile where nectar drops is usually found.

On the other hand, at least four insect species may be considered as probable pollinators in *E. aestivalis* (Fig. 6). One of them is an unidentified species of weevil (Curculionidae) that was observed feeding on nectar during both flowering seasons but carrying the four pollinia attached to its thorax only once. Other species that may be considered probable pollinators are the native *Melangyna novaezealandiae* (Syrphidae), *Calliphora quadrimaculata* (Calliphoridae), the Ichneumonid wasp *Aucklandella* sp. (Ichneumonidae) and the native Colletidae, *Hylaeus* sp.

Figure 6: *Melangyna novaezealandiae* (Diptera: Syrphidae) (left), *Hylaeus* sp. (Hymenoptera: Colletidae) (centre) and *Aucklandella* sp. (Hymenoptera: Ichneumonidae) (right) foraging on flowers of *Earina aestivalis*.

The pollinating efficiency of these last four species was tested in the laboratory as described in Methods in this chapter. From all insects included in this experiment, *Melangyna novaezealandiae* achieved the higher number of pollinia deposition (25%), followed by *Calliphora quadrimaculata* (19.23%) and the native *Hylaeus* sp. (10.52%). The Ichneumonid wasp was responsible for only one pollinia deposition (4%). To some extent, all four species may be considered "probable pollinators".

Finally, in *W. cunninghamii* the only insect species observed were *Prosalius advena* (Formicidae), *Apis mellifera* and the syrphid *Helophilus antipodus* and *M. novaezealandiae* (Fig. 3). These last two are considered as "probable pollinators" of *W. cunninghamii*.

Nectar Composition

The average amount of sugar per flower varied from 0.007 to 1.199 mg in *E. aestivalis* and *E. mucronata*, respectively (Table 1). Dilution factors used in the anthrone assays; absorbance and interpolated sugar for each species are presented in the Appendix 8.

Table 1: Nectar standing crop in four epiphytic New Zealand orchids (mean ± standard error) expressed as milligrams sugar equivalents (n = 10).

Species	Sucrose (mg)
E. aestivalis	0.007 ± 0.001
E. autumnalis	0.716 ± 0.219
E. mucronata	1.199 ± 0.148
W. cunninghamii	0.211 ± 0.196

Breeding system and genetic incompatibility barriers

The figures obtained from the hand-pollination treatments showed that all *E. autumnalis* and *E. mucronata* are totally self-compatible whereas *E. aestivalis* and *W. cunninghamii* seems to be partially self-incompatible (Fig. 7). The S.C.I. (a ratio between fruits set after self-hand pollination and number of fruits yielded after hand-cross pollination) obtained for all the taxa studied are shown in the Table 2. Detailed figures regarding treatments, flowers and fruits yielded are presented in the Appendix 2.

Table 2: Self-compatibility index (S.C.I.) obtained in four epiphytic New Zealand orchids studied.

Taxa	S.C.I.
E. aestivalis	0.4
E. autumnalis	0.9
E. mucronata	1.6
W. cunninghamii	0.5

Although the four species are scented and nectariferous, natural fruits-set is considerable low in the wild, in particular the taxa *E. mucronata* and *E. aestivalis* (4.19 and 6.9%, respectively) (Fig. 7). The highest fruit-set was observed in the autumn flowering species *E. autumnalis*, from 324 flowers tagged in the wild only 28.8% of them (91 flowers) set fruit. Regarding the success of the pollination treatments, fruit set was significantly augmented in all four species after hand-cross pollination (χ^2= 585.36, 7 df, $p< 0.0001$) (Fig. 7, Appendix 5).

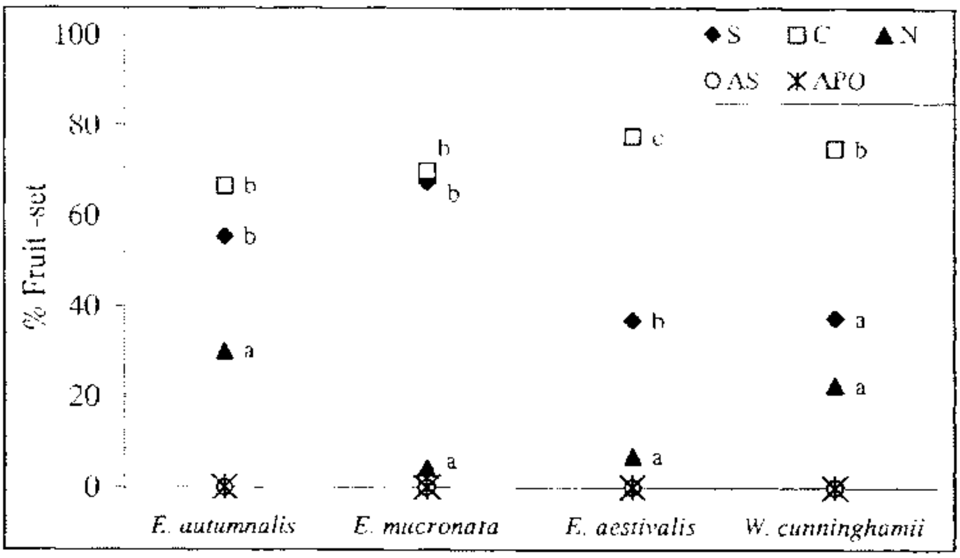

Figure 7: Percentage of fruit-set obtained in the four taxa studied (**S**: hand self-pollination; **C**: hand-cross pollination; **N**: natural pollination; **AS**: automatic self-pollination; **APO**: apomixis). Shared letters within species indicate means that are not significantly different.

Fruit-set following hand cross-pollination was above 60% in all species. Conversely, fruit-set after hand self-pollination, was rather variable and lower than fruit-set through hand cross-pollination for all species but *E. mucronata*, in which almost 70% of hand self-pollinated flowers formed fruits (29 flowers out of 43) (Fig 5).

None of these species set seed through agamospermy or automatic self-pollination, therefore they depend entirely on pollinating agents for their reproduction (Fig. 7).

Pollen and ovule numbers and P/O ratios

All species studied are monandrous orchids (*i.e.* one anther per flower), therefore the number of pollen presented in the Table 3 corresponds to the number of pollen grains per flower. The pollinarium of all taxa comprise four hard pollinia that are removed by insects as a single unit since they are kept together by the viscidial disc. In *W. cunninghamii* pollinia are unconnected. In all four taxa pollen grains are joined into tetrads.

W. cunninghamii presented the highest number of pollen grains per flower (464 711 ± 43373), whilst the spring-summer flowering taxa *E. mucronata* and *E. aestivalis* present the lowest (Table 3). Regarding to number of ovules per flower, again *W. cunninghamii* presented the highest values, 13 335 ± 411.90. The autumn flowering species *E. autumnalis* showed the lowest number of ovules per flower (Table 3). Pollen/ovule ratios (P/O) calculated for these species fluctuated from 46: 1 to 20:1 (Table 3).

Table 3: Pollen and ovule numbers, P/O ratios and tetrads size of four epiphytic New Zealand orchids.

Species	Pollen # per flower	Ovule # per flower	P/O Ratio	Tetrad & Monad size (μm)
E. aestivalis	113 400 ± 6120	5 749 ± 506.70	20: 1	32.3 X 20.0
E. autumnalis	274 275 ± 8148	5 930 ± 139.36	46: 1	33.2 X 18.3
E. mucronata	142 285 ± 11490	6 793 ± 271.31	20: 1	33.3 X 18.9
W. cunninghamii	464 711 ± 43373	13 335 ± 411.90	34: 1	39 X 22.4

Osmophores

The areas of the perianth presenting a positive reaction after being treated with neutral red are presented in the Table 4. From all taxa examined, only the results obtained in *E. autumnalis* were unclear. In this species, the perianth showed a generalised positive reaction to the stain; but since the flower epidermis is rather waxy it was not clear whether the positive reaction observed was actually tissue imbibed of stain or precipitated stain on the epidermis.

In general, the positive staining of the structures was observed after the first two hours of treatment. Areas with a positive reaction were commonly around the column, particularly around the stigma, and the anther cap and the labellum. It is interesting to note that areas stained by neutral red were different in *E. mucronata* and *E. aestivalis*. The positive staining of structures such as stigmatic surface and pollinia were not considered because of their absorbent nature.

Table 4: Absorbance of neutral red by floral structures of four New Zealand epiphytic orchids.

Species	Column	Labellum	Petals	Lateral sepals	Dorsal sepal
E. autumnalis	-------	-------	-------	-------	-------
E. mucronata	Top of column (around anther cap) & base of column	Tip of the lip, underneath nectary	No reaction	No reaction	No reaction
E. aestivalis	Top of column (around anther cap)	No reaction	Entire petal	The entire sepal	The entire dorsal sepal
W. cunninghamii	Column wings and around stigma	Lateral lobes and ridges	No reaction	No reaction	No reaction

DISCUSSION

Reproductive Biology

Contrary to Thompson (1927) and Clements *et al.* (1997), pollination treatments in this study have demonstrated that all four orchids are incapable of autonomous selfing and completely dependent on pollinators. Moreover, the species *Earina autumnalis* and *E. mucronata* have proved to lack of self-incompatibility barriers. Ackerman (1986) explains self-compatibility is highly advantageous in epiphytic species since they usually are sparsely distributed in the forests and geitonogamous pollinations are more likely to occur.

The taxa *E. aestivalis* and *W. cunninghamii*, on the contrary, seem to be partially self-incompatible. Self-incompatibility has been described in several epiphytic orchids (Ackerman 1989, Johansen 1990, Bartareau 1995, Parra-Tabla 2000, Borba *et al.* 2001) and in almost 70% of *Dendrobium* species (Johansen 1990), to which *W. cunninghamii* is closely related and had been previously ascribed. The gradual anthesis observed in the flowers of *W. cunninghamii* and the floral polymorphisms (*i.e.* colour variability) observed within the flowering patch are well known strategies to promote cross-pollination.

Self-incompatibility in flowering plants prevents fertilisation by pollen from the same plant or clone, and ultimately inbreeding depression. It is possible that *E. aestivalis* and *W. cunninghamii* are currently developing self-incompatibility barriers. *E. aestivalis* forms dense clumps on its host tree, with easily up to 1000 flowering spikes per patch; and it is likely that the evolution of self-incompatibility in this epiphytic plant has been indirectly triggered by changes in the population's demography. Ackerman (1986) proposes that when normally sparsely distributed self-compatible species form dense populations inbreeding depression can occur, and this may exert strong selection for developing incompatibility mechanisms or against extensive clumping.

It is likely that this taxon is in a process of reproductive isolation from the spring flowering self-compatible *E. mucronata*, this may also explained the different phenological patterns observed in *E. aestivalis*. The *in situ* evolution of incompatibility barriers from compatible progenitors is likely in New Zealand since the latter is rather generalised in the flora of the country (Godley 1979). Furthermore, the long isolation of the country and the existence of unspecialised pollinating fauna in New Zealand might constrain the establishment of self-incompatible taxa (Lloyd 1985, Webb & Kelly 1993), and favour those with an efficient clonal reproduction.

Despite the rewarding nature of these four orchid taxa, fruit set was low in all of them. Sabat & Ackerman (1996) suggest that xenogamous orchids usually have a low fruit-set often less than 10%. Fruit-set in xenogamous plants may be limited by the amount of resources allocated to reproduction or by pollinator availability (Primack & Hall 1990, Kearns & Inouye 1993). Hand cross-pollinations in this study significantly increased fruit-set, indicating that resources are not limited. However, it is important to note that the artificial increment of fruit-set may influence the performance of the plant the following season either reducing the growth or the chances of flowering (Primack & Hall 1990).

The generally low fruit-set observed across these orchids may be explained by the poor effectiveness of the pollinators that visit them. As mentioned before, New Zealand stands out by the disproportionate reliance on unspecialised insect groups (Primack 1983, Lloyd 1985). Moreover the disproportionate importance of Diptera as pollinators in New Zealand is well known (Primack 1978, 1983, Lloyd 1985). The pollinator surveys were limited (*e.g.* only diurnal observations) but despite this, the number of insects associated with to these four orchids was noticeable; 18 species, 2 of them non-previously described (Appendix 9). The order Diptera (7 families) and Hymenoptera (4 families) were the most represented. The families associated to these orchids are indeed frequent visitors to flowers of many species in New Zealand (Primack 1978, 1983, Lloyd 1985).

Not all the insects observed in this study are regarded as pollinators but "probable pollinators". Adams & Lawson (1993) described as "probable pollinators" those insects that are observed taking up pollinia of the plant but with no later deposition of them on a stigma of a co-specific flower, and "suggested" pollinators those that are observe visiting the flowers but never taking up pollinia.

In *Earina autumnalis* the only species regarded as a probable pollinator was *Eristalis tenax*. Pollination studies conducted overseas have reported *Eristalis tenax* is a regular pollinator of the families Rosaceae, Brassicaceae and Alismataceae (Arya 1982-1983, Tanaka 1985, Kevan *et al.* 1990, Dashad *et al.* 1994). So far, the foraging behaviour of this species has been considered rather labile; changing from generalist to specialist, and a mixture of them, according to the flowering density in the population (Kunin 1993). It is likely that the inconsistency in the foraging behaviour of *E. tenax* may be causing the low-fruit observed in *Earina autumnalis*.

The detrimental effect generalist insects might have on the reproductive success of these orchids may be added to by the constant visitation of nectar thieves. Although nectar thieves might be able to remove pollinia from the flowers while searching for nectar, they will not necessarily deposited them on the stigma. An example of this is the native syrphid *Melanostoma fasciatum* observed in *E. autumnalis*.

In *E. mucronata*, on the contrary the only species that may be considered as "suggested" pollinator is *Dilophus nigrostigmus* (Diptera: Bibionidae). This based on the size of the insect and the highly modified proboscis that may facilitates the insects' access to the nectary. However, the pollinating efficiency of *Dilophus* species has been considered low (Limonta & Antignati 1994). More thorough observations should be conducted to determine the function of this fly in the pollination of *E. mucronata*.

E. aestivalis is pollinated by Diptera and Hymenoptera and likely Coleoptera. The order Diptera in *E. aestivalis* is represented by the species *M. novaezealandiae* (Diptera: Syrphidae) and flies of the family Calliphoridae, both considered frequent floral visitors of many plants in New Zealand (Primack 1978, 1983, Lloyd 1985). Species of the Calliphoridae have been regarded as pollinators of several plant families *e.g.* Hamamelidaceae, Nepenthaceae, Asteraceae and Polygonaceae (Schedl 1992, Momose & Inoue 1993, Meynie & Bernard 1997, Chua 2000) but not Orchidaceae. In general, they have been considered generalists and rather polylectic (Schedl 1992). The order Hymenoptera in *E. aestivalis* was represented by the genus *Hylaeus* (Colletidae) and an

Ichneumonid wasp. Orchid pollination by these insects has been previously reported in the literature (Nilsson 1981, Gumprecht 1980, Bartareau 1995, Pedersen 1995, Lehnebach 1998). It is interesting to point out that pollination by ichneumon wasps has often been linked to syndromes of pseudocopulation (Nilsson 1981, Graham 1983). The order Coleoptera is represented by one unidentified species of weevil that was collected in both flowering season inside of flowers of *E. aestivalis*. Pollination by weevil has only been described for species of the family Palmae (Mora 1982, Henderson *et al.* 2000) and in the custard-apple family Annonaceae (Irvine & Armstrong 1988). However, more observations should be conducted to explore the real function of this insect in the pollination of *E. aestivalis*.

Finally, the syrphid flies *Melangyna novaezealandiae* and *Helophilus antipodus* and the common honeybee (*Apis mellifera*) were regarded as "probable" pollinators of *W. cunninghamii*. It is likely that before the introduction of *A. mellifera* to New Zealand *M. novaezealandiae* and *H. antipodus* were the main pollinators of this orchid and now are sharing the pollinating service or perhaps being displaced by the honeybee. Honeybees have also been reported as "probable" pollinators *Dendrobium kingianum* (Orchidaceae) in Australia (Adams & Lawson 1993).

The floral advertisement, reward system and taxonomic groups visiting the species complex *E. mucronata-E. aestivalis*, seems to parallel the pattern of the terrestrial orchid *Listera ovata* (L.) R. Br. (Nilsson 1981) and the epiphytic orchid *Dendrochilum longibracteatum* Pfitzer (Pedersen 1995). The orchids studied by Nilsson (1981) and Pedersen (1995) often grow in dense clones; produce long racemes with numerous small flowers emitting a distinct scent and nectar of easily access. A wide range of insects visits these two orchids, especially of the order Diptera, Hymenoptera and Coleoptera-but in both orchids only a small selection of the visitors actually act as pollinators. Nilsson (1981) and Pedersen (1995) suggest that these frequent visits by non-legitimate insects suggest a great loss of the nectar and pollinia. Nilsson (1981) points out that these losses are one of the outcomes of a pollination strategy incorporating a complete anthecological specialisation to insects that are not strictly anthophilous.

The numerous insects visiting these flowers might result from the rich rewarding offered by these orchids. Nectar is the primary floral reward for many pollinators but nectar thieves may appreciate it as well. Indeed, the energetic content of the nectar per flower produce by these orchids is substantial, 3 to 20 Joules (1mg sucrose = 16.8 Joules). Similar values have been observed previously Castro & Robertson (1997) and Perrot (1997) in New Zealand plants that have birds (honeyeaters) as visitors. However, it is important to note that the nectar standing crop only measures the availability of resources at a single point in time (Possingham 1989), and this values may be affected by variability in nectar production among flowers and/or the selective foraging by pollinators (Pleasants & Zimmerman 1983). The importance of these orchids as nectar source for the local insect community is significant; *E. autumnalis* was the only rewarding species observed flowering during autumn to early winter in the area along with *Metrosideros excelsa* (Myrtaceae).

P/O ratios have been proposed as an alternative way to infer the group of pollination insects in orchids (Neiland & Wilcock 1995). Primarily, P/O ratios in plants have been used to predict the plant reproductive strategy (Cruden 1977). Thus, a small P/O indicated autogamy whereas a higher P/O is indicative of xenogamy. However in orchids, and other plants with pollen clumped in pollinia, P/O ratios do not follow Cruden's principle, and they are usually even lower than those observed in cleistogamous plants (Cruden 1977). Neiland & Wilcock (1995) suggested that lower P/O ratios are more likely to be found in orchids pollinated by more efficient pollinator such as Hymenoptera whereas higher P/O ratios to species pollinated by Diptera. The P/O ratios of the orchids included in this study ranged from 20:1 to 46: 1; values within those previously reported for terrestrial orchids (Neiland & Wilcock 1995, Nazarov & Gerlach 1997 and chapter II). However, they are very high when compared to the P/O of 1.2: 1 in the epiphytic orchid *Coryanthes senghasiana*, which is the only P/O ratio record in the literature for an epiphytic orchid (Nazarov & Gerlach 1997). This species is strictly pollinated by Euglossine bees (Hymenoptera: Apidae) (Gerlach & Schill 1989). In this study the highest P/O belongs to *Earina autumnalis*, pollinated by *Eristalis tenax* and *W. cunninghamii* pollinated by *H. antipodus* and *M. novaezealandiae*, all of them flies from the Syrphidae family.

Contrary to tropical epiphytic orchids, pollination strategies in New Zealand epiphytic orchids are not species-specific and pollination may occur after visitation by several species of insects. Furthermore, contrary to the typical scattered distribution of epiphytic plants some of these orchids are densely distributed within the forests canopy and exhibit major floral displays. For instance, in Marima Domain Park, plants of *E. aestivalis* are almost on every tree and when flowering, they can produce over 300 flowering panicles per clump. In New Zealand massed flowering is considered a common condition evolved in plants with inconspicuous flowers (Lloyd 1985). *W. cunninghamii*, however, seems to fit better the trend observed in its tropical counterparts.

The information gathered in this study does not reject the existence of the dispersion-specialisation hypothesis but questions the applicability to species of *Earina* Lindl. in New Zealand. The reproductive biology of the New Zealand flora has been strongly affected by selective forces imposed by several peculiarities such as long isolation from other elements of Gondwanaland, oceanic climate, unusual combination of ancient Gondwanic, tropical and recently arrived elements in its flora and the paucity of specialised pollinators (Lloyd 1985, Webb & Kelly 1993). Regarding the latter, the most outstanding feature of New Zealand pollinating fauna is the large proportion of plants pollinated by dipterans, more than in any other landmass of continental origin in the world (Lloyd 1985). Bees are represented by *ca.* 40 species, all short-tongued and primitive (Webb & Kelly 1993). Carlquist (1974) and Primack (1978, 1983) suggest that the principal consequence of the anomalous representation of pollinating groups in New Zealand is that the flowers rely to an unusual degree on unspecialised insects that promiscuously visit a wide range of flowers and that operate in an imprecise manner (Lloyd 1985).

The number of New Zealand plants pollinated by promiscuous and unspecialised pollinators is considerable. Primack (1983) observed that only four of the 82 montane species that he examined exhibit a specialised pollinator relationship with a "*single order*" of insects. So far, specialised pollinations systems in New Zealand had been reported only in bird-and bat-pollinated species (Webb & Lloyd 1993). Lloyd (1985) suggest that the total effect of these various selective forces have promoted a floral syndrome that enables the flowers to be promiscuously pollinated in order to set-fruit by whichever insects are available on any occasion, but with the concomitant energetic cost pointed out before by Nilsson (1981).

Conservation Implications

As mentioned before in chapter II, the probability of extinction due to pollinator failure may be anticipated by evaluating three main aspects of this interaction; the chances of mutualisms failing, the degree of reproductive dependence on the mutualism and the importance of seeds in the demography of the plant studied (Bond 1994,1995). The four orchids included in this study have an absolute dependence on their pollinators to produce fruits. Furthermore *Earina aestivalis* and *Winika cunninghamii* seem to be partially self-incompatible, a feature that increases the dependence on pollinators and therefore the risk of extinction by pollinator failure.

Pollinator failure may occur when native pollinators are displaced by exotic species or due to extinction of the pollinator. Generalist nectar-feeding insects impose a potential threat to the reproduction of these nectariferous orchids by competing and displacing their native pollinators. Murphy & Robertson (2000) have already pointed out the threat *A. mellifera* may impose to the local native insect community. These authors observed that the activity of *A. mellifera* strongly negatively influences the abundance and diversity of Diptera in two common alpine shrubs (*Hebe stricta* and *Leptospermum scoparium*). Perhaps, the low fruit-set observed in *W. cunninghamii* is likely due to the displacement of the native pollinators *M. novaezealandiae* and *H. antipodus* by *A. mellifera*.

Habitat fragmentation has been considered an equally important threat to the efficient functioning of the plant-pollinator interaction, either changing the composition of the local insect community and natural enemies, the neighbourhood of potential mates, the availability of resources, microclimate, or by causing the further extinction of the pollinators (Aizen & Feinsinger 1994, Cunningham 2000). As with other ecological interactions, the impact it may have on the survival of the species may not be noticed immediately (Janzen 1974). Therefore, monitoring the reproductive success of orchid populations in contrasting habitats is recommended. Reproductive failure has a cumulative effect and it may be only notice after several years of monitoring. For instance, the effect of habitat fragmentation in the reproductive success of the Mexican epiphytic orchid *Oncidium ascendens* Lindl. was only noticed after three years of monitoring female and male pollination success (Parra-Tabla *et al.* 2000).

Fortunately, New Zealand epiphytic orchids have several compensatory features that may facilitate their survival. First, they do not have species-specific pollination syndromes, which reduces the chances of reproductive failure by extinction of the pollen vector. Secondly, they do not seem to present host-tree specificity, which allows them to grow as epiphyte in different species of trees (native or exotic) or as lithophytes. Lastly, they exhibit profuse vegetative growth and are apparently long-lived. Considering these features, protective management of these species should principally aim at maintaining a habitat supporting a diverse native flora and an active community of insects, avoiding the introduction of generalist nectar feeding insects. Also, special attention should be paid to the effects of successional changes and disturbance regimes. Succession and disturbance in epiphytic plants have remained largely unstudied and future research is essential to understand the regeneration dynamics and colonisation strategies of these orchids.

IV

Pollen Limitation of Fruiting Success in Rewarding and Non-rewarding New Zealand Orchids

INTRODUCTION

Reproductive success may be constrained by the availability of pollinators and the amount of resources plants allocate for reproduction (Wilson 1983, Primack & Hall 1990, Kearns & Inouye 1993, Primack & Stacy 1998) or both. Although resources limit fruit production in many species of flowering plants, there is evidence that the reproductive output of other species is limited instead by pollen availability (see review in Ackerman & Montalvo 1990 and Burd 1994).

Pollen limitation has been considered widespread among some plant communities such as tropical trees (Bawa 1974), sclerophyllous vegetation in Chile (Arroyo & Uslar 1993) and monocotyledonous wildflowers (including orchids) of the Cape region of South Africa (Johnson & Bond 1997). In the Orchidaceae, pollen limitation has been observed in both terrestrial and epiphytic species (Ackerman 1989, Zimmerman & Aide 1989, Ackerman & Montalvo 1990, Burd 1994, Proctor & Harder 1994, Johnson & Bond 1997, Hansen & Olesen 1999).

The evolutionary importance of pollen limitation is considerable. Stebbins (1970) and Arroyo & Squeo (1990) consider pollen limitation as a cause of floral radiation when plants colonise regions in which their pollinators are scarce or absent, evolving into more effective insect-attraction strategies, making a pollinator shift or developing an autogamous breeding systems. Benzing & Atwood, cited in Ackerman (1989), suggest that orchid pollination systems might have evolved largely in response to competition for pollinators when they are scarce.

The presence of pollinia in orchids makes them an ideal model system for studying pollen limitation and pollinator effectiveness. In this family, pollen grains are joined together by elastoviscin forming a compact and coherent structure called the pollinium (*pl.* pollinia). The cohesion of the pollen grains is highly variable within the family but hard pollinia are the most common among orchids (Dressler 1993). Hard pollinia represent the greatest degree of cohesion, and are deposited as an entire unit on the stigma (Johnson & Edwards 2000). Therefore, both pollinator visitation history and pollination success are recorded in the removals and depositions of pollinia.

Despite the simplicity of these observations and the numerous orchid studies, reports on pollinia removal (PR) and pollinia deposition (PD) ratios are scarce, especially in rewarding species (Proctor & Harder 1995). Surveys of pollinia removal and deposition may be useful to assess orchid pollination success and in monitoring the functioning of plant-pollinator interactions by measuring the success of the male (pollen donation) and the female function (with two components fruit-set and pollen deposition) (Broyles & Wyatt 1990, Proctor & Harder 1995, Parra-Tabla *et al.* 2000). These measurements are especially useful when assessing the effect predation, habitat disturbance and fragmentation has on pollination success (Ackerman & Moya 1996, Parra-Tabla *et al.* 2000, Malo *et al.* 2001). These studies are particularly important considering that low reproductive success may promote rarity and increase the risk of extinction of some orchids (Tremblay *et al.*1998).

In New Zealand, the only records of pollinia removal and pollinia depositions found in the literature date from late 1800's and early 1900's (Darwin 1877, Thompson 1879, 1927). Unfortunately, these observations were fairly limited and never related to pollinator efficiency. This chapter aims to determine whether fruit-set is pollen limited in some terrestrial and epiphytic New Zealand orchids; and secondly explore the effect contrasting rewarding strategies (nectar v/s deception) have on the pollination success of these orchids. The occurrence of a diverse orchid flora in New Zealand, with numerous epiphytic and terrestrial species and contrasting reward strategies and flowering periods makes it possible to study orchid pollination biology from a complementary approach.

METHODS

Species description and study sites

Four rewarding epiphytic (*Earina autumnalis*, *E. mucronata*, *E. aestivalis* and *Winika cunninghamii*) and three non-rewarding terrestrial orchids (*Pterostylis alobula*, *P. patens* and *P. australis* Hook. f.) occurring in the south of the North Island were studied during 2001 and 2002. All seven species rely on pollinators for their pollination (see chapter II and III). Pollinia in the epiphytic species are hard and waxy, while in *Pterostylis* they are soft and easily break apart. The species studied belong to two flowering guilds, spring-summer (*Earina mucronata*, *E. aestivalis*, *Winika cunninghamii*, *P. patens* and *P. australis*) and autumn-winter flowering (*E. autumnalis* and *P. alobula*).

The endemic *P. australis* is a 10 – 25 cm tall herb, with 4-5 leaves; the single flower has an elongated dorsal sepal and lateral sepals that are greatly extended in their points, considerably overtopping the rest of the flower. It flowers from November to late December, and is predominantly insect pollinated. The species occurs in the southeast of the North Island and the west coast of the South Island (St. George 1999).

Descriptions of the remaining species and study sites are given in chapters II and III.

Pollination experiments

To determine whether fruit-production is limited by pollen availability, supplementary hand-pollinations were performed in all species except *P. australis*. Pollen from distant plants was always used to pollinate the experimental group. After pollination, capsules were allowed to develop under natural conditions, and then harvested before dehiscence occurred. Pollinator behaviour when visiting the flowers was also observed; later they were captured and identified. Results were compared with the natural fruit-set from un-manipulated plants. Pollination experiments are explained in detail in Chapters II and III.

The intensity of pollen limitation on each species was summarised by the intensity of pollination index following Baker *et al.* 2000, $P = 1 - (F_o/F_s)$, where F_o is the fertility of un-manipulated flowers (seed-set) and F_s is the fertility of the flowers that received supplemental hand cross-pollen. A score of P= 0 or less indicates that no pollen limitation was detected. In this study, fruit-set will be used to assess fertility (Baker *et al.* 2000).

Pollination assessment in the population

In each population, un-manipulated flowers of the species mentioned above were randomly surveyed for pollinia removal and deposition following Thien (1969), Johnson & Bond (1992) and Lehnebach (1998). From the flowers that had their pollinia removed or/and deposited, the success of the male (pollen removal) and female function (pollen deposition) was determined. In order to understand the relationship between pollinia removal and deposition and the species rewarding system in a broader context, values obtained in this study were compared with information from the international literature (Appendix 10).

Data analysis

Fruit-set was analysed by means of generalised linear model with a binomial error distribution and logit link function using SAS (SAS Institute 2001). Difference between pollinia removal and deposition of rewarding and non-rewarding orchids was assessed by applying an ANOVA tests using SPSS (SPSS Inc. 1999). Since PD/PR ratio data for New Zealand and remaining species were not normally distributed, the non-parametric Mann-Whitney *U*-test was used to assess the difference between the PD/PR ratio of rewarding and no-rewarding species using SPSS (SPSS Inc. 1999).

RESULTS

Levels of natural fruit-set were low in both rewarding and non-rewarding species ($F_{1,4}$ = 0.172, P<0.70; Appendix 11), fluctuating from 4.3% (*P. alobula*) to 40% (*P. patens*) (Fig. 1). These low values appear to be produced by low pollen availability, as supplementary hand-pollinations increased fruit set above 40% in all species and significantly so in all but *P. patens* (χ^2= 585.36, 7 df, P< 0.0001) (Fig. 1). *W. cunninghamii* and *E. aestivalis* set the highest percentage of fruits following hand-pollinations, 75 and 77% respectively. The intensity of pollen limitation varied from 0.32 to 0.94 in *P. patens* and *P. alobula*, *E. mucronata*, respectively.

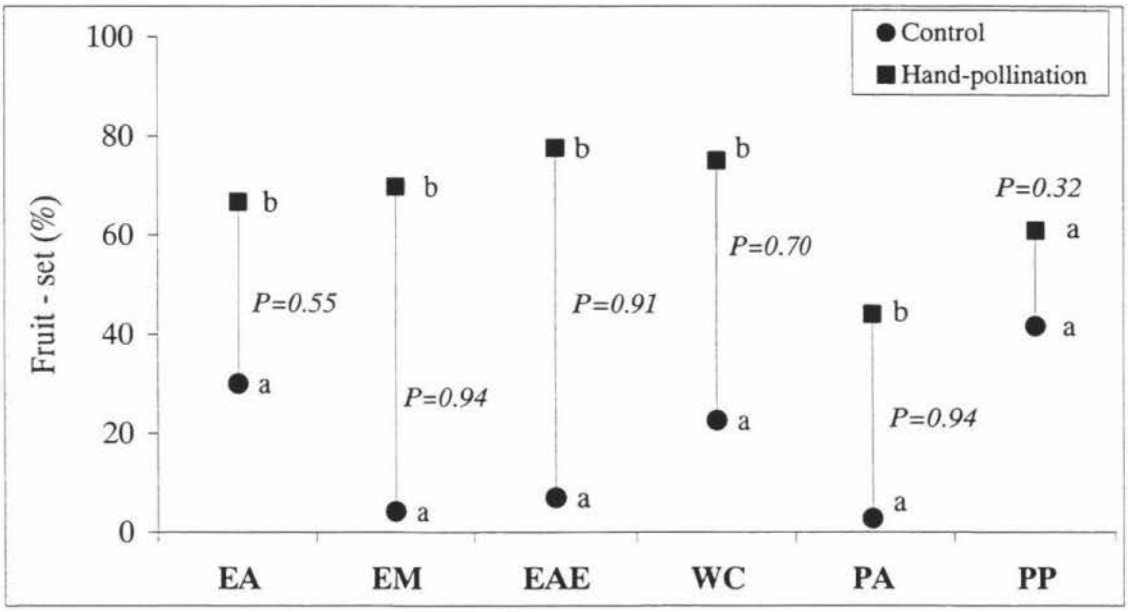

Figure 1: Percent of fruit-set produced in un-manipulated (control) and hand cross-pollinated flowers and intensity of pollen limitation in six New Zealand orchids (**EA**: *Earina autumnalis*, **EM**: *E. mucronata*, **EAE**: *E. aestivalis*, **WC**: *Winika cunninghamii*, **PA**: *Pterostylis alobula* and **PP**: *P. patens*. Shared letters within species indicate means that are not significantly different).

Pollinia removal (PR) was generally higher than pollinia deposition (PD) in all species surveyed except *P. australis* (PR<PD) and *P. alobula* (PR = PD) (Fig. 2). In general pollinia removal was significantly higher in rewarding (*i.e. E. aestivalis*, *E. mucronata*, *E. autumnalis* and *W. cunninghamii*) than in non-rewarding species (*Pterostylis* species)

($F_{1,5}$ = 6.641, P=0.05; Appendix 11), in the latter less than 15% of flowers had their pollinia removed. The highest values of pollinia removal were observed in the epiphytic species *W. cunninghamii* and *E. autumnalis* with 48.64 and 91.52%, respectively.

In contrast, pollinia deposition was low in all taxa, ranging from 0.91% in *E. mucronata* to 34% in *P. australis*. Pollination success, measured as pollinia deposition, was similar in rewarding and non-rewarding species ($F_{1,5}$. = 1.387, P=0.292; Appendix 11). Within non-rewarding species, *P. australis* showed the highest number of flowers with pollinia deposited (Fig. 2).

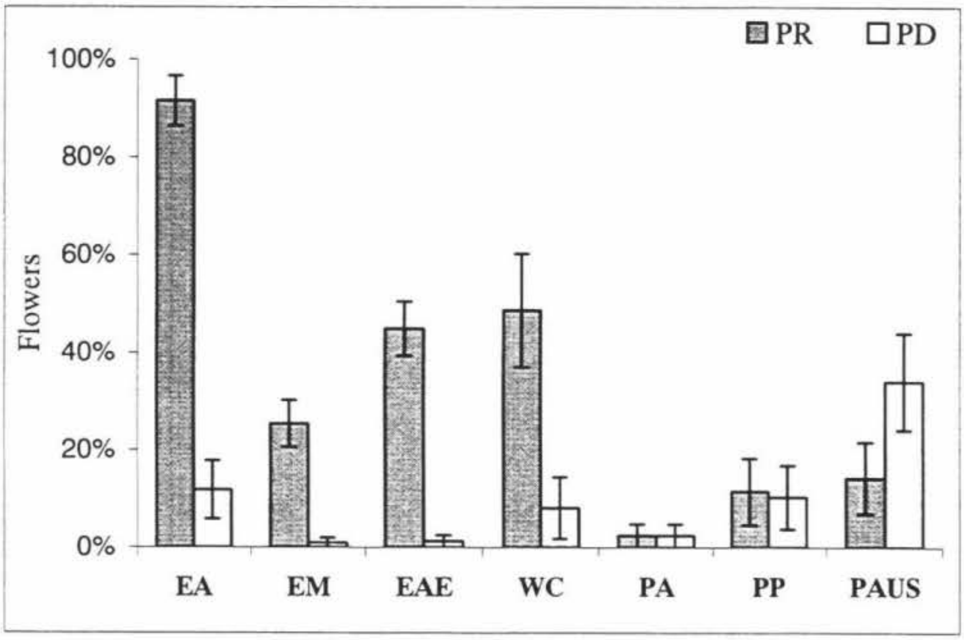

Figure 2: Percent of flowers with pollinia removed (PR) and pollinia deposited (PD) in the rewarding and non-rewarding orchids in New Zealand (**EA**: *Earina autumnalis*, **EM**: *E. mucronata*, **EAE**: *E. aestivalis*, **WC**: *Winika cunninghamii*, **PA**: *Pterostylis alobula*, **PP**: *P. patens* and **PAUS:** *P. australis*).

Pollinia removal is higher in the autumn flowering *Earina* than in those taxa flowering in the spring-summer period. Pollinia removal and pollinia deposition are higher in *E. aestivalis* (summer flowering) than in *E. mucronata* (spring flowering), pollinia deposition was almost twice the rate of *E. mucronata*. The contrary is observed when comparing the winter flowering *P. alobula* with the summer flowering species *P. australis* and *P. patens,* which both have higher removal and deposition rates.

The ratio of deposition to removal varied considerably among species, from 2.39 to 0.03 in *P. australis* and *E. aestivalis* respectively (Appendix 10). Overall, non-rewarding species had a higher PD/PR ratio than rewarding species (*U*= 0.00, *P*= 0.034; Appendix 12). The relationship of these two measurements are summarised in the Figure 3

Pollinia removal may be considered here as a measure of visitation and the PD/PR ratio as a measure of pollination efficiency - the higher the value the more efficient the pollination system is. The four nectariferous orchids included in this study presented significantly lower PD/PR ratios, indicating that floral visitors are removing considerable amounts of pollinia but few are actually reaching the stigmas (Fig. 3) and these species, therefore, seem to have a less efficient pollination system.

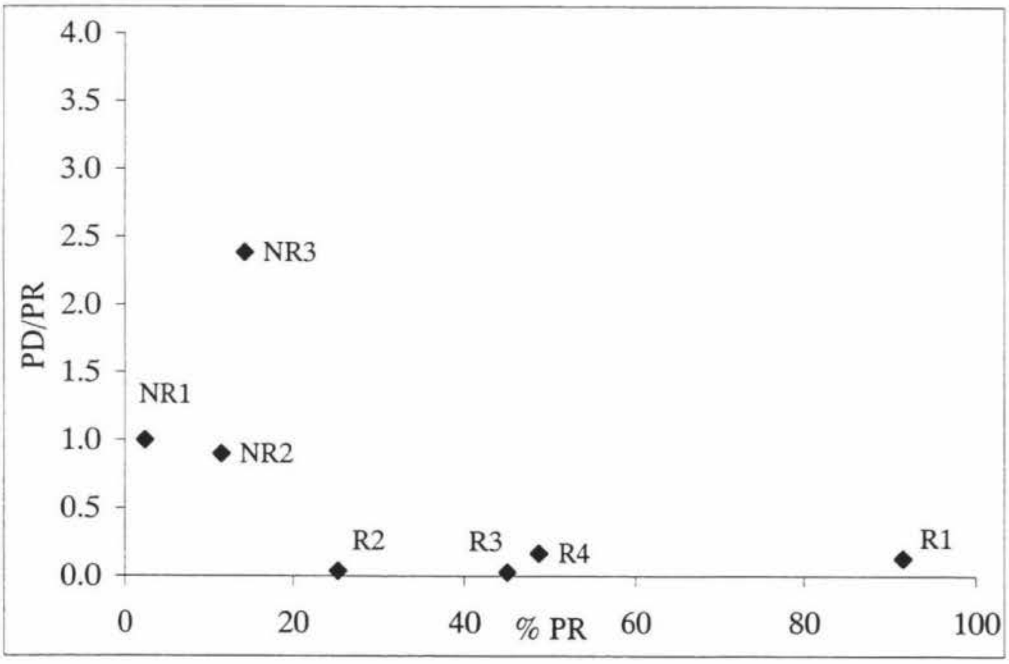

Figure 3: Pollination efficiency in rewarding (R) and non-rewarding (NR) orchids in New Zealand (**R1**: *Earina autumnalis*, **R2**: *E. mucronata*, **R3**: *E. aestivalis*, **R4**: *Winika cunninghamii*, **NR1**: *Pterostylis alobula*, **NR2**: *P. patens* and **NR3**: *P. australis*).

This trend is inconsistent when the PD/PR of New Zealand species are analysed together with PD/PR ratios calculated from removal and depositions reported elsewhere in the literature (Fig. 4, Appendix 10). The ratio of deposition to removal in rewarding and non-rewarding species is similar (U = 38.000, P = 0.364) and rewarding and non-rewarding species are indiscriminately scattered throughout the graph (Fig. 4).

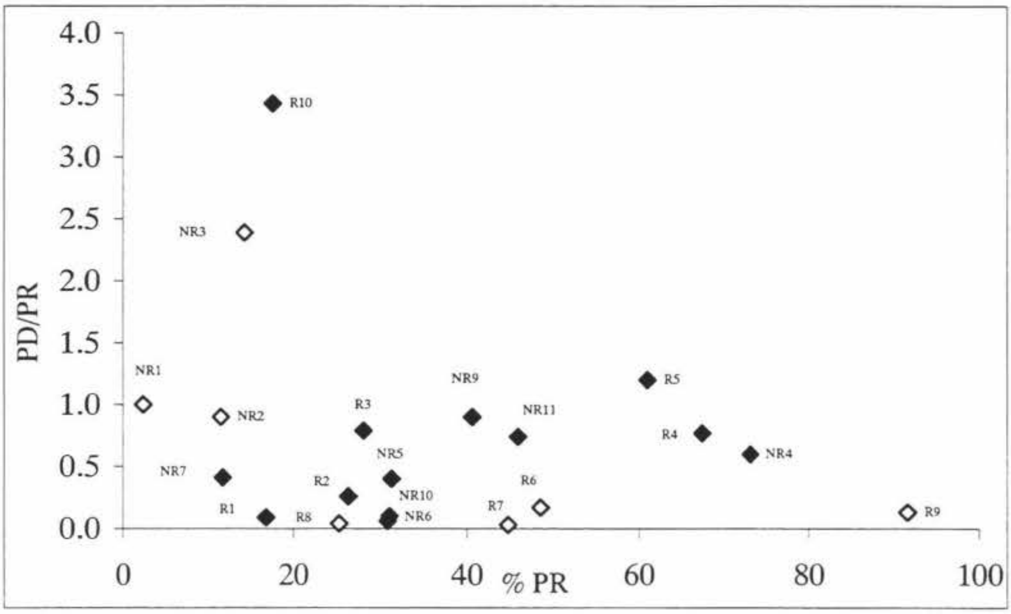

Figure 4: Pollination efficiency in rewarding (R1 – R10) and non-rewarding (NR1 – NR11) orchids. Clear rhomboids represent New Zealand species (Values of PD, PR and PD/PR ratio are given in the Appendix 10) (R1: *Dactylorhiza fuchsii*; R2: *Anacampis pyramidalis*; R3: *Comparettia falcata*; R4: *Platanthera stricta*; R5: *Disa uniflora*; R6: *Winika cunninghamii*; R7: *Earina aestivalis*; R8: *E. mucronata*; R9: *E. autumnalis*; R10: *Goodyera foliosa* var. *maximowicziana*; NR1: *Pterostylis alobula*; NR2: *P. patens*; NR3: *P. australis*; NR4: *Chloraea lamellata*; NR5: *Epidendrum ciliare*; NR7: *Pogonia japonica*; NR6: *Eulophia cristata*; NR9: *Caladenia tentaculata*; NR10: *Diuris maculata*; NR11: *Chloraea virescens*).

DISCUSSION

Pollen limitation seems to be responsible for the low fruit-set observed in these orchids - fruit-set was significantly increased by supplementary hand-pollinations in all species except *P. patens*. Low reproductive success in orchids is common; fruit set of 10 - 5% or below is usual in orchids (Nilsson 1983, Zimmerman & Aide 1989, Tremblay *et al.* 1998), this poor reproductive success has been generally attributed to the limited pollen delivery epiphytic and terrestrial orchids may experience (Gill 1989, Johnson & Bond 1997, Johnson & Steiner 1997, Hansen & Olesen 1999). Low reproductive success due to pollen limitation also seems to be common in many out-crossing flowering plants (Campbell 1987, Burd 1994, Larson & Barrett 1999, Baker *et al.* 2000, Goodwillie 2001) and is widespread in several plant communities of the world (Bawa 1974, Campbell 1987, Arroyo & Uslar 1993, Johnson & Bond 1997).

The causes of pollen limitation are various and they may be related to abiotic or biotic factors. Pollinator paucity, habitat preferences of pollinators and environmental conditions during flowering are the most commonly reported causes in the literature (Campbell 1987, Burd 1994, Johnson & Bond 1997, Larson & Barrett 1999, Baker *et al.* 2000) and more recently the breakdown of mutualistic relationships plant-pollinators (Robertson *et al.* 1999, Cunningham 2000, Parra-Tabla *et al.* 2000, Malo *et al.* 2001). Pollen limitation of the orchids studied in this chapter may be effected by two general components of their pollination biology, floral traits and pollinators. The disturbance of these components by human activities may also have a significant influence on the intensity of pollen limitation and hence in their reproductive success (see chapters II and III).

The presence of floral rewards, either nectar or pollen, has a significant effect on the reproductive success of out-crossing species. Neiland & Wilcock (1998) have demonstrated that the provision of nectar doubles the probability of fruit-set of orchids in both temperate and tropical areas and high reproductive success is associated with nectar reward. On the other hand, low fruit-set is common in orchids with pollination mechanisms based on deception, *i.e.* nectarless (Gill 1989, Proctor *et al.* 1996). In contrast to this, natural fruit-set in the orchids studied here was equally low in both rewarding and non-rewarding species. The same situation is observed when contrasting the intensity of pollen limitation in rewarding and non-rewarding species, except *P. patens*. Moreover, in contrast to the findings of Johnson & Bond (1997), pollinia deposition was also low in rewarding orchids. The low fruit-set in these nectariferous native orchids is somewhat unexpected. Nectar production within the family is generally considered as a mean of escaping the reproductive limitations of low pollinator visitation frequencies (Neiland & Wilcock 1998), and then a higher fruit-set in rewarding than deceptive species may be expected. The cause of low pollination success in these rewarding orchids may lie in the simplicity of their flowers and/or the poor efficiency of their pollinators in depositing pollinia.

All four nectariferous orchids lack complicated morphological adaptations for specific pollination systems with great accessibility to the reproductive structures and nectar. This general simplicity in the floral structures is a common feature of the New Zealand flora (Lloyd 1985). Floral simplicity promotes visitation by a wide range of insects that will not necessarily achieve pollination (see Chapter III) hence the lower efficiency of these species. In fact, although generalised attraction mechanisms may result in frequent visits, they also increase the risk of illegitimate depositions of pollinia and therefore wastage of pollen (Nilsson 1981, Pedersen 1995, Rasmussen 1995).

Species–specific pollination systems, such as the pollination by sexual deception proposed in the species of *Pterostylis* (chapter II), may be a more cost-effective system. In *P. alobula*, pollinia removal is low but the likelihood of each pollinia removed fertilising a conspecific flower is high, and pollen loss is minimal despite low visitation. Nevertheless, specialised pollination systems are also vulnerable to pollen limitation as legitimate visits may be rare (Larson & Barrett 1999). Species with specialised pollination mechanisms are more dependent on their pollinators; therefore plant fertility is most susceptible to pollinator stochasticity and the latter subject to the environmental fluctuations (Larson & Barrett 1999). Hence, species flowering during harsh and fluctuating environmental conditions (*e.g.* winter or early spring) are prone to pollen limitation because of the unpredictability of pollinator service (Baker *et al* 2000). Infrequent visitation by male fungus gnats may be responsible of pollen limitation in the winter flowering *P. alobula*.

Snow & Whigham (1989) suggest that if orchids have evolved under condition of infrequent pollinator visits, they may not have the resources to sustaining high levels of fruit-set. Under these circumstances, the primary role of unvisited flowers is to attract pollinators and enhance pollinia import and the female function of the visited flowers (Snow & Whigham 1989). Large floral display by synchronous anthesis or dense inflorescences in order to increase floral visitation is a common feature of New Zealand flora (Lloyd 1985) and is observed in these epiphytic orchids. Although large floral displays generally promote insect visitation and therefore fruit-set (Kearns & Inouye 1993), the excess of flowers relative to the number of pollinators visiting the populations may constrain reproductive success (Fritz & Nilsson 1994, Larson & Barrett 1999, Baker *et al.* 2000). In large flowering populations, pollination service is regulated by the size of the pollinator population, which in turn is regulated by abiotic seasonal factors (Inouye & Pyke 1988, Herrera 1995, Ishii & Higashi 2001).

Under conditions of infrequent visitation, pollinators will visit only flowers with certain floral traits, *i.e.* those attracting them most. Consequently, only a few individuals within the population will set fruit. Fixation of these floral traits is facilitated by the great dispersal ability of orchid seeds. The selected genotype(s) can easily become establish at a long distance from the mother population, making further exchange of pollen unlikely, and stimulating speciation by processes such as founder effect, differential selection pressure or genetic drift (Rasmussen 1995). If pollen limitation is a widespread condition in some New Zealand orchid genera, floral polymorphism and those narrowly distributed "forms" described within the genus *Pterostylis* (St. George 1999) are likely to be product of selective forces imposed by pollen limitation. The role of pollen limitation in promoting floral radiation, speciation, evolution of new mating strategies and specialisation to specific pollinators has been previously documented by Stebbins (1970), Arroyo & Squeo (1990) and Johnson & Bond (1992).

The flowering pattern observed in the species complex *E. mucronata*/*E. aestivalis* may be explained, to certain extent, as a result of a limited pollen delivery. It is possible that this species complex is adjusting its reproductive phenology to a season with a better pollination service. The direction of this change is difficult predict with the data here gathered. The spring flowering taxa *E. mucronata* seems to have a more efficient pollination system (PD/PR= 0.004) than *E. aestivalis* (PD/PR= 0.003) but also a lower fruit-set (4 %) and more pollen limitation (p= 0.94) than *E. aestivalis*.

Although this chapter demonstrated the occurrence of pollen limitation in six species of native orchids regardless of their rewarding systems, it is important to remark that these results do not necessarily indicate a widespread condition. These results represent the local response of some orchid populations under determined environmental conditions that prevailed during the season when this study was conducted. It is totally likely that the intensity of pollen-limitation will be different along the distributional range of these species and/or through the years (Campbell 1987, Gill 1989, Campbell & Halama 1993, Johnson & Bond 1997, Larson & Barrett 1999, Baker *et al.* 2000). Ideally, future studies should include as many populations as possible and covering the distributional range of the species as well as considering several flowering seasons; if resource limitation has an accumulative effect on fruit-set its effect will not be evident until several fruiting episodes have occurred.

V

Concluding Remarks

The main goal of any conservation effort is to maintain self-sustaining populations in the wild in the long-term. Eliminating the threats to these populations, or reducing their effect, and increasing the knowledge of their biology and ecology may significantly contribute to this goal. However, when endangered or rare plants are under permanent or immediate threat, management of the population is the only alternative. Population management may involve tactics such as reintroduction, translocation and improvement of the seed-set by hand-pollination treatments (Cropper 1993, Given 1994). Unfortunately, when the basic biology of the target taxa is unknown or poorly studied, these attempts may be unsuccessful and sometimes disadvantageous, changing the genetic structure of the populations and therefore their evolutionary development (Cropper 1993).

This lack of information may have a dramatic effect on plants, especially when the target taxa depend on the presence of other organisms for their survival, that hence must be preserve at the same time (Given 1994). In orchids, species survival may be limited by the normal functioning of at least three interactions, plant-pollinator, plant-mycorrhiza and, in epiphytes, plant-host tree. The presence of these interactions, usually species-specific, makes orchids extremely vulnerable to disturbances in the ecosystem and therefore useful indicator species (Wells 1981). Despite knowing the range of ecological interactions orchids may have, translocations and manipulation of orchid populations in New Zealand have already taken place (de Lange 1991, Clarkson *et al.* 1993), even though the biology and reproductive strategies of these, and many other orchids in the country, are still unknown.

This thesis studied the pollination ecology of a range of terrestrial and epiphytic orchids in the lower portion of the North Island. The reproductive biology of these orchids was studied by using hand-pollination treatments, comparative assessment of fruiting success and assessing their reproductive features (pollen and ovule numbers and P/O), also the causes limiting their reproductive success were explored. Furthermore, the plant-pollinator interactions observed in these orchids were studied by assessing the specificity of the interaction and the orchid reproductive dependence on this mutualism.

The research conducted in this thesis is the first detailed work on the pollination of New Zealand orchids and many of the results are novel when put into context of the rest of the vascular flora. First, specialised pollination systems are considered uncommon in New Zealand, and restricted to bird and bat pollinated plants (Webb & Kelly 1993). Pollination syndromes observed in some of the orchids included in this study may prove the contrary. For instance, in *Pterostylis alobula*, pollination occurs by male fungus gnats probably attracted by sexual deception. This appears to be the first record of a species-specific pollination system in insect-pollinated flowers in New Zealand. Pollination by fungus gnats had been suggested before in some New Zealand's *Pterostylis* but never confirmed. Furthermore, pollination *Earina* species by records of visiting insects such as the Ichneumonidae wasps, usually linked to pollination by pseudocopulation, and weevil (this has never been described in any native New Zealand flowering plant nor in any orchid species worldwide) are very interesting observations requiring further and detailed studies.

Secondly, the rich insect fauna associated with the nectariferous orchids was unexpected especially when considering past records indicating poor insect visitation. In this study a total of 18 species belonging to 13 families were observed visiting these orchids, two of these are undescribed. Epiphytic orchids appear to be an important source of nectar for native and exotic insect species, especially during autumn and early winter (*E. autumnalis*). Unfortunately, their rewarding nature also makes them more vulnerable to visitation by exotic or generalists nectar-feeding insects that may compete and displaced their native pollinators (*e.g. Apis mellifera*) and also promote pollen loss by illegitimate visitation (nectar thieves).

Finally, the occurrence of two partially self-incompatible orchid species is also a novelty. Self-incompatibility is rare in New Zealand, and it has been experimentally demonstrated only in seven species (Webb & Kelly 1993). If this reproductive barrier has originated *in situ*, studying the forces promoting self-incompatibility may be helpful to understand orchid speciation processes, diversification of mating systems and systematic relationships with other genera (*e.g. Winika - Dendrobium*). However, self-incompatibility also increases the risk of low reproductive success when populations are highly fragmented, since individuals are likely then to be sparsely distributed and plant-pollinator interactions may be disrupted.

Finally, this study demonstrates a poor reproductive and pollination success in all the out-crossing orchids, except *P. patens*. Although low reproductive success is common in orchids its causes are not clearly understood. This study has shown that low fruit-set in these widespread orchids is caused by the NZ orchids may be affected by low pollen availability, and therefore by pollination failure. There may be several causes of pollination failure in these orchids due perhaps to harsh climatic conditions, pollinator displacement by exotic insects, altered pollination effectiveness, pollinator extinction and/or habitat fragmentation. Identifying the cause(s) of pollination failure in these orchids should be the next step towards their conservation.

A thought

If fruit-set is low in these widespread species, what can we expect fruit-set in rare or narrowly distributed orchids to be like?

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Appendix 1: Checklist of native New Zealand orchids (only taxonomically valid names were considered).

1. *Acianthus sinclairii*
2. *Adenochilus gracilis*
3. *Aporostylis bifolia*
4. *Bulbophyllum pygmaeum*
5. *Bulbophyllum tuberculatum*
6. *Caladenia alata*
7. *Caladenia atradenia*
8. *Caladenia barlettii*
9. *Caladenia carnea*
10. *Caladenia catenata*
11. *Caladenia chlorostyla*
12. *Caladenia lyallii*
13. *Caladenia minor*
14. *Caladenia nothofageti*
15. *Caleana minor*
16. *Calochilus herbaceous*
17. *Calochilus paludosus*
18. *Calochilus robertsonii*
19. *Chiloglottis cornuta*
20. *Chiloglottis formicifera*
21. *Chiloglottis valida*
22. *Corybas acuminatus*
23. *Corybas carsei*
24. *Corybas cheesemanii*
25. *Corybas cryptanthus*
26. *Corybas dienemus*
27. *Corybas iridiscens*
28. *Corybas macranthus*
29. *Corybas oblongus*
30. *Corybas orbiculatus*
31. *Corybas papa*
32. *Corybas rivularis*
33. *Corybas rotundifolious*
34. *Corybas trilobus*
35. *Cryptostylis subulata*
36. *Cyrtostylis oblonga*
37. *Cyrtostylis reniforme*
38. *Danhatchia australis*
39. *Drymnoanthus adversus*
40. *Drymnoanthus flavus*
41. *Earina autumnalis*
42. *Earina aestivalis*
43. *Earina mucronata*
44. *Gastrodia cunninghamii*
45. *Gastrodia minor*
46. *Gastrodia sesamoides*
47. *Genoplesium nudum*
48. *Genoplesium pumilum*
49. *Microtis arenaria*
50. *Microtis oligantha*
51. *Microtis parviflora*
52. *Microtis unifolia*
53. *Orthoceras novae-zelandiae*
54. *Prasophyllum colensoi*
55. *Prasophyllum patens*
56. *Pterostylis agathicola*
57. *Pterostylis alobula*
58. *Pterostylis areolata*
59. *Pterostylis australis*
60. *Pterostylis banksii*

61. *Pterostylis brumalis*
62. *Pterostylis cardiostigma*
63. *Pterostylis cernua*
64. *Pterostylis foliata*
65. *Pterostylis graminea*
66. *Pterostylis humilis*
67. *Pterostylis irsoniana*
68. *Pterostylis irwinii*
69. *Pterostylis micromega*
70. *Pterostylis montana*
71. *Pterostylis nutans*
72. *Pterostylis oliveri*
73. *Pterostylis paludosa*
74. *Pterostylis patens*
75. *Pterostylis porrecta*
76. *Pterostylis puberula*
77. *Pterostylis tanypoda*
78. *Pterostylis tasmanica*
79. *Pterostylis tristis*
80. *Pterostylis trullifolia*
81. *Pterostylis venosa*
82. *Spiranthes sinensis*
83. *Thelymitra aemula*
84. *Thelymitra carnea*
85. *Thelymitra circumsepta*
86. *Thelymitra cyanea*
87. *Thelymitra hatchii*
88. *Thelymitra intermedia*
89. *Thelymitra longifolia*
90. *Thelymitra malvina*
91. *Thelymitra matthewsii*
92. *Thelymitra media*
93. *Thelymitra nervosa*
94. *Thelymitra pauciflora*
95. *Thelymitra pulchella*
96. *Thelymitra sanscilia*
97. *Thelymitra tholiformis*
98. *Townsonia deflexa*
99. *Waireia stenopetala*
100. *Winika cunninghammi*

Appendix 2: Pollination treatments, number of individuals, number of flowers treated and fruit-set in four terrestrial orchids (*Thelymitra longifolia*, *Gastrodia cunninghamii*, *Pterostylis alobula* and *P. patens*) and four epiphytic orchids (*Winika cunninghamii*, *Earina autumnalis*, *E. mucronata* and *E. aestivalis*) (**APO**: apomixis, **AS**: automatic self-pollination, **N**: natural fruit-set, **C**: hand cross-pollination and **S**: hand self-pollination).

T. longifolia

Treatment	No. Individuals	No. Flowers	No. Capsules	% Fruit-set
APO	5	14	0	0
AS	8	25	20	80
C	7	31	25	83.87
S	7	24	17	70.83
N	5	46	36	78.26

G. cunninghamii

Treatment	No. Individuals	No. Flowers	No. Capsules	% Fruit-set
APO	6	28	17	60.71
AS	5	49	40	81.63
C	6	34	6	17.64
S	6	29	0	0
N	6	197	182	92.38

P. alobula

Treatment	No. Individuals	No. Flowers	No. Capsules	% Fruit-set
APO	52	52	0	0
AS	30	30	0	0
C	50	50	22	44
S	50	50	17	34
N	73	73	2	2.73

P. patens

Treatment	No. Individuals	No. Flowers	No. Capsules	% Fruit-set
APO	30	30	0	0
AS	30	30	0	0
C	23	23	14	60.86
S	27	27	18	66.66
N	24	24	10	41.66

W. cunninghamii

Treatment	No. Clumps	No. Flowers	No. Capsules	% Fruit-set
APO	3	35	0	0
AS	3	39	0	0
C	3	32	24	75
S	3	32	12	37.5
N	3	62	14	22.58

E. autumnalis

Treatment	No. Clumps	No. Flowers	No. Capsules	% Fruit-set
APO	8	42	0	0
AS	8	51	0	0
C	8	66	40	66.66
S	8	65	36	55.38
N	10	303	91	30.03

E. mucronata

Treatment	No. Clumps	No. Flowers	No. Capsules	% Fruit-set
APO	5	45	0	0
AS	5	33	0	0
C	5	43	30	69.76
S	5	43	29	67.44
N	3	191	8	4.19

E. aestivalis

Treatment	No. Clumps	No. Flowers	No. Capsules	% Fruit-set
APO	3	45	0	0
AS	3	33	0	0
C	3	49	38	77.5
S	3	46	17	36.95
N	5	230	16	6.9

Appendix 3: Results from the binomial generalised linear model analysis for natural fruit-set, comparison between species of the same genus are highlighted ($\chi^2 = 585.36$, 7 df, $p<0.0001$; threshold for significance: 0.002).

Species	EAU	EMUC	GC	PA	PP	TL	WC
EAE	**< 0.0001**	**0.2274**	< 0.0001	0.2004	< 0.0001	< 0.0001	0.0007
EAU		**< 0.0001**	< 0.0001	0.0002	0.2391	< 0.0001	0.2396
EMU			< 0.0001	0.5841	< 0.0001	< 0.0001	< 0.0001
GC				< 0.0001	< 0.0001	0.0066	< 0.0001
PA					**< 0.0001**	< 0.0001	0.0020
PP						0.0031	0.0811
TL							< 0.0001

EAE: *Earina aestivalis*, EAU: *E. autumnalis*, EMU: *E. mucronata*, GC: *Gastrodia cunninghamii*, PA: *Pterostylis alobula*, PP: *P. patens*, TL: *Thelymitra longifolia* and WC: *Winika cunninghamii.*

Appendix 4: Results from the binomial generalised linear model analysis for fruit-set through pollination treatments in New Zealand terrestrial orchids. Threshold for significance in A: 0.008 and B: 0.017. **AS**: automatic self-pollination; **APO**: apomixis; **S**: hand self-pollination; **C**: hand cross-pollination; **N**: natural pollination).

Species	*G. cunninghamii*^{A}	*T. longifolia*^{A}	*P. alobula*^{B}	*P. patens*^{B}
χ^2	90.06	0.86	38.44	3.47
df	3	3	2	2
P	< 0.0001	= 0.8353	< 0.0001	< 0.0001
Contrasting				
AS– APO	0.0482	--------	--------	--------
AS – S	--------	0.4578	--------	--------
AS – N	0.0278	0.8639	--------	--------
AS – C	<0.0001	0.9518	--------	--------
S – APO	--------	--------	--------	--------
S – C	--------	0.3982	0.3063	0.6706
S – N	--------	0.4928	0.0002	0.0766
N – APO	< 0.0001	--------	--------	--------
N – C	< 0.0001	0.8004	<0.0001	0.1908
C - APO	0.0009	--------	--------	--------

Appendix 5: Results from the binomial generalised linear model analysis for fruit-set through pollination treatments in New Zealand epiphytic orchids. Threshold for significance: 0.017. (**C**: hand cross-pollination, **S**: hand self-pollination; **N**: natural pollination, **APO**: apomixis; **S**: hand self-pollination).

Species	*E. aestivalis*	*E. autumnalis*	*E. mucronata*	*W. cunninghamii*
χ^2	112.27	30.19	133.10	24.70
df	2	2	2	2
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Contrasting				
APO - AS				
C – S	0.0001	0.5451	0.8163	0.0033
C – N	< 0.0001	< 0.0001	< 0.0001	< 0.0001
N - S	0.0001	0.0001	< 0.0001	0.1288

Appendix 6: Procedure for the anthrone colorimetric assay for total carbohydrate (modified from Kearns & Inouye 1993).

The total carbohydrate determination in the samples was conducted in four stages:

1. Extraction of sugar from wicks previously placed in plants. Each filter paper wicks was placed in a vial containing 5mL of distilled water, swirled and leave for 30 mins.

2. Determination of the appropriate dilution factor to use for each species. Sub samples of 1, 0.5, 0.1 and 0.01mL of the solution were removed from one of the vials and poured into boiling tubes previously labelled. Volumes were brought to 1mL using distilled water. 2mL of anthrone reagent (250mL of H_2SO_4, 12.5mL of distilled water and 0.5g of anthrone) was added to the tube and gently vortex to mix the reagents. Tubes were then placed for 10 min. in a water bath (~80°C). When cool, tubes were compared with the standard sugar series previously prepared. The best dilution factor was selected based on the closest match with the standard near the lighter end of the scale.

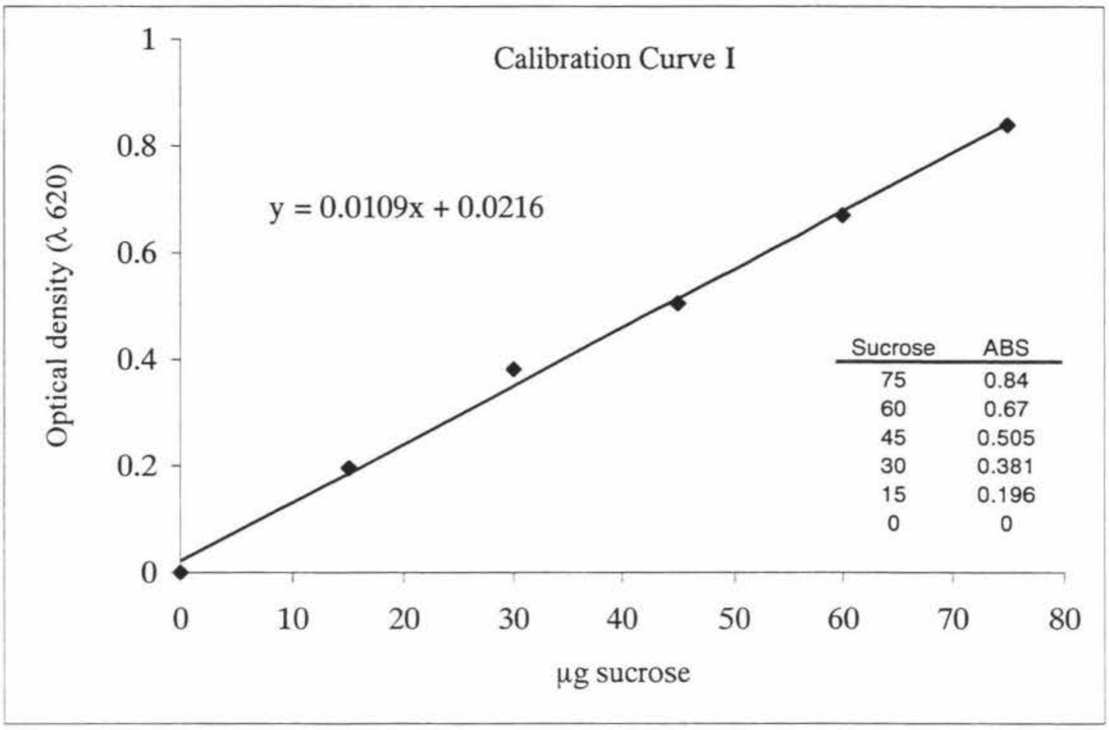
3. Analysis of the replicate samples. The remaining samples were prepared as mentioned in the step 2 but using only the best dilution factor. Sugar concentration was read in the spectrophotometer at λ 620nm (here the equipment Jenway 6105 U.V./Vis. Spectrophotometer, Biolab Scientific Ltd. was used).

4. Sugar concentration in the sample was estimated using the calibration curve previously prepared. To calculate the actual sugar content, sugar concentration in the sample was multiplied by the dilution factor to give total amount of sugar in the vial.

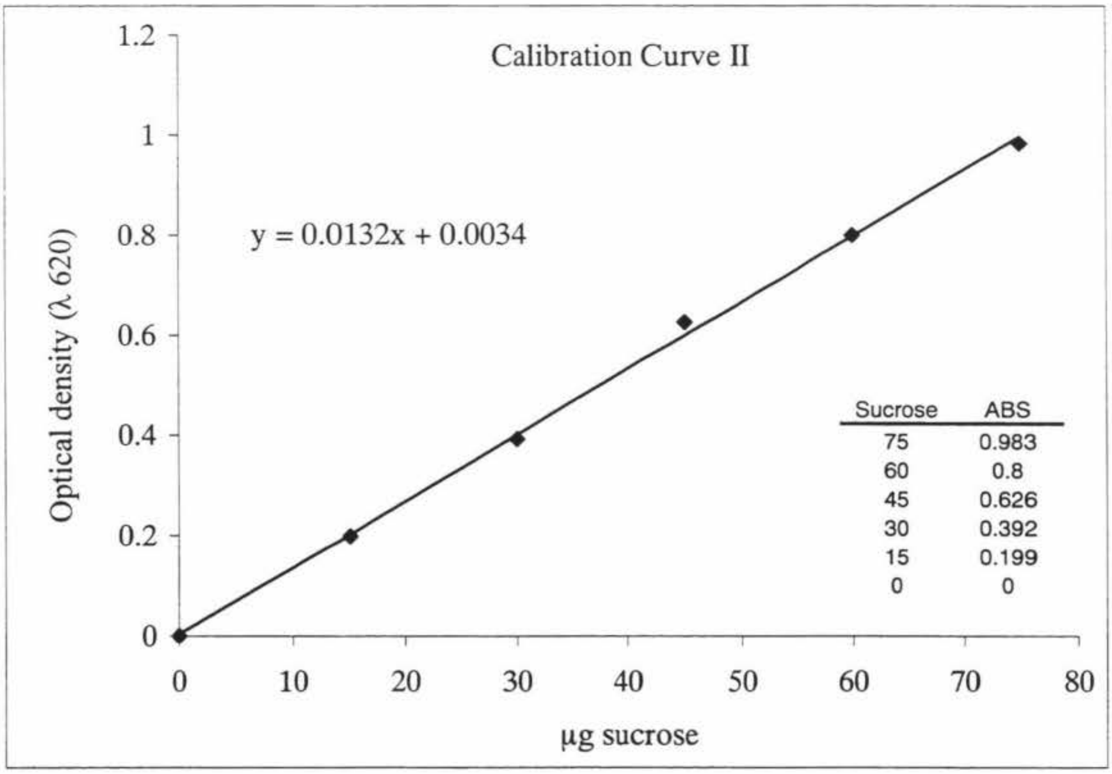
Sugar standards

50mL of a stock solution of 75mg of sucrose (Analar) was prepared in a volumetric flask. Then dilutions of the stock were prepared taking 2.5, 2, 1.5, 1 and 0.5mL into 50mL volumetric flasks and top up with distilled water. A blank of distilled water only was prepared too.

Appendix 7: Calibration curves for calculating µg sucrose. Sugar content was determined in two rounds, a new anthrone and sugar standards had to be prepared (Calibration curve II).



Sucrose	ABS
75	0.84
60	0.67
45	0.505
30	0.381
15	0.196
0	0



Sucrose	ABS
75	0.983
60	0.8
45	0.626
30	0.392
15	0.199
0	0

Appendix 8: Nectar standing crop expressed in milligrams of sucrose in *Earina aestivalis*, *E. autumnalis*, *E. mucronata* and *Winika cunninghamii*.

E. aestivalis (nectar dilution: 1mL)

Sample	Absorbance	Interpolated Sugar	Sucrose equiv. (μg)
1	0.047	2.3302	11.66
2	0.057	3.2477	16.23
3	0.026	0.4036	2.018
4	0.051	2.6972	13.486
5	0.027	1.7878	8.939
6	0.020	1.2575	6.288
7	0.066	0.0208	0.104
8	0.014	0.8030	4.015
9	0.020	1.2575	6.288
10	0.018	1.1060	5.53
	Average sugar content and standard error		7.4558 ± 1.616302

E. autumnalis (nectar dilution: 0.1mL)

Sample	Absorbance	Interpolated Sugar	Sucrose equiv. (μg)
1	0.176	14.1651	708.25
2	0.078	5.1743	258.72
3	0.387	32.6972	1634.86
4	0.176	14.1651	708.26
5	0.240	20.0366	1001.83
6	0.079	5.2660	263.30
7	0.023	0.1284	6.42
8	0.090	6.2752	313.76
9	0.480	42.5137	2125.68
10	0.052	2.7889	139.45
	Average sugar content and standard error		716.053 ± 219.2176

E. mucronata (nectar dilution: 0.1mL).

Sample	Absorbance	Interpolated Sugar	Sucrose equiv. (μg)
1	0.238	19.8532	992.68
2	0.233	19.3944	969.72
3	0.420	36.5504	1827.70
4	0.300	25.5412	1277.06
5	0.297	25.2660	1263.30
6	0.150	11.7798	588.99
7	0.480	42.5137	2125.68
8	0.240	20.0366	1001.83
9	0.185	14.9908	749.90
10	0.283	23.9816	1199.08
	Average sugar content and standard error		1199.594 ± 148.0255

W. cunninghamii (nectar dilution: 1mL).

Sample	Absorbance	Interpolated Sugar	Sucrose equiv. (μg)
1	0.850	76.0000	380
2	0.323	27.6513	138.065
3	0.143	11.1376	55.68
4	0.854	76.3669	381.83
5	0.351	47.7889	238.89
6	0.387	33.5229	167.61
7	0.176	14.1651	70.8255
8	0.350	30.1284	150.642
9	0.750	66.8256	334.128
10	0.450	39.3027	196.5135
	Average sugar content and standard error		211.4184 ± 37.78725

Appendix 9: List of insects associated with some of the orchids included in this study (MD: Marima Domain, RFP: Ruahine Forest Park, FHR: Forest Hill Rd.; Palmerston North and P: Putara; Tararua Forest Park). *Exotic species. #Undescribed species.

Species	Insect taxa	Family	Site
E. aestivalis	*Prolasius advena*	Formicidae	MD
	Melangyna novaezealandiae	Syrphidae	MD
	Spilogonoa melas	Muscidae	MD
	Calliphora quadrimaculata	Calliphoridae	MD
	Aucklandella sp. (*ursula*?)	Ichneumonidae	MD
	Hylaeus sp.	Colletidae	MD
	Unidentified weevil	Curculionidae	MD
E. autumnalis	*Limonia tarsalba*	Tipulidae	P
	Hilara sp. #	Empidae	P
	Melanostoma fasciatum	Syrphidae	P
	Eristalis tenax *	Syrphidae	P
	Bombus terrestris *	Apidae	P
	Heteria sp.	Tachinidae	P
	Zealandotachina sp.	Tachinidae	P
E. mucronata	*Dilophus nigrostigmus*	Bibionidae	P
	Ceratochilus sp.	Tipulidae	P
	Hilara sp. #	Empidae	P
	Unidentified beetle	Coleoptera	MD
W. cunninghamii	*Melangyna novaezealandiae*	Syrphidae	P
	Helophilus antipodus	Syrphidae	P
	Apis mellifera *	Apidae	P
	Prolasius advena	Formicidae	P
P. australis	*Aneura longipalpis* (♂)	Mycetophilidae	RFP
	Cerotelion sp. (♂)	Keroplatidae	RFP
P. alobula	*Zygomyia sp.* (♂)	Mycetophilidae	FHR
	Cnephasia jactatana	Tortricidae	FHR

Appendix 10: Percentages of pollinia removal (PR) and pollinia deposition (PD) and PD/PR ratio calculated in rewarding (R1-10) and non-rewarding (NR1 – 11) orchids.

Code	Species	%PR	%PD	PD/PR	References
R1	*Dactylorhiza fuchsii*	16.75	1.5	0.09	Waite *et al.* 1991
R2	*Anacampis pyramidalis*	26.4	6.96	0.26	Waite *et al.* 1991
R3	*Comparettia falcata*	28.15	22.32	0.79	Rodriguez-Robles *et al.* 1992
R4	*Platanthera stricta*	67.5	52	0.77	Patt *et al.* 1989
R5	*Disa uniflora*	61	73	1.2	Johnson & Bond 1992
R6	*Winika cunninghamii*	48.64	8.1	0.17	This work
R7	*Earina aestivalis*	44.85	1.24	0.03	This work
R8	*Earina mucronata*	25.38	0.91	0.04	This work
R9	*Earina autumnalis*	91.52	11.86	0.13	This work
R10	*Goodyera foliosa var. maximowicziana*	17.5	60.1	3.43	Naoto & Tsutomu 1997
NR1	*Pterostylis alobula*	2.39	2.39	1	This work
NR2	*Pterostylis patens*	11.49	10.34	0.9	This work
NR3	*Pterostylis australis*	14.28	34.06	2.39	This work
NR4	*Chloraea lamellata*	73.07	44	0.6	Lehnebach 1998
NR5	*Epidendrum ciliare*	31.44	12.7	0.4	Ackerman & Montalvo 1990
NR7	*Pogonia japonica*	11.7	4.75	0.41	Ushimaru & Nakata 2001
NR6	*Eulophia cristata*	31	1.97	0.06	Lock & Profita 1975
NR9	*Caladenia tentaculata*	40.6	36.6	0.9	Peakall & Beattie 1996
NR10	*Diuris maculata*	31.25	3.01	0.1	Beardsell *et al.* 1986
NR11	*Chloraea virescens*	46	34	0.74	Riveros & Lehnebach 2000

Appendix 11: Results of ANOVA analyses for natural fruit set (Table 1), pollinia removal (Table 2) and pollinia deposition (Table 3) between New Zealand rewarding and non-rewarding orchids.

Table 1

Source	SS	DF	MS	F	P
Between	52.417	1	52.417	.172	0.700
Within	1220.173	4	305.043		
Total	1272.591	5			

Table 2

Source	SS	DF	MS	F	P
Between	3199.022	1	3199.022	6.641	0.050
Within	2408.705	5	481.741		
Total	5607.727	6			

Table 3

Source	SS	DF	MS	F	P
Between	174.240	1	174.240	1.387	0.292
Within	628.289	5	125.658		
Total	802.529	6			

Appendix 12: Results of Mann-Whitney *U*- test on pollinia deposition/pollinia removal ratio (PD/PR) in New Zealand rewarding and non-rewarding orchids (Table 1) and New Zealand species together with PD/PR obtained from the literature (Table 2).

Table 1

Group	N	Mean rank	Sum of the Ranks	U	P
Rewarding	4	2.50	10.00	0.000	0.034
Non-rewarding	3	6.00	18.00		
Total	7				

Table 2

Group	N	Mean rank	Sum of the Ranks	U	P
Rewarding	10	9.30	93.00	38.000	0.364
Non-rewarding	10	11.70	117.00		
Total	20				

Appendix 13: Number of rain days per month in the years 2000, 2001 and 2002 as recorded at Palmerston North Airport (data missing between March and May 2001 is due to interruption in the measuring).