Pollination Ecology of Four Epiphytic Orchids of New Zealand

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• *Background and Aims* In New Zealand epiphytic orchids are represented by four genera and eight species. The genera *Earina* (three species) and *Winika* (one species) are the most conspicuous and widespread. These are likely to be some of the southernmost distributed genera of epiphytic orchids in the world.
• *Methods* To identify the pollination strategies that have evolved in these orchids, hand-pollination treatments were done and floral visitors were observed in several wild populations at two areas of southern North Island (approx. 40°S). Pollen : ovule ratio and osmophores were also studied and the total carbohydrate content of the nectar produced by each species was measured.
• *Key results Earina autumnalis* and *Earina mucronata* are self-compatible, whereas *Earina aestivalis* and *Winika cunninghamii* appear to be partially self-incompatible. All four orchids are incapable of autonomous selfing and therefore completely dependent on pollinators to set fruits. Floral visitors observed in the genus *Earina* belong to Diptera, Coleoptera and Hymenoptera and to Diptera and Hymenoptera in *W. cunninghamii*.
• *Conclusions* Contrary to many epiphytic orchids in the tropics, the orchid–pollinator relationship in these orchids is unspecialized and flowers are visited by a wide range of insects. Putative pollinators are flies of the families Bibionidae, Calliphoridae, Syrphidae and Tachinidae. All four orchids display anthecological adaptations to a myophilous pollination system such as simple flowers, well-exposed reproductive structures, easily accessed nectar and high pollen : ovule ratios.   © 2004 Annals of Botany Company

Key words: Bibionidae, Calliphoridae, Diptera, *Earina aestivalis*, *Earina autumnalis*, *Earina mucronata*, epiphytic orchids, New Zealand, Orchidaceae, generalist pollination, Syrphidae, Tachinidae, *Winika cunninghamii*.

INTRODUCTION

The evolution of pollination strategies in epiphytic flowering plants have been strongly influenced by their habitat and spatial distribution (Madison, 1977; Ackerman, 1986; Benzing, 1989, 1990). Epiphytes are often small and hyper-dispersed, features that directly affect floral display and size of the reward per patch for pollinators (Ackerman, 1986; Benzing, 1990). Consequently, there may be selection pressure for pollination systems of epiphytes to become more specialized than densely distributed terrestrial species—the dispersion–specialization hypothesis (Ackerman, 1986). Benzing (1990) suggests that if epiphytic plants utilized generalist pollinators, or were less attractive to pollinators, reproductive output may be dramatically reduced.

In many of the tropical and subtropical areas of the world, orchids are one of the most numerous groups of epiphytes (Benzing, 1989). Studies regarding their pollination strategies are abundant in the literature (for reviews, see Tremblay, 1992; Neiland and Wilcock, 1998). Highly specialized pollination mechanisms and species-specific attraction of pollinators appear to be common strategies in several of the epiphytic orchids (e.g. Dodson, 1965; Ackerman, 1983; Nilsson *et al.*, 1985, 1987; Slater and Calder, 1988; Gerlach and Schill, 1989; Devries and Stiles,

1990; Ackerman *et al.*, 1994; Bartareau, 1995; Bush and Beach, 1995; Parra-Tabla *et al.*, 2000; and others); but not a rule (Proctor *et al.*, 1996). This kind of limited pollinator diversity and specialization on pollinators not only reduces the cost of successfully transferring pollen between individuals (Tremblay, 1992) but also promotes speciation. In fact, the great diversity of epiphytic orchids currently observed in the tropics has been linked to the evolution of specialized pollination strategies (Benzing, 1989, 1990; Borba and Semir, 2001).

Although epiphytic orchids are generally restricted to tropical or subtropical areas, four genera (*Bulbophyllum*, *Drymoanthus*, *Earina* and *Winika*) and at least eight species of epiphytic orchids occur in the temperate rain forest of New Zealand (St George, 1999). The genera *Earina* and *Winika* are the most conspicuous and widespread throughout New Zealand and offshore islands, including the Stewart Island (approx. 47°S). These are likely to be some of the southernmost distributed genera of epiphytic orchids in the world. Contrary to their tropical counterparts, pollination syndromes of epiphytic orchids occurring in such temperate areas of the world are unknown (Neiland and Wilcock, 1998). Insect pollination has been suggested for New Zealand *Earina* and *Winika* (Thomson, 1881; Johns and Molloy, 1983; Molloy and St George, 1994; St George, 1999), but never confirmed. Except for observations of a long-legged crane fly on *Earina autumnalis* and *Earina mucronata* (Scandel, 1996), insect visitation has not been

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further observed. The breeding system of *Earina* is also unclear; Thomson (1927) reported the lack of fruit-set after hand cross- and hand self-pollination in *E. autumnalis*, whereas Molloy (1990) suggested self-pollination as the main source of fruit-set. Similarly, the reproductive biology of *Winika cunninghamii* is far from understood. Johns and Molloy (1983) suggested this orchid relies on pollinators, as plants in insect-proof cages do not set seeds; yet Clements *et al.* (1997) indicate that automatic self-pollination is likely to be responsible for fruit-set. Unfortunately, none of these authors explain the observations conducted and/or the data collected to support their claims.

This paper aims to identify the pollination strategies that have evolved in these two New Zealand epiphytic orchid genera by studying their breeding system, pollen : ovule ratios, nectar sugar content and associated flower visitors at two areas of southern North Island (approx. 40°S).

METHODS

Species description

The genus *Earina* (tribe Epidendreae: subtribe Glomerinae; sensu Dressler 1993) comprises about ten species occurring in Fiji, Vanuatu, Samoa, New Caledonia and New Zealand. In New Zealand it is represented by three endemic species: *E. autumnalis* (Forst.f.) Hook.f., *E. mucronata* Lindl. and *E. aestivalis* Cheesem. The species *E. autumnalis* (Fig. 1A) has either short erect stems or larger drooping stems. When flowering, it forms a panicle approx. 5–10 cm long. The flowers are relatively small (approx. 13 mm 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 and Edgar, 1976). The closely related species *E. mucronata* (Fig. 1B) and *E. aestivalis* (Fig. 1C) usually have pendulous stems. When flowering, both species form a panicle of 10 cm. The flowers are yellow to orange and 1 cm in diameter. The labellum is much broader and showier than the rest of the floral elements. In both species, the 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 and Edgar, 1976; St George, 1999). The taxonomic status of *E. aestivalis* is still unclear, and it has been variously considered as a late-flowering form of *E. mucronata*, or as a separate species (Cheeseman, 1919; Moore and Edgar, 1976; Parsons *et al.*, 1995; St George, 1999). AFLP data do not support the split of these two orchids into different species (C. A. Lehnebach, unpubl. res.). These taxa will be treated here as two species; they differ in flowering phenology, which may have profound consequences for the pool of flower visitors and pollinators.

The genus *Winika*, formerly regarded as *Dendrobium*, is now treated as a monotypic endemic genus. *Winika cunninghamii* (Lindl.) M.A.Clem., D.L.Jones et Molloy (Fig. 1D) bears flowers that are almost 3 cm across. The sepals and petals range from white to green. The three-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. Although *W. cunninghamii* is fairly common, it has apparently been over-collected near population centres (St George, 1999).
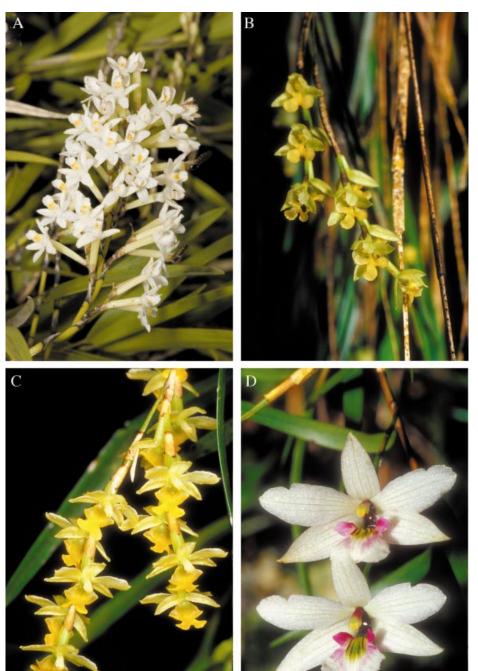
Study sites

This study was conducted in two areas of the lower south portion of the North Island, the Tararua Forest Park (40°41′S/175°32′E) and Marima Domain (40°30′S/175°42′E). The Tararua Forest Park protects mountain landscapes, considerable tracts of indigenous forests and the upper catchments of many rivers. At low altitudes, species such as *Nothofagus fusca* (Fagaceae) and *Dicksonia squarrosa* (Pterophyta: Dicksoniaceae) dominate the forests. Ten populations of *Earina autumnalis*, five populations of *E. mucronata* and three populations of *W. cunninghamii* were studied at this site. Marima Domain is a small rural park located 30 km from the city of Palmerston North; it is surrounded by farmland and supports a forest dominated by *Podocarpus totara* (Podocarpaceae) and *Melicytus ramiflorus* (Violaceae). Three populations of *Earina mucronata* and eight of *E. aestivalis* were studied in this park.

Reproductive biology

Breeding system and self-incompatibility barriers were determined by performing the following treatments in wild populations. (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: pollinarium was removed and flowers bagged until stigma receptivity. Flowers were considered receptive when the stigmatic surface was sticky and moist. Flowers were then hand-pollinated with the whole pollinarium from another flower on the same inflorescence and bagged again until wilting. (4) Cross-pollination: same as previous treatment, but flowers were pollinated with pollen from another individual. (5) Natural pollination: floral buds were tagged and allowed to develop to fruit under natural condition of pollination.

Fruit-set after hand self-, hand cross- and natural pollination was analysed by means of generalized linear model with a binomial error distribution and logit link function using SAS (SAS Institute, 2001).

All four species are clonal, forming dense clumps and making identification of independent individuals impossible. To avoid crossing between siblings, pollination treatments were done on inflorescences distantly located within the clump and always using pollen from a different population. Distances between conspecific populations (clumps) ranged from 15 m to 55 km.

F IG . 1. New Zealand endemic epiphytic orchids included in this study: (A) *Earina autumnalis*; (B) *E. mucronata*; (C) *E. aestivalis*; (D) *Winika cunninghamii*.

Pollen : ovule ratio (P/O)

All the pollinia from ten flowers from each species, each from a different individual, were collected and soaked in KOH 10 % for 5 h before macerating. Pollen grains were sub-sampled in 200 µL of Alexander's stain (Alexander, 1980) 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

At least ten 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 (1986) and Vogel (1990) to reveal the presence of

TABLE 1. *Putative osmophore locations in three New Zealand epiphytic orchids as indicated by absorbance of neutral red* (Earina autumnalis *not included, see Results*)

Species	Column	Labellum	Petals	Lateral sepals	Dorsal sepal
E. mucronata (*n* = 10 flowers)	Top of column (around anther cap) and base of column	Tip of the lip, underneath nectary	No reaction	No reaction	No reaction
E. aestivalis (*n* = 10 flowers)	Top of column (around anther cap)	No reaction	Entire petal	The entire sepal	The entire dorsal sepal
W. cunninghamii (*n* = 10 flowers)	Column wings and around stigma	Lateral lobes and ridges	No reaction	No reaction	No reaction

osmophores (scent glands). According to these authors, those tissues with presumptive osmophoric function stain deep red while other tissues remain unstained.

Phenology and pollinator observations

Flowering and fruiting periods were observed weekly in plants tagged both in the field and at a shade-house at Massey University, in Palmerston North. Insect activity was observed in the wild. Several days were spent in the field at each site during the flowering period, usually 3–4 h between approx. 1000 h and 1800 h, during which pollination observations were made. A representative sample of the insects 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 and Lawson (1993). These authors described as 'probable pollinators' in orchids as 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 as those that are observed visiting the flowers but not seen taking up pollinia.

Nectar standing crop

Using filter-paper wicks (McKenna and Thompson, 1988), nectar was collected from at least ten flowers of each of the species. Wicks were air-dried at room temperature and then used to determine nectar sugar contents using the anthrone colorimetric assay for total carbohydrate (Kearns and Inouye, 1993). This method does not allow nectar concentration to be measured, but does give a measure of total carbohydrate content of the nectar produced.

RESULTS

Phenology and pollinators

Flowering period of all four orchids extended for almost 3 months. *Earina mucronata* began to flower in October, *E. aestivalis* in December and *E. autumnalis* in late February. In these orchids, the leafy shoot takes almost 3 months to complete development and initiate flowering. Capsule ripening takes over 3 months. Seed dispersal is assisted by the presence of elaters in the capsules.

In *W. cunninghamii*, the floral buds begin their development during early October, and flowering occurs about 2 months later, December–January. Blooming in this species is not as synchronized as in *Earina*, and it is usual to find flowers at all stages of development or anthesis during the flowering period. Also, unlike *Earina*, the capsules ripen just 1 month after pollination. When ripe, the capsules of *W. cunninghamii* develop three openings at the distal end, where the column base was situated. Capsules are wind-shaken and seeds wind-dispersed.

Osmophores

These orchids showed a positive staining by neutral red, indicating the presence of osmophores (Table 1). Osmophoric areas were different in each species. Areas with a positive reaction were commonly obscure around the column wings, particularly around the stigma, and the anther cap and the labellum. The positive staining of structures such as stigmatic surface and pollinia were not considered osmophoric because of their absorbent nature. In *E. autumnalis* the results were unclear – the perianth showed a generalized 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.

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 corresponds to the number of pollen grains per flower. The pollinarium of all taxa comprise four hard pollinia. These are removed by insects as a single unit in *Earina* species; they are kept together by the viscidial disc. In *W. cunninghamii*, pollinia are unconnected and can be individually removed. In all four taxa, pollen grains are joined into tetrads. Pollen : ovule ratios calculated for these orchids fluctuated from 20 : 1 to 46 : 1 (Table 2).

Nectar standing crop

The average amount of sugar per flower varied both within the genus *Earina* and between the genera. The smallest amounts of sucrose equivalents were observed in *E. aestivalis* (0·007 ± 0·001 mg) and *W. cunninghamii*

(0·211 ± 0·196 mg), while the largest occurred in *E. autumnalis* and *E. mucronata* (0·716 ± 0·219 mg and 1·199 ± 0·148 mg, respectively).

Breeding system and genetic incompatibility barriers

Hand-pollination treatments showed that *E. autumnalis* and *E. mucronata* are self-compatible, whereas *E. aestivalis* and *W. cunninghamii* seem to be partially self-incompatible (Table 3). In the latter two species, fruit-set after cross-pollination was significantly higher than after hand self-pollination. Although these four orchids are scented and nectariferous, natural fruit-set was low in the wild, particularly in *E. mucronata* (4·1 %) and *E. aestivalis* (6·9 %) (Table 3). The highest fruit-set in the wild was observed in the autumn-flowering species *E. autumnalis* (30 %). Fruit-set following hand cross-pollination was above 60 % in all species. None of these species set seed through agamospermy or autonomous self-pollination; therefore they depend entirely on pollinating agents for their reproduction. The self-compatibility index (a ratio between fruits set after hand self-pollination and number of fruits yielded after hand cross-pollination) obtained for all the taxa studied are shown in the Table 3.

Floral visitors

The insects visiting these orchids belong to the order Diptera (seven families), Hymenoptera (four families) and Coleoptera (two families) (Table 4). Nectar seems to be the only reward offered, since the pollinia are strongly packed and waxy, and very difficult to break apart mechanically so are unlikely to be harvested by flower visitors. Although a wide range of insects visited these orchids, only a few of them were observed carrying pollinia. *Earina autumnalis* was visited by seven insect species, mainly dipterans (Table 4). Only the cosmopolitan syrphid fly *Eristalis tenax* was captured on two occasions carrying the four pollinia attached to the mouthparts.

Earina mucronata was visited mainly by dipterans of three families: Bibionidae, Empidae and Tipulidae (Table 4). But also, a small beetle was observed on a single occasion feeding on nectar from the underneath of the mesochile where a nectar drop is usually found (a possible extrafloral nectary). On the other hand, at least seven insect species visited *E. aestivalis*: Hymenoptera (three species), Diptera (three species) and Coleoptera (one species) (Table 4). The latter is an unidentified species of weevil (Curculionidae) that was observed feeding on nectar during two flowering seasons but carrying the four pollinia attached only once. *Winika cunninghamii* was visited by four insect species belonging to three families; Apidae, Syrphidae and Formicidae (Table 4). Unfortunately, none of them was observed carrying or depositing pollinia.

DISCUSSION

In contrast to the suggestions of Thomson (1927) and Clements *et al*. (1997), all four orchids studied here are incapable of autonomous selfing and are completely dependent on pollinators to set fruits. Moreover, our data show a significantly lower fruit-set following self-pollination compared with cross-pollination in *E. aestivalis* and *W. cunninghamii*. This suggests some form of incompatibility in these two species. However, the mode of this incompatibility, i.e. the site of the inhibition of self-pollen, was not identified. This inhibition may occur on the stigmatic surface or in the style as in sporophytic and gametophytic self-incompatibility systems (de Nettancourt, 1977; Kearns and Inouye, 1993), or later in the ovary where selfed capsules may be more likely to abort than crossed ones as in cryptic self-incompatibility (de Nettancourt, 1977; Bertin and Sullivan, 1988; Becerra and Lloyd, 1992). The latter form of incompatibility thus grades into inbreeding depression (Bertin and Sullivan, 1988; Becerra and Lloyd, 1992).

TABLE 2. *Pollen and ovule numbers (average ± s.e.) and P/O ratios of four epiphytic New Zealand orchids (n = 10 flowers for each species)*

	Pollen nos per flower	Ovule nos per flower	P/O ratio
E. aestivalis	113 400 ± 6120	5 749 ± 506·70	20 : 1
E. autumnalis	274 275 ± 8148	5 930 ± 139·36	46 : 1
E. mucronata	142 285 ± 11490	6 793 ± 271·31	20 : 1
W. cunninghamii	464 711 ± 43373	13 335 ± 411·90	34 : 1

TABLE 3. *Percentage of fruit-set after pollination treatments and self-compatibility index (SCI) in four New Zealand epiphytic orchids*

	N	S	C	AS	APO	SCI
E. autumnalis (n = 29)	30·0 (303)[a]	55·3 (65)[b]	60·6 (66)[b]	0·0 (51)	0·0 (42)	0·9
E. mucronata (n = 21)	4·1 (101)[a]	67·4 (43)[b]	69·7 (43)[b]	0·0 (33)	0·0 (45)	0·9
E. aestivalis (n = 34)	6·9 (230)[a]	36·9 (46)[b]	77·5 (49)[c]	0·0 (33)	0·0 (45)	0·4
W. cunninghamii (n = 19)	22·5 (62)[a]	37·5 (32)[a]	75·0 (32)[b]	0·0 (39)	0·0 (35)	0·5

APO, apomixis; AS, autonomous self-pollination; C, hand cross-pollination; S, hand self-pollination; N, natural fruit-set; *n*, total number of inflorescences used in this study.

Number of flowers used in each treatment in parentheses.

Shared letters within species indicate means that are not significantly different.

Only hand self-, hand cross- and natural pollination were tested (threshold of confidence 0·017).

TABLE 4. *Insects visiting four epiphytic orchids of New Zealand*

Order	Family	Genus/species	E. aestivalis	E. autumnalis	E. mucronata	W. cunninghamii
Coleoptera	Carabidae	Unidentified beetle			X	
	Curculionidae	Unidentified weevil	X			
Diptera	Bibionidae	*Dilophus nigrostigmus**			X	
	Calliphoridae	*Calliphora quadrimaculata*	X			
	Empidae	*Hilara* sp. 1‡		X		
		Hilara sp. 2‡			X	
	Muscidae	*Spilogonoa melas*	X			
	Syrphidae	*Melangyna novaezealandiae**	X			X
		Melanostoma fasciatum		X		
		Eristalis tenax†*		X		
		*Helophilus antipodus**				X
	Tachinidae	*Heteria* sp.*		X		
		Zealandotachina sp.*		X		
	Tipulidae	*Limonia tarsalba*		X		
		Ceratochilus sp.			X	
Hymenoptera	Apidae	*Apis mellifera*†				X
		Bombus terrestris†		X		
	Colletidae	*Hylaeus* sp.	X			
	Formicidae	*Prolasius advena*	X			X
	Ichneumonidae	*Aucklandella* sp. (*ursula*?)*	X			

* Putative pollinators.
† Species exotic to New Zealand.
‡ Undescribed species.

It is possible that *E. aestivalis* and *W. cunninghamii* are currently evolving self-incompatibility barriers triggered by changes in population demography and floral display. Ackerman (1986) noted 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. Indeed, *E. aestivalis* forms dense clumps on its host tree, with up to 1000 or more flowering spikes per patch. This species may be in a process of reproductive isolation from the self-compatible *E. mucronata*, which may explain the differences observed in the flowering periods and location of osmophoric areas. Self-incompatibility has been described in several epiphytic orchids (Ackerman, 1989; Bartareau, 1995; Parra-Tabla *et al.*, 2000; Borba *et al.*, 2001) and in almost 70 % of *Dendrobium* species (Johansen, 1990), to which *W. cunninghamii* is related and had been previously ascribed (Morris *et al.*, 1996). The gradual anthesis observed in the flowers of *W. cunninghamii* and the colour variability observed within the flowering patch of this orchid are well-known strategies to promote cross-pollination. *Earina autumnalis* and *E. mucronata* appear to lack significant self-incompatibility barriers, a strategy which is generally considered advantageous in epiphytic species since they are often sparsely distributed in the forests and geitonogamous pollinations are more likely to occur (Ackerman, 1986). The *in situ* evolution of self-incompatibility from compatible progenitors is perhaps more likely to occur in New Zealand than the establishment of self-incompatible taxa after long-distance dispersal events (Lloyd, 1985; Webb and Kelly, 1993). Unfortunately, pollination of the remaining *Earina* species is unknown,

thus further discussion on the evolution of breeding systems or dispersal events of this genus to New Zealand is limited.

Not all the insects observed as flower visitors in this study are regarded as confirmed pollinators but are better described as 'probable or suggested pollinators'. In *Earina autumnalis*, the only species regarded as a probable pollinator was the cosmopolitan *Eristalis tenax*. This syrphid fly is also a regular visitor to flowers of some New Zealand plants in the Onagraceae, Asteraceae and Rosaceae (Primack, 1978). The foraging behaviour of *E. tenax* has been considered rather labile; changing from generalist to specialist, and a mixture of them, depending on the flower density of the visited species (Kunin, 1993). The remaining insect species visiting this orchid (e.g. *Bombus terrestris* or *Melanostoma fasciatum*) are less likely to remove or deposit pollinia because of their size or their behaviour while visiting the flowers, which was usually from the side and away from the reproductive structures. However, tachinid flies should not be discarded as suggested pollinators for *E. autumnalis*. Tachinid flies are regular floral visitors to a number of New Zealand plants (Primack, 1983) and, unlike *E. tenax*, they forage on cold and rainy days (Primack, 1983), which are the prevailing weather conditions when this orchid flowers.

As for *Earina mucronata*, the only species considered as a suggested pollinator was the March fly, *Dilophus nigrostigmus* (Bibionidae). This species was never actually observed carrying pollinia, but the size of its body and the highly modified proboscis suggest this species is likely to successfully pollinate *E. mucronata*. Pollinating efficiency of other *Dilophus* species has been considered low (Limonta and Antignati, 1994). This may be one of the reasons to the poor fruit-set of this orchid in the wild (4·1 %). March flies

have also been reported as floral visitors of native species belonging to the Asteraceae, Schrophulariaceae, Onagraceae and Myrtaceae (Primack, 1983). Because of their small size, the remaining two insect taxa (*Ceratochilus* sp. and *Hilara* sp.) can only be regarded as floral visitors.

Only *Melangyna novaezealandiae* (Syrphidae) and flies of the family Calliphoridae can be regarded as suggested pollinators of *E. aestivalis*. In general, these species have been considered generalists and rather polylectic (Primack, 1983; Schedl, 1992). Primack (1978, 1983) and Lloyd (1985) note these species are important floral visitors since they visit flowers of more plant species than any other insect species in New Zealand. Although the remaining insects may be able to pollinate *E. aestivalis*, and orchid pollination by Colletidae and Ichneumonid wasps has been previously reported in the literature (Nilsson, 1981; Gumprecht, 1980; Bartareau, 1995; Pedersen, 1995; Lehnebach and Riveros, 2003), it is unlikely they are legitimate pollinators. Primack (1983) suggests that Hymenopterans of the genus *Hylaeus* are uncommon flower visitors to New Zealand native flora, and to date they have only been considered co-pollinators of New Zealand mistletoes (Kelly *et al.*, 1996), and pollination by ichneumonid wasps has often been linked to syndromes of pseudocopulation (Nilsson, 1981; Graham, 1983), which is not the case in *E. aestivalis*. The visits of a weevil are of interest. Floral visitation by species of Curculinidae have been reported before in New Zealand, but their efficiency as pollinators is considered poor (Primack, 1983). Overseas, pollination by weevils has been described only for species of the family Palmae (Mora, 1982; Henderson *et al.*, 2000) and the custard-apple family Annonaceae (Irvine and Armstrong, 1988). Notwithstanding, this insect was the only species observed carrying pollinia; further observations should be conducted to clarify whether it is a legitimate pollinator. The group of insects visiting *E. aestivalis* seems to parallel the pattern of the terrestrial orchids *Listera ovata* (Nilsson, 1981) and *Dendrochilum longibracteatum* (Pedersen, 1995). A wide range of insects visit these two orchids, particularly Diptera, Hymenoptera and Coleoptera, but in both orchids, only a small selection of the visitors actually acts as pollinators.

Although *W. cunninghamii* is visited by native syrphids and *Apis mellifera*, it is likely that before the introduction of the honeybee to New Zealand, the syrphids *Melangyna novaezealandiae* and *Helophilus antipodus* were the main pollinators of this orchid and now are sharing the pollinating service or perhaps being displaced by the honeybee. Indeed, Murphy and Robertson (2000) have observed the strong negative influence *A. mellifera* has on the abundance and diversity of dipterans in two common native shrubs. The native ant, *Prolasius advena*, is deemed as a nectar robber since it is too small to successfully pollinate *W. cunninghamii*.

The numerous insects visiting the flowers of these four orchids may be a result of the rich reward they offer, but also it makes them more vulnerable to visitation by exotic or generalist nectar-feeding insects, promoting pollen loss by illegitimate visitation (nectar thieves, see below). Indeed, the energetic content of the nectar per flower produced by these orchids is substantial, 3–20 J (1 mg sucrose = 16·8 J).

Similar values have been observed previously by Castro and Robertson (1997) and Perrott (1997) in New Zealand plants that attract birds (honeyeaters) as visitors. 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 value may be affected by variability in nectar production among flowers and/or the selective foraging by pollinators (Pleasants and Zimmerman, 1983). The importance of these orchids as nectar sources for the local insect community may be significant; especially in *E. autumnalis*. This orchid, together with *Metrosideros excelsa* (Myrtaceae), was the only rewarding species observed flowering from autumn to early winter in the vicinity of the population studied.

The P/O ratios of the orchids included in this study are within those previously reported for terrestrial fly-pollinated orchids, 10 : 1 to 24 : 1 (Neiland and Wilcock, 1995); but they are very high when compared with the P/O of the epiphytic orchid *Coryanthes senghasiana* (Nazarov and Gerlach, 1997), which was the only P/O ratio record found in the literature for an epiphytic orchid. *Coryanthes senghasiana* has a P/O ratio of 1·2 : 1 and is strictly pollinated by Euglossine bees (Hymenoptera: Apidae) (Gerlach and Schill, 1989). Although, pollen : ovule ratios have been considered a useful indicator of plant's reproductive strategy and breeding system (Cruden, 1977), in orchids and other plants with pollen clumped in pollinia, P/O ratios do not follow Cruden's principle (e.g. Wyatt *et al.*, 2000). Neiland and Wilcock (1995) have used P/O ratios in orchids to predict the group of pollinating insects. These authors suggest that lower P/O ratios are more likely to be found in orchids pollinated by more efficient pollinators such as Hymenoptera while higher P/O ratios may be likely in species pollinated by Diptera. In the study reported here the highest P/O belongs to *E. autumnalis* and *W. cunninghamii* which are primarily pollinated by syrphid flies. In addition to this, the high P/O ratios observed in these orchids may explain the consistency of their pollinia and the method by which pollinia are deposited on the stigmatic surface. Nazarov and Gerlach (1997) observed that in orchids with hard or sectile pollinia only the apical part of the pollinia comes in contact with the stigmatic secretion, while in those with soft pollinia a larger part of it is presented to the stigma. Thus, orchids with hard pollinia have a higher P/O ratio than those with soft ones (Nazarov and Gerlach 1997). Although these theories are consistent with our observations and the results of Lehnebach and Riveros (2003), orchid P/O ratios are scant in the literature and a further detailed and extensive revision is required to understand the significance and evolution of the P/O ratios in the Orchidaceae (Wyatt *et al.*, 2000).

Unlike several of their tropical counterparts, pollination of New Zealand epiphytic orchids is neither highly specialized nor species-specific. Information gathered in this study suggests all four orchids are primarily pollinated by generalist Dipterans. These orchids have light-coloured flowers (white-yellowish), simple scented flowers with an easy access to the nectary, well-exposed reproductive structures and high P/O ratios; all considered as floral adaptations to this pollination syndrome (Faegri and van der

Pijl, 1979; Neiland and Wilcock, 1995). Pollination by dipterans is a common syndrome in New Zealand flora. Indeed, the most outstanding feature of the reproductive biology of New Zealand flora is the large proportion of plants pollinated by dipterans, more than any other landmass of continental origin in the world (Lloyd, 1985; Webb and Kelly, 1993). Carlquist (1974) and Primack (1978, 1983) suggest that the principal consequence of this anomalous representation of pollinating groups in New Zealand is that the flowers rely on unspecialized insects that promiscuously visit a wide range of flowers and that operate in an imprecise manner. For instance, Primack (1983) observed that only four of the 82 montane species that he examined exhibit a specialized pollinator relationship with a single order of insects. However, for these four New Zealand epiphytic orchids, visitation by generalist and non-legitimate pollinators implies a great loss of the nectar and pollinia. Pollinium loss in these orchids is significant; records of pollination success measured by C. A. Lehnebach (unpubl. res.) in all four orchids show that pollinium deposition may be as low as 1 %, while pollinium removal may reach over 70 %. Lloyd (1985) and Webb and Kelly (1993) suggest that the absence of specialized pollination systems in New Zealand may be the result of strong selective forces imposed by several biogeographic factors such as long isolation from other elements of Gondwanaland, oceanic climate, unusual combination of ancient Gondwanic, tropical and recently arrived elements in its flora (e.g. epiphytic orchids), and the paucity of specialized pollinators.

Although Diptera are the second most important group of orchid pollinators (van der Pijl and Dodson, 1969; Christensen, 1994), till now, fly-pollinated orchids had been only described in most of the tropical and almost exclusively epiphytic orchids of the tribes Dendrobieae (subtribe Bulbophyllinae) and Epidendreae (subtribe Pleurothallidinae) (Christensen, 1994; Borba and Semir, 2001; Borba *et al*., 2001). Moreover, in nectarless species of the latter, the orchid–pollinator relationship has been described as species-specific (Borba and Semir, 2001). Pollination by dipterans in other epiphytic orchids of such tribes, such as those in the subtribe Dendrobiinae (*Winika*) and Glomerinae (*Earina*), is scant or unknown (Christensen, 1994). Thus, our observations are an important contribution to understand the occurrence of this syndrome within epiphytic orchids. Further studies on the pollination efficiency of the insects observed here and on the pollination ecology of other epiphytic orchids from other temperate areas, e.g. *Sarcochilus australis* and *Dockrillia striolata* in Tasmania, would be significant to understand the evolution of pollination strategies in epiphytic orchids occurring outside tropical or sub-tropical areas.

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