

## Pollination studies of four New Zealand terrestrial orchids and the implication for their conservation

CARLOS A. LEHNEBACH  
Allan Wilson Centre for Molecular Ecology and  
Evolution  
Massey University  
Private Bag 11 222  
Palmerston North, New Zealand  
c\_lehnebach@hotmail.com

ALASTAIR W. ROBERTSON  
Ecology Group  
Institute of Natural Resources  
Massey University  
Private Bag 11 222  
Palmerston North, New Zealand

DUNCAN HEDDERLEY  
Crop & Food Research  
Private Bag 11 600  
Palmerston North, New Zealand

species and was observed only in *G. cunninghamii* and *P. alobula*. Aphids were usually found inside the flowers of *G. cunninghamii*, but the role they may have as pollinators is undetermined. In *P. alobula*, male fungus gnats of *Zygomysia* (Mycetophilidae: Diptera) were considered pollinators. Hypotheses on the attraction system(s) used by these greenhouse orchids are discussed. These two species are more likely to be affected by disruption of the plant-pollinator mutualism because of the specialist nature of the plant-pollinator interaction.

**Keywords** conservation; disruption of mutualisms; *Diplodinium*; *Gastrodia*; Mycetophilidae; New Zealand; Orchidaceae; pollination; *Pterostylis*; *Thelymitra*; terrestrial orchids; sexual deception; shelter flowers

**Abstract** The New Zealand orchid flora comprises 25 genera and over 100 species. Most of the species are terrestrial and are found throughout the country. Terrestrial orchids are the most threatened group within the family. We studied the pollination ecology of four terrestrial orchids: *Gastrodia cunninghamii*, *Thelymitra longifolia*, *Pterostylis alobula*, and *P. patens*. Reproduction of these orchids relies on contrasting reproductive strategies. *Thelymitra longifolia* is predominantly self-pollinated, whereas both *Pterostylis* species are cross-pollinated and have an absolute dependence on pollinators. Hand-pollination treatments showed *T. longifolia*, *P. alobula*, and *P. patens* to be self-compatible. Results for *G. cunninghamii* were unclear and need further study. Insect flower visitation is uncommon in these

### INTRODUCTION

The New Zealand orchid flora comprises 25 genera and over 100 species. The family represents almost 5% of the total native vascular species in the flora and 17.4% of the monocot flora (updated from Johns & Molloy 1983). Most of the genera are terrestrial, which as a group contains over 90 species. Nearly half of the 21 genera in this group are represented by just one species. Terrestrial orchids are the most threatened group within the family. Currently the list of threatened and uncommon New Zealand plants includes 33 described species of ground orchids and c. 17 taxonomically indetermined taxa (de Lange et al. 2004).

Habitat destruction, modification, fragmentation, and over-collection are thought to be the main threats to New Zealand terrestrial orchids (Norton et al. 1994; St George 1999). The effect that disruption of ecological interactions with pollinators and/or associated fungi may have on their survival has not been examined. Disruption of plant-pollinator relationships potentially poses a serious threat to species survival, and the negative effect on species reproductive success has already been observed in various

bird-pollinated New Zealand plants (Anderson 1997; McNutt 1998; Robertson et al. 1999; Montgomery et al. 2001; Newstrom & Robertson 2005).

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 the 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. Despite the prevalence of self-pollination in New Zealand terrestrial orchids, the occurrence of insect-dependent species has also been reported (Cheeseman 1873; Darwin 1877; Thomson 1927; Molloy 1990; St George 1999). 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 inside the flower. Later, the insect escapes by passing through a "tunnel" formed by the column wings and, in doing so, removes a pollinium, if one is still intact, and carries it to a second flower where pollinium deposition takes place during the escape (Johns & Molloy 1983; Proctor et al. 1996).

Pollination studies in New Zealand orchids are still far from complete. Besides the study on the pollination ecology of a selected group of common native epiphytic and terrestrial orchids by Lehnebach (2002) and Lehnebach & Robertson (2004) and the reviews of Godley (1979) and Newstrom & Robertson (2005), most of the information available is based on morphological and phenological observations, little of it in great detail.

Understanding orchid pollination systems is essential for their effective conservation (Sipes & Tepedino 1995). Furthermore, it helps to anticipate their extinction probability should the plant-pollinator interaction be disrupted. To assess species extinction after pollinator failure, Bond (1994, 1995) suggested consideration of three main aspects of the interaction: the likelihood of mutualisms failing, the degree of reproductive dependence on the mutualism, and the importance of seeds in the demography of the plant studied. In this paper, we concentrate on the first two aspects by studying the breeding system

and pollinator-dependence of four common terrestrial orchids occurring in southern North Island.

## METHODS

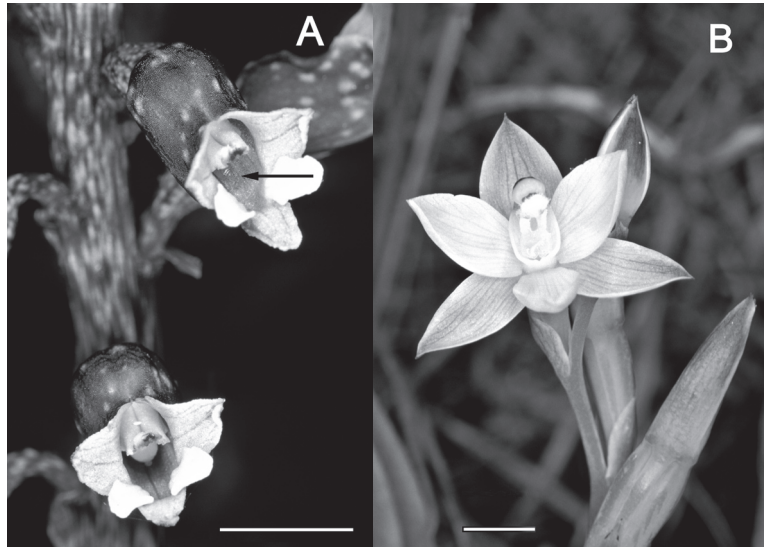
### Species studied

The saprophytic orchid *Gastrodia cunninghamii* (Fig. 1A) belongs to a genus of about 15 species distributed throughout the Australasian region (Johns & Molloy 1983). The plant can reach 1 m in height and bear up to 70 flowers on its single stem. The flowers are knobby and tubular and formed by the fusion of the sepals and petals, which are separated only at their tips. It occurs both in the North and South Islands, 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).

In New Zealand *Thelymitra longifolia* (Fig. 1B) is one of the most widespread orchid species. It occurs in the North and South Islands and also Stewart, Chatham, and Auckland Islands (Moore & Edgar 1976; Johns & Molloy 1983). It is usually found on clay banks, in grasslands, or 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 c. 1.5 cm across. The genus *Thelymitra* is distributed throughout Australasia and Malaysia and is regarded as being primitive in the Orchidaceae due to its actinomorphic flowers (van der Pijl & Dodson 1966).

The genus *Pterostylis* contains over 180 species and several natural hybrids, and is found throughout the Australasian region (St George 1999; Jones & Clements 2002). Recently, based on morphological and molecular evidence, this genus has been taxonomically reassessed and split into 17 genera (Jones & Clements 2002). This revision has resulted in 5 genera for New Zealand species of *Pterostylis*: *Diplodium*, *Hymenochilus*, *Linguella*, *Plumatichilos*, and *Pterostylis*. This classification affects only one of the species included in this study: *P. alobula* (Hatch) L.B. Moore has been transferred to *Diplodium* and is now referred as *Diplodium alobulum* (Hatch) D.L. Jones, Molloy et M.A. Clem. The former name of this orchid, *P. alobula*, will be used in this paper. Species included in this study are *P. alobula* and *P. patens* Colenso; both endemic to New Zealand. *Pterostylis alobula* is a plant of c. 15 cm height when flowering. It has sessile, bracteate leaves. The plant usually bears a solitary and erect flower.

**Fig. 1** Flowers of *Gastrodia cunninghamii* (A) and *Thelymitra longifolia* (B). Arrow in *G. cunninghamii* indicates aphid crawling inside the flower. Scale bars = 5 mm.



The labellum is narrowed in its upper half, with slightly swollen, truncate or crenulated tip. The second species, *P. patens*, is a robust grass-leaved plant of montane and subalpine forests. When flowering the plant may reach 15 to 100 cm tall, topped by a single flower which is considerably bigger than that of *P. alobula*, at 5 × 3 cm. Both species may form clonal populations by the production of daughter tubers on stolonoid roots (Jones & Clements 2002) yet isolated individuals of *P. patens* are often found in the wild.

### Study sites

**Paengaroa Scenic Reserve** (39°38'S, 175°43'E): The reserve occupies 101 ha. It is a forest remnant on the banks of the Hautapu River in the Rangitikei Ecological Region, North Island. 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 while the understorey vegetation comprises mainly divaricating shrubs (see Ogle et al. (2000) for further details). The orchid *G. cunninghamii* was studied at this site.

**Takapari Road, Ruahine Forest Park** (40°4'S, 175°58'E): This park includes the boundary between Hawke's Bay and Manawatu-Wanganui regions, North Island. Takapari Rd is in the southern Ruahine Range. On this range are three altitudinal vegetational belts: podocarp-hardwood forest, *Weinmannia* forest or its scrub replacement, and *Plagianthus regius* scrub (van Essen 1992). The populations of

*Pterostylis patens* studied here occur along the road, in the first two vegetation belts.

**Forest Hill Road** (40°21'S, 175°32'E): This is a public road, c. 10 km from Palmerston North city, North Island. There are several populations of *Pterostylis alobula* along the roadside, some of them among remnants of native shrubs of *Kunzea ericoides* and the exotic *Cytisus scoparius* and under pine tree plantations.

**Pahiataua Track** (40°24'S, 175°42'E): The study site is on the roadside c. 15 km from Palmerston North city. This site supports a secondary growth scrub dominated by *Cytisus scoparius*. A *Pinus radiata* plantation tops the bank. The sun orchid *T. longifolia* was studied at this site.

### Reproductive biology

The breeding system of these orchids was studied by performing the following hand-pollination treatments:

1. *Direct autogamy*: flowers were bagged before anthesis until senescence, excluding pollinators. This treatment tests whether fruit-set occurs by autonomous self-pollination.
2. *Apomixis*: before anthesis, the entire pollinarium was removed with forceps, and the flowers were then bagged until senescence. The treatment allowed determination of agamospermy.
3. *Self-compatibility*: the pollinarium was removed and flowers bagged until stigma receptivity. Flowers were then hand-pollinated with the whole pollinarium

from another flower on the same inflorescence and bagged again until wilting. Pollinia from the same flower were used to hand self-pollinate *Pterostylis* species.

4. *Cross-pollination*: same as previous treatment, but flowers were pollinated with pollen from another individual. Since *P. alobula* and *P. patens* are colony forming species, pollen from different colonies were used for this treatment.

5. *Natural pollination*: floral buds were tagged and allowed to develop to fruit under natural conditions of pollination.

To reach the reproductive structures of *G. cunninghamii*, the entire perianth was removed before anthesis using a scalpel. Flowers were then emasculated using forceps and bagged until stigmas were receptive. This was done for all treatments except direct autogamy and natural pollination.

Before dehiscence, capsules from all species were collected and stored individually in paper bags. The presence of self-incompatibility was scored using a self-compatibility index (SCI: number of fruits produced by hand self-pollination/number of fruits produced by hand cross-pollination). Fruit-set was analysed by generalised linear model with a binomial error distribution and logit link function using SAS (SAS Institute 2001). Likelihood Ratio Tests were used to test for significant differences between treatments.

### Pollen:ovule ratio (P/O)

To determine the P/O ratio, the entire pollinia of 10 flowers, each from a different individual, were collected. Due to the hardness of pollinia in *G. cunninghamii*, they were soaked in 10% KOH for 5 h before observation. Pollen grains were sub-sampled

in 200 µl of Alexander's (1980) stain and counted using a haemocytometer. Capsules in early ripening stage were used to determine ovule numbers. Ovules from one carpel were sub-sampled in 500 µl of water and counted under the stereomicroscope. P/O ratios were obtained following Cruden (1977).

### Osmophores

Since osmophores (scent glands) may or may not be morphologically and anatomically distinguished from adjacent tissues in the perianth, at least 10 fresh flowers of each species, each from a different individual, were submerged in a stain bath of 1:1000 neutral red:tap water for 2–12 h following Stern et al. (1986) and Vogel (1990) to reveal the presence of osmophores. According to these authors, tissues with presumptive osmophoric function stain deep red while other tissues remain unstained.

### Phenology and pollinators observations

Flowering and fruiting periods were observed weekly in plants tagged in the field. Flower life span was measured in *T. longifolia* and *P. alobula*. Several days were spent in the field at each site during the flowering period, usually 3–4 h between c. 10.00 a.m. and 6.00 p.m., during which pollination observations were made. A representative sample of the insects visiting these orchids was collected and identified. The activity of nocturnal or crepuscular visitors was not assessed. Floral visitors were ranked according to their pollinator efficiency following Adams & Lawson (1993). These authors considered insects to be "confirmed pollinators" in orchids when pollinia are observed being picked up as well as deposited, "probable pollinators" as those insects that are observed taking up pollinia of the plant but

**Table 1** Putative osmophore locations in four New Zealand terrestrial orchids as indicated by absorbance of neutral red ( $n = 10$  flowers for each species).

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

with not observed deposition on a stigma of a co-specific flower, and “suggested pollinators” as those that are observed visiting the flowers but not seen taking up pollinia.

## RESULTS

### Phenological observations

The saprophytic orchid *G. cunninghamii* started flowering in early January and continued for almost 2 months. In *T. longifolia* flowering occurred from early November to early January. Flower life-span was short,  $\pm 5$  days. As reported by others (e.g., Catling 1990), anthesis in this species appeared to be subject to environmental conditions, i.e., the flowers only open on sunny and warm days. The flowering period of *P. alobula* extended for almost 4 months (mid May to early August). Flowering within the population is gradual, with individuals at different developmental stages observed for several months. Floral life-span in this species is also long; tagged flowers often lasted over a month even after being hand-pollinated. The capsules of this species complete their development after 3 months. The flowering period of *P. patens* is short, only about 1 month (December). Two months later capsules were fully developed and seed dispersal occurred during mid February.

### Osmophores

These four species are all scentless to the human perception. However, neutral red positively stained several areas of the perianth suggesting possible scent production (Table 1). The presumed 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*. Scent glands appear to be located in the same areas of the perianth in both species (Table 1).

### Pollen and ovule numbers and P/O ratios

The highest number of pollen grains per flower was recorded in *P. alobula* and *G. cunninghamii* (Table 2). The type of pollinia observed in these four orchids varies in cohesion and aggregation. In *G. cunninghamii*, each pollinium is formed by several massulae weakly held together by viscid threads. However, tetrads within the massulae are strongly bound together. Pollinia in *T. longifolia* are soft and powdery, and weakly joined to the viscidial disc. They may be removed either as a coherent unit or each as a single pollinium. In this orchid, pollen grains are found as monads. Pollen grains of *Pterostylis* species are presented as tetrads (*P. alobula*) and monads (*P. patens*). The soft pollinia of both species may be withdrawn as single units or a group. Pollen: ovule ratios calculated for these orchids fluctuated from 24:1 to 320:1 (Table 2).

### Breeding system

Hand-pollination treatments showed that *T. longifolia*, *P. alobula*, and *P. patens* are self-compatible (Table 3). The responses of *G. cunninghamii* to the pollination treatments were somewhat puzzling. This orchid did not produce seeds when flowers were hand self-pollinated but did so after automatic self-pollination, hand cross-pollination, and apomixis (Table 3). Natural fruit-set was extremely high in *T. longifolia* and *G. cunninghamii*, reaching 78 and 92%, respectively, and automatic self-pollination occurred only in these two. Conversely, in both species of *Pterostylis* natural fruit-set was low, remarkably so in the winter flowering *P. alobula*. Only 2 individuals, each from a different population, produced seeds (Table 3). This orchid had also the lowest fruit-set after hand self-pollination, 34%.

### Floral visitors

Although the pollinator activity was surveyed in all four orchids, insect visitation was observed only in *P. alobula* and *G. cunninghamii*. Male fungus gnats

**Table 2** Pollen and ovule numbers (average  $\pm$  SE) and P/O ratios of four New Zealand terrestrial orchids.  $n = 10$  flowers per species.

Species	Pollen grains per flower	Number of ovules per flower	P/O ratio
<i>G. cunninghamii</i>	283 821 $\pm$ 3721.3	11 400 $\pm$ 191.2	25:1
<i>T. longifolia</i>	114 165 $\pm$ 9099.9	4842 $\pm$ 213.1	24:1
<i>P. alobula</i>	366 857 $\pm$ 3831.1	1147 $\pm$ 7.7	320:1
<i>P. patens</i>	262 837 $\pm$ 3419.6	6333 $\pm$ 801.3	42:1

of the genus *Zygomysia* (Mycetophilidae: Diptera) were found both alive and dead inside the flowers of *P. alobula* on several occasions, and some of them were found imprisoned by the column wings and in a position ready to remove pollinia (Fig. 2A). Aphids were usually found inside the flowers of *G. cunninghamii*.

## DISCUSSION

### Reproductive biology

Hand-pollination treatments confirmed the absence of genetic incompatibility in three of the species studied, *T. longifolia*, *P. alobula*, and *P. patens*. 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). Self-compatibility seems to be a common condition in the New Zealand flora (Godley 1979; Newstrom & Robertson 2005). This is probably a consequence of the unspecialised pollinating fauna and the long isolation of the country, which may constrain dispersal and establishment of self-incompatible taxa (Webb & Kelly 1993; Newstrom & Robertson 2005).

Despite these three orchids being self-compatible, their reproduction relies on contrasting reproductive strategies. *Thelymitra longifolia* is predominantly self-pollinated, whereas *Pterostylis* species have an absolute dependence on insects to achieve pollination. Self-pollination in the genus *Thelymitra* is not new, and it is considered likely that more than half the species in this genus are autogamous (Jones 1981). Regardless of the high fruit-set that can occur through automatic self-pollination in *T.*

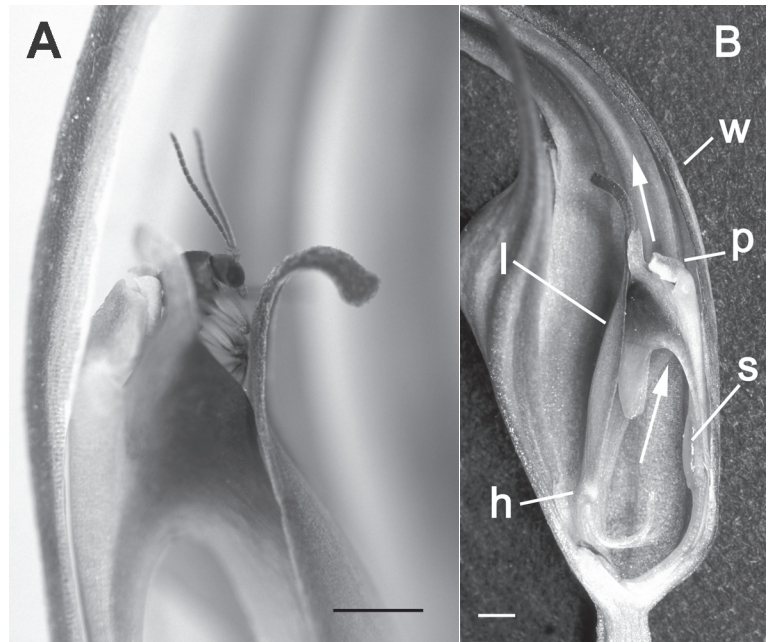
*longifolia*, cross-pollination may also occur at times. The presence of olfactory and visual cues to attract pollinators and records of burrowing bees (*Leoproctus fulvescens*) visiting and removing pollinia of *T. longifolia* in northern New Zealand (Jones 1981) suggest that this species has a mixed selfing-outcrossing strategy. Furthermore, floral senescence seems not to be triggered by pollen depositions, as flowers with pollinia deposited on the stigma and still in total anthesis were observed on numerous occasions during this study. This mixed pollination system may have evolved in *T. longifolia* as a "fail-safe" mechanism due to the unpredictable climatic conditions during flowering, the lack of suitable pollinators, or high competition for pollinators at certain times of the year (e.g., Hogan 1983). Consequently, if cross-pollination does not occur in *T. longifolia*, flowers can always self-pollinate. This mechanism is known as delayed self-pollination (Lloyd 1979; Lloyd & Schoen 1992) and has been observed previously in other New Zealand plant species (Robertson & Lloyd 1991).

In the autumn–winter flowering *P. alobula*, pollination seems to be achieved by male fungus gnats of the genus *Zygomysia*, and although no insects were observed visiting or pollinating the summer-flowering *P. patens*, pollination by male fungus gnats is suspected here too. This is supported by observations on a third species in the area, *P. australis*, on which we found two male fungus gnats, *Aneura longipalpis* (Mycetophilidae: Diptera) and *Cerotelion* sp. (Keroplastidae: Diptera), inside the flowers. Indeed, this genus appears to be pollinated exclusively by microdipterans in the families Culicidae, Phoridae, and Mycetophilidae (Cheeseman 1873; Darwin 1877; Thomson 1927; Jones 1981; Christensen 1994; Bernhardt 1995a; Jones & Clements 2002). Pollination by fungus gnats is not

**Table 3** Percentage of fruit set after pollination treatments and self-compatibility index (SCI) in four New Zealand terrestrial orchids. N, natural fruit-set; S, hand self-pollination; C, hand cross-pollination; AS, autonomous self-pollination; APO, apomixis. Number of flowers used in each treatment in brackets. Fruit-set within column followed by the same letter are not significantly different at  $P < 0.05$  according to the Likelihood Ratio test.

Treatments	<i>G. cunninghamii</i>	<i>T. longifolia</i>	<i>P. alobula</i>	<i>P. patens</i>
N	92.3 (197) <sup>d</sup>	78.2 (46) <sup>b</sup>	2.7 (73) <sup>a</sup>	41.6 (24) <sup>b</sup>
S	0.0 (29) <sup>a</sup>	70.8 (24) <sup>b</sup>	34.0 (50) <sup>b</sup>	66.6 (27) <sup>b</sup>
C	17.6 (34) <sup>b</sup>	83.7 (31) <sup>b</sup>	44.0 (50) <sup>b</sup>	60.8 (23) <sup>b</sup>
AS	81.6 (49) <sup>cd</sup>	80.0 (25) <sup>b</sup>	0.0 (30) <sup>a</sup>	0.0 (30) <sup>a</sup>
APO	60.7 (28) <sup>c</sup>	0.0 (14) <sup>a</sup>	0.0 (52) <sup>a</sup>	0.0 (30) <sup>a</sup>
SCI	0.0	0.6	0.7	1.1

**Fig. 2** *Pterostylis alobula* with *Zygomysia* sp. (Mycetophilidae) imprisoned by the column wings and the tip of the labellum (A) and details of the internal floral structures of a “greenhood” orchid (B). Lateral sepal has been removed for the photograph. w, window-panes; p, pollinia; s, stigma; h, hinge; l, labellum. Arrows indicate the path used by the fungus gnat when escaping from the trap. Scale bars = 1 mm.



limited to *Pterostylis* species and it seems to have evolved in riparian plants and understorey herbs inhabiting cool, moist, evergreen, and fungus-rich forests where floral resources are scarce (Mesler et al. 1980; Goldblatt et al. 2004; Okuyama et al. 2004). Many of these conditions are also typical in the habitats these two orchids occupy.

Two pollination systems have been described for insect-pollinated *Pterostylis*. Pseudocopulation has been suggested to occur in many species with an exposed labellum and deflexed lateral sepals (Jones & Clements 2002). The labellum in this group is ornamented, coloured, and also scented to resemble a female gnat (Bernhardt 1995a,b). Pollination by pseudocopulation in this group is also supported by observations of male fungus gnats flying directly towards the labellum with their genitalia exerted when close to the labellum (Jones & Clements 2002), and 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). Pollination by pseudocopulation has been previously suggested to occur in some New Zealand *Pterostylis* species (Jones & Clements 2002); however, it is unlikely to occur in *P. alobula* and *P. patens*. These two species belong to a different section of the genus in which the labellum is neither exposed nor insectile in the same way as

those observed by Bates (1977), Beardsell & Bernhardt (1983), and Jones & Clements (2002) (cf. *P. tristis* or *P. tanypoda* in New Zealand).

The attractant mechanism used by the species in this section is still unclear, and several hypotheses have been proposed. Hyett (cited in van der Pijl & Dodson 1966) suggested sapromyophily, pointing out the strongly unpleasant odour of some Australian species such as *P. mutica*. However, emission of carrion-like or fungous scent has never been reported in any of the New Zealand *Pterostylis* and was never perceived in *P. alobula* or *P. patens*. Moreover, female gnats are usually found inside sapromyophilous flowers since they mimic oviposition sites (Dafni 1984), and only male fungus gnats were recorded in this study.

Alternatively, Jones (1981) suggested 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 in the first place, only their movements once inside the trap. The presence of colourless translucent areas in the perianth such as those of *P. alobula* and *P. patens* has been reported in other

trap flowers (Dafni 1984; Vogel & Martens 2000). These authors explained that since flies are positively phototropic once inside the floral 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 to the reproductive structures.

Sexual deceit is a likely explanation for attraction of male fungus gnats by these orchids. Sexual deception exploits the reproductive stimuli of pollinators by the emission of odours that probably act as a species-specific sexual attractant (Dafni 1984). Pollination by sexual deception is an intermediate stage in pseudocopulatory systems toward true pseudocopulation (Dafni & Bernhardt 1990). This idea is also supported by the presence of osmophores in the sepal tips, labellum, and column wings of *P. alobula* and *P. patens*, and the fact that only male fungus gnats were recorded visiting these flowers. Thus, it is possible that scent glands emit volatile stimulating signals that attract only male fungus gnats to the flowers.

An alternative hypothesis is the use of primary and secondary attractants by these orchids: protection and temperature attraction, respectively. If this is the case, the presence of male fungus gnats inside these flowers may be only coincidental and due to limited sampling. Fungus gnats may be using *Pterostylis* flowers as "warm shelter" during the night or during harsh environmental conditions. It is known that temperature inside the flowers of some plant species may rise due to scent production or absorption of energy from the sun, resulting in striking differences with the external temperature (see references in Faegri & van der Pijl 1979). Although this mechanism seems to be exploited by some plant species occurring in temperate and arctic regions of the world (Kevan 1975; Faegri & van der Pijl 1979), it has not been thoroughly studied and information is scanty.

Whatever is the case, the events leading to the pollination of these species appear to include attraction of fungus gnats, the insect entering the flower, and triggering of the labellum to swing back in the flower, imprisoning the insect in the floral chamber. Subsequently, the colourless translucent areas in the perianth guide the insect deep into the trap where the stigma is located. From here, the events are as described by Johns & Molloy (1983) and Proctor et al. (1996): the insect escapes by passing through a "tunnel" formed by the column wings and, in doing

so, removes a pollinium if one is still intact and carries it to a second flower where pollinium deposition takes place during the escape (Fig. 2B). If this sequence is confirmed for these species, it would be one of the first species-specific insect pollinator systems recorded in New Zealand (Webb & Kelly 1993; Newstrom & Robertson 2005).

The pollination system of the saprophytic *G. cunninghamii* is difficult to assess from the data obtained in this study. In general, this species has been considered as autonomously autogamous (Molloy 1990), and morphological adaptations such as an extremely short column that places the pollinia immediately above the stigma reinforce the idea. Our results confirm a high rate of fruit set inside pollinator-exclusion bags as well as in un-manipulated flowers, though attempts at emasculation followed by hand self-pollinations failed. These failures may be attributed to the inappropriate handling of the pollen or the stress caused by removal of the entire perianth to pollinate the flowers. Damage to floral parts results in the release of ethylene which may have caused tissue degradation (D. L. Jones pers. comm.). The function aphids may have in the pollination of *G. cunninghamii* is unknown and needs further study. They may help to self-pollinate the flowers transferring pollen when crawling over the reproductive structures, perhaps attracted to the pseudopollen this orchid forms at the base of the labellum or the stigmatic secretion. The importance of aphids (and thrips) in the self-pollination of facultatively xenogamous species and their ability to transfer pollen has been noticed before by Baker & Cruden (1991). Unfortunately, aphids observed inside the flowers of *G. cunninghamii* were neither collected nor identified; therefore their status as pollinators or pests is unknown.

### Conservation implications

Of the four species considered here, *Pterostylis alobula* and *P. patens* are the more likely to be affected by pollinator failure. These species, and especially *P. alobula*, seem to have a species-specific pollination syndrome by male fungus gnats; both species are obligate xenogamous and depend absolutely on pollinators to set fruit, and their reproductive success in the wild is very limited, especially in *P. alobula*. Only 2 individuals, each from a different population, produced seeds under natural conditions of pollination (72 individuals; 3 colonies).

A number of factors may limit the reproductive success of *P. alobula*. First, *P. alobula* flowers in winter (June-July) when climatic conditions are



harsh and insect activity can be dramatically reduced. However, it is important to note that flowers of *P. alobula* have several features considered as adaptations to overcome low pollinator availability, e.g., long floral persistence (c. 1 month, even after pollination had occurred), high P:O ratio (320:1), and the aggregation of pollen grains into tetrads. A high count of pollen grains in this species is highly advantageous as usually only a single pollinium is removed by the gnats (Jones 1981); this may allow fertilisation of a large number of ovules in a single visit. Second, floral and capsule predation by the leaf-roller caterpillar *Cnephasia jactatana* damaged some of the pollination treatments and may have had an effect on the final natural fruit-set in this population. This native lepidopteran is a polyphagous feeder and can be an important orchard pest in New Zealand, dramatically damaging kiwifruit soon after fruit-set (Steven 1990). Floral herbivory directly affects reproductive success of plants by the consumption of reproductive organs and indirectly through the reduction of floral attractiveness (Malo et al. 2001) and, in the particular case of *P. alobula*, by damaging the trap system. Third, accumulation of heterospecific pollen may have reduced the reproductive output of *P. alobula*. Pollen of the exotic species *Pinus nigra* was observed on stigmas of *P. alobula* in several of the populations studied here. Although the effect of heterospecific pollen accumulation in the reproductive success was not quantified in this study, it is known to be detrimental in other orchid species for fruit set by reducing the stigmatic area available to receive conspecific pollen (Neiland & Wilcock 1999). Finally, habitat transformations may have a considerable effect on the reproductive success of these species. Indeed, it is likely that the lower fruit-set observed in *P. alobula* in contrast to *P. patens* may be due to habitat clearance and fragmentation; *P. alobula* was studied in a highly disturbed area whereas *P. patens* was in a protected area. To determine whether one or a combination of these factors, may threaten wild populations of *P. alobula* or *P. patens*, further studies are needed.

*Thelymitra longifolia* and *Gastrodia cunninghamii* appear to be less vulnerable to the disruption of the plant-pollinator mutualism. These orchids are facultative xenogamous or completely autogamous, and are not dependant on pollinators to set fruits. Although self-pollination may assure fruit-set and maintain genotypes already adapted to the local environment, it might be a disadvantageous strategy when progeny face changes in the environmental conditions (see Cropper & Calder 1990).

The protection of these orchids may depend rather more on the maintenance of a stable habitat or successional stage.

Finally, despite the potential for mechanisms such as clonal propagation (e.g., *Pterostylis*) and the typically relatively large seed production per flower to buffer these species through episodes of reproductive failure, it is important to consider that orchid colonisation may often be microsite-limited (Calvo 1993). Orchid seeds are heterotrophic and will germinate only if a precise mycorrhizal infection takes place; thus, establishment will be restricted only to sites where the suitable mycorrhiza is present. Consequently, survival of these species will depend on the correct functioning of another ecological interaction, mycorrhizal dependency and nutrition, a field largely unstudied in New Zealand orchids.

#### ACKNOWLEDGMENTS

We thank the Public Good Science and Ecology Group Fund (Massey University) for the financial support for this study; Cathy Lake, Tracy Harris, Amy Trass, Viv Nichols, and Paul Peterson for help in the field; Ian Andrews for helping with insect identification, and D. L. Jones and an anonymous reviewer for their helpful comments on drafts of the manuscript. Carlos Lehnebach was supported in New Zealand by a scholarship from New Zealand Overseas Development Assistance Programme.

#### REFERENCES

- Adams PB, Lawson SD 1993. Pollination in Australian orchids: a critical assessment of the literature 1882–1992. *Australian Journal of Botany* 41: 553–575.
- Anderson S 1997. Changes in native ecosystem processes: the dynamics of pollination and dispersal in New Zealand Forest. Unpublished MSc thesis, University of Auckland, Auckland, New Zealand.
- Alexander MP 1980. A versatile stain for pollen, fungi, yeast and bacteria. *Stain Technology* 55: 13–18.
- Baker JD, Cruden RW 1991. Thrips-mediated self-pollination of two facultatively xenogamous wetland species. *American Journal of Botany* 78: 959–963.
- Bates R 1977. Pollination of orchids. *Native Orchid Society of South Australia Newsletter* 1: 7.
- Beardell DV, Bernhardt P 1983. Pollination biology of Australian terrestrial orchids. In: Williams EG, Knox RB, Gilbert JH, Bernhardt P ed. *Pollination '82*. Parkville, Victoria, University of Melbourne Press. Pp. 166–183.

- Beardsell DV, Clements MA, Hutchinson JF, Williams, EG 1986. Pollination of *Diuris maculata* R. Br. (Orchidaceae) by floral mimicry of the native legumes *Daviesia* spp. and *Pultenaea scabra* R. Br. Australian Journal of Botany 34: 165–173.
- Bernhardt P 1995a. Notes on the anthecology of *Pterostylis curta* (Orchidaceae). Cunninghamia 4: 1–8.
- Bernhardt P 1995b. Biogeography and floral evolution in the *Geoblasteae* (Orchidaceae). In: Arroyo MTK, Zedler PH, Fox MD ed. Ecology and biogeography of mediterranean ecosystems in Chile, California and Australia. New York, Springer-Verlag. Pp. 116–134.
- Bond WJ 1994. Do mutualism matter? Assessing the impact of pollinator and disperser disruption on plant extinction. Philosophical Transactions of the Royal Society of London, series B 344: 83–90.
- Bond WJ 1995. Assessing the risk of plant extinction due to pollinator and disperser failure. In: Lawton J, May R ed. Extinction rates. Oxford University Press. Pp. 131–146.
- Calvo RN 1993. Evolutionary demography of orchids: Intensity and frequency of pollination and the cost of fruiting. Ecology 74: 1033–1042.
- Catling P 1990. Auto-pollination in the Orchidaceae. In: Arditti J. ed. Orchid biology, reviews and perspectives, Vol. 5. Portland, Timber Press. Pp. 121–158.
- Cheeseman T 1873. On the fertilisation of the New Zealand species of *Pterostylis*. Transactions and Proceedings of the New Zealand Institute 5: 352–357.
- Cheeseman T 1881. On the fertilisation of *Thelymitra*. Transactions and Proceedings of the New Zealand Institute 13: 291–296.
- Christensen DE 1994. Fly pollination in the Orchidaceae. In: Arditti J ed. Orchid biology: reviews and perspectives VI. New York, John Wiley & Sons. Pp. 415–454.
- Clayton S, Aizen M 1996. Effects of pollinia removal and insertion on flower longevity in *Chloraea alpina* (Orchidaceae). Evolutionary Ecology 10: 653–660.
- Cropper S, Calder D 1990. The floral biology of *Thelymitra epipactoides* (Orchidaceae) and the implication of pollination by deceit on the survival of this rare orchid. Plant Systematics and Evolution 158: 11–22.
- Cruden R 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.
- Dafni A 1984. Mimicry and deception in pollination. Annual Review of Ecology and Systematics 15: 259–278.
- Dafni A, Bernhardt P 1990. Pollination of terrestrial orchids of southern Australia and the mediterranean region; systematic, ecological, and evolutionary implications. Evolutionary Biology 24: 193–253.
- Darwin C 1877. The various contrivances by which orchids are fertilised by insects. London.
- de Lange PJ, Norton DA, Heenan PB, Courtney SP, Molloy BPJ, Ogle CC, Rance BD, Johnson PN, Hitchmough R 2004. Threatened and uncommon plants of New Zealand. New Zealand Journal of Botany 42: 45–76.
- Faegri K, van der Pijl L 1979. The principles of pollination ecology. USA, Pergamon Press. 244 p.
- Galetto L, Bernardello G, Rivera GL 1997. Nectar, nectaries, flower visitors, and breeding system in five terrestrial Orchidaceae from Central Argentina. Journal of Plant Research 110: 393–403.
- Godley E 1979. Flower biology in New Zealand. New Zealand Journal of Botany 17: 441–466.
- Goldblatt P, Bernhardt P, Vogan P, Manning JC 2004. Pollination by fungus gnats (Diptera: Mycetophilidae) and self recognition sites in *Tolmiea menziesii* (Saxifragaceae). Plant Systematics and Evolution 244: 55–67.
- Hogan K 1983. The pollination biology and breeding system of *Aplectrum hyemale* (Orchidaceae). Canadian Journal of Botany 61: 1906–1910.
- Johns H, Molloy B 1983. Native orchids of New Zealand. Wellington, New Zealand.
- Jones DL 1981. The pollination of selected Australian orchids. In: Lawler L, Kerr RD ed. Proceedings of the Orchids Symposium. 13th International Botanical Congress, Sydney, Australia. Pp. 40–43.
- Jones DL, Clements MA 2002. A review of *Pterostylis* (Orchidaceae). Australian Orchid Research 4: 1–168.
- Kevan PG 1975. Sun-tracking solar furnaces in high Arctic flowers: significance for pollination and insects. Science 189: 723–726.
- Lehnebach CA 2002. Pollination ecology of New Zealand orchids. Unpublished MSc thesis, Massey University, Palmerston North, New Zealand.
- Lehnebach CA, Robertson AW 2004. Pollination ecology of four epiphytic orchids of New Zealand. Annals of Botany 93: 773–781.
- Lloyd DG 1979. Some reproductive factors affecting the selection of self-fertilization in plants. American Naturalist 113: 67–79.
- Lloyd DG, Schoen DJ 1992. Self- and cross-fertilization in plants. I. Functional dimensions. International Journal of Plant Sciences 153: 358–369.

- Malo JE, Leirana-Alcocer J, Parra-Tabla V 2001. Population fragmentation, florivory, and the effects of flower morphology alterations on the pollination success of *Myrmecophila tibicinis* (Orchidaceae). *Biotropica* 33: 529–534.
- McNutt KL 1998. Impacts of reduced bird densities on pollination and dispersal mutualisms in New Zealand forests. Unpublished MSc thesis, Massey University, Palmerston North, New Zealand.
- Mehrhoff L 1983. Pollination in the genus *Isotria* (Orchidaceae). *American Journal of Botany* 70: 1444–1453.
- Mesler RM, Ackerman JD, Lu KL 1980. The effectiveness of fungus gnats as pollinators. *American Journal of Botany* 67: 564–567.
- Molloy B 1990. Pollination systems of New Zealand native orchids. In: St George I, McCrae D ed. *The New Zealand orchids: natural history and cultivation*. Dunedin, Southern Colour Print. Pp. 36–56.
- Montgomery BR, Kelly D, Ladley JJ 2001. Pollinator limitation of seed set in *Fuchsia perscandens* (Onagraceae) on Banks Peninsula, South Island, New Zealand. *New Zealand Journal of Botany* 39: 559–565.
- Moore L, Edgar E. 1976. *Flora of New Zealand*. Vol. II. Wellington, Government Printer.
- Neiland MR, Wilcock CC 1999. The presence of heterospecific pollen on stigmas of nectariferous and nectarless orchids and its consequences for their reproductive success. *Protoplasma* 208: 65–75.
- Newstrom L, Robertson A 2005. Progress in understanding pollination systems in New Zealand. *New Zealand Journal of Botany* 43: 1–59.
- Norton D, Lord J, Given D, de Lange P 1994. Over-collecting: an overlooked factor in the decline of plant taxa. *Taxon* 43: 181–185.
- Ogle CC, La Cock GD, Arnold G, Mickleson N 2000. Impact of an exotic vine *Clematis vitalba* F. (Ranunculaceae) and of control measures on plant biodiversity in indigenous forest, Taihape, New Zealand. *Austral Ecology* 25: 539–551.
- Okuyama Y, Kato M, Murakami N 2004. Pollination by fungus gnats in four species of the genus *Mitella* (Saxifragaceae). *Botanical Journal of the Linnean Society* 144: 449–460.
- Proctor M, Yeo P, Lack A 1996. *The natural history of pollination*. USA, Harper Collins. 479 p.
- Robertson AW, Lloyd DG 1991. Herkogamy, dichogamy, and self-pollination in six species of *Myosotis* (Boraginaceae). *Evolutionary Trends in Plants* 5: 53–63.
- Robertson AW, Kelly D, Ladley JJ, Sparrow AD 1999. Effects of pollinator loss on endemic New Zealand mistletoes (Loranthaceae). *Conservation Biology* 13: 499–508.
- SAS Institute 2001. *SAS/STAT Guide for Personal Computers*, Version 8. Cary, NC, SAS Institute Inc.
- Sipes S, Tepedino VJ 1995. Reproductive biology of the rare orchid, *Spiranthes diluvialis*: breeding system, pollination and implications for conservation. *Conservation Biology* 9: 929–938.
- St George I 1999. *The natural guide to New Zealand orchids*. Auckland, New Zealand, Random House. 176 p.
- Stern W, Curry K, Whitten W 1986. Staining fragrance glands in orchid flowers. *Bulletin of the Torrey Botanical Club* 113: 288–297.
- Steven D 1990. Entomology in kiwifruit. In: Warrington IJ, Weston GC ed. *Kiwifruit science and management*. Auckland, New Zealand, Ray Richards in association with the New Zealand Society for Horticultural Science. Pp. 362–412.
- Sydes M, Calder D 1993. Comparative reproductive biology of two sun-orchids; the vulnerable *Thelymitra circumsepta* and the widespread *T. ixioides* (Orchidaceae). *Australian Journal of Botany* 41: 577–589.
- Thien L, Marcks B 1972. The floral biology of *Arethusa bulbosa*, *Calopogon tuberosus*, and *Pogonia ophioglossoides* (Orchidaceae). *Canadian Journal of Botany* 50: 2319–2325.
- Thomson G 1927. The pollination of New Zealand flowers by birds and insects. *Transactions and Proceedings of the New Zealand Institute* 57: 106–125. [issued separately 1926]
- van Essen PR 1992. Ecology of the *Olearia colensoi* dominated sub-alpine scrub in the Southern Ruahine Range, New Zealand. Unpublished MSc thesis, Massey University, Palmerston North, New Zealand.
- van der Pijl L, Dodson C 1966. *Orchid flowers: their pollination and evolution*. Coral Gables, USA, University of Miami Press. 214 p.
- Vogel S 1990. *The role of scent glands in pollination*. New Delhi, India, Amerind Publishing Ltd. 202 p.
- Vogel S, Martens J 2000. A survey of the function of the lethal kettle traps of *Arisaema* (Araceae), with records of the pollinating fungus gnats from Nepal. *Botanical Journal of the Linnean Society* 136: 61–100.
- Webb CJ, Kelly D 1993. The reproductive biology of the New Zealand flora. *Trends in Ecology and Evolution* 8: 442–447.