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Pollen limitation of fruit set in Western Australian terrestrial orchids

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Abstract

Hand pollination was used to test whether fruiting success in six Western Australian terrestrial orchid species is limited by the availability of pollen. Open pollination controls all had a lower production of fruit than hand pollinated flowers. Species offering nectar had a higher open pollinated fruiting success (*Lyperanthus serratus*, *Prasophyllum elatum* and *Prasophyllum fimbria*) than those that did not (*Caladenia flava* and *Diuris brumalis*). One species (*Pterostylis recurva*) had almost equivalent fruit production for hand and open pollination, and may be resource limited as its habitat was the most heavily shaded. The results of this investigation confirm that similar processes seem to be operating in the environmentally similar, but widely separated, areas of the Cape of South Africa and the south-west of Western Australia.

Keywords: pollination, fruit limitation, necter reward, orchid, terrestrial, Western Australia

Introduction

Fruit set in many plants is considerably less than the number of flowers apparently available for fertilization. Many studies have examined this phenomenon and provided proximate and ultimate reasons for why this might occur (Bierzychudek 1981*;* Ayre & Whelan 1989). Two main areas were identified. The first is resource limitation, where the number of fruits is limited by the amount of nutrients or carbohydrates that can be supplied by the maternal plant. The second is pollen limitation where there is a lack of suitable pollen supplied to flowers to ensure fertilization (Burd 1994; Johnson & Bond 1997).

In some mediterranean ecosystems, pollinators seem to be very abundant (*e.g.* Whelan & Burbidge 1980; Coetze & Giliomee 1985; Horskins & Turner 1999) so that low soil nutrients may be limiting for species that heavily invest their seeds with scarce mineral nutrients (Pate *et al*. 1986). However there are also systems in which pollinators seem relatively rare, particularly at some times of the year, so that receipt of appropriate pollen may limit successful fruit set (Dafni 1984; Johnson & Bond 1997). Several studies on the pollination of eastern Australian orchids (both sexually and florally deceptive species, and those with rewards) have revealed that some are pollen limited (Bartareau 1995; Dafni & Calder 1987; Jones 1972; Neiland & Wilcocks 1998; Sydes & Calder 1993).

In Cape fynbos, pollen limitation is a very common cause of low seed set (Johnson & Bond 1997). Pollinators, at least for some groups, seem to be scarce. As an example, at least 15 red-flowered fynbos species rely for pollination on the butterfly *Meneris tulbaghia* (Cowling & Richardson 1995). In south-west Western Australia, the vegetation is very similar to that of the Cape, with many parallels in structure and function (Cowling *et al*. 1994). If pollen limitation in fynbos is prevalent, then is this also the case in the south-west of Western Australia? This study aimed to examine this question using a set of six orchid species of common occurrence in the Perth area of Western Australia.

Methods

Six species, from five genera with a range of pollination systems, were chosen from areas close to the Perth metropolitan region in Western Australia where orchid species were known to flower in reasonable numbers (Table 1). Orchid population density was determined by the T-square sampling procedure (Krebs 1989) or from quadrats. Canopy projective cover was determined from analysis of digital images taken vertically upwards with a camera 1 m from the ground, at ten randomly chosen points along a transect through the area where each species grew (Table 1).

Orchid pollen coheres to form a pollinium, generally found near the top of the column, attached to a small sticky button called the viscid disc (Hoffman & Brown 1998). The structure was easy to remove with tweezers and place on another flower's stigma. Xenogamous pollinations were between flowers on plants at least several metres apart to ensure outcrossing beyond clones.

Between 10 to 20 plants were tagged in a population, with 40 flowers being randomly assigned to a control or experimental group. As this study only aimed to test if pollen delivery was limiting fruit production, flowers were not bagged. None of the species spontaneously self-pollinated. Fruit set was recorded about 5 weeks after pollination. In some cases the flowers on the plant died, reducing the number of flowers available for assessment. Fruit set is the most efficient measure of female success in orchids, which have thousands of microscopic seeds (Johnson & Bond 1997).

Table 1

Location and attributes of the orchid populations.

	Caladenia flava	***Diuris brumalis***	***Diuris brumalis***	***Pterostylis recurva***	***Lyperanthus serratus***	***Prasophyllum elatum***	***Prasophyllum fimbria***
Location	32° 3' 1.4" 115° 56' 4.0" Canningvale, corner Nicholson Rd and High Rd	31° 53' 17.0" 116° 13' 9.7" Mt Helena, Dowie Rd	31° 53' 14.0" 116° 13' 21.4" Mt Helena, Dowie Rd	32° 5' 23.7" 116° 3' 45.3" Rolystone, Douglas Rd	32° 6' 2.7" 116° 3' 23.5" Rolystone, Robinson Rd	32° 5' 46.3" 116° 2' 51.9" Rolystone, Canning Mills Rd	32° 3' 0.5" 115° 56' 1.9" Canningvale, corner Nicholson Rd and High Rd
Habitat	Degraded open eucalypt woodland	Degraded semirural (mostly cleared)	Degraded open eucalypt woodland	Casuarina – eucalypt forest	Degraded eucalypt open forest	Eucalypt woodland	Recently burnt heathy swamp
Date of pollination	9 October	8 July	8 July	9 October	9 October	9 October	9 October
Time to fruit set	5 weeks	6 weeks	6 weeks	5 weeks	5 weeks	5 weeks	5 weeks
Canopy cover (%)	8.2 ± 4.4	7.8 ± 5.5	29.6 ± 10.5	57.4 ± 7.2	49.4 ±8.9	3.5 ± 2.3	0
Density (m^{-2})	1.5	3.6	3.0	0.1	0.3	Two colonies of 4 - 5	0.6
Range (m^{-2})	1.0 – 2.9	2.5 – 6.1	1.7 – 13.7	0.1 – 0.2	0.2 – 1.3	-	-
Number of plants used	17	40	40	40	24	10	10
Number of flowers used	34	40	40	44	40	40	40
Flower death (%)	0	10	0	36	10	55	0
Nectar	-	-	-	-	+	+	+
Perfume	-	-	-	-	+	+	+
Flower visitors*	beetles	Native bees	Native bees	Mosquitoes, flies	Weevils, bees, moths	Bees, flies, beetles, wasps	Bees, flies, beetles, wasps
Flower visitors (this study, [1] = pollinium adhereing)	-	-	-	Midge[1]	Brown weevils	Black beetles	Gnats, bupestrid beetle[1]

* (Bernhardt 1990; G Brockman, personal communication; A Brown, CALM, personal communication; Brown *et al.* 1997; Hoffman & Brown 1998).

Results

The orchid species had a range of pollination systems and pollinators, and grew in a number of vegetation types, from forest to wet heathland (Table 1). Three species (*Prasophyllum elatum, P. fimbria* and *Lyperanthus serratus*) offered a food reward (nectar) to pollinators whereas the other species did not (Bernhardt & Burns-Balogh 1986; Coleman 1933; Hoffman & Brown 1998; Jones 1972). In *Prasophyllum* and *Lyperanthus* the nectar collects at the base of the labellum (Bernhardt & Burns-Balogh 1986; Coleman 1933; Hoffman & Brown 1998; Jones 1972). As well as nectar, these species have a strong fragrance.

Caladenia flava has a general attraction to pollinators via colour, while *Diuris brumalis* is considered to mimic Fabaceae species (Adams & Lawson 1993; Beardsell *et al.* 1986; Dafni & Bernhardt 1988; Nilsson 1992). Nothing specifically is known about the method of attraction to *Pterostylis recurva*, except that it may exploit flies which are dependent on winter mushrooms, or may attract flies via its opaque colour (Bernhardt 1990; A Brown, CALM, personal communication)

Plant densities ranged from approximately three plants m^{-2} for colonial species to 0.1 plants m^{-2} for solitary species (Table 1). Most of the species do not have episodic flowering. However, *P. elatum* and *P. fimbria* are stimulated to flower after fire (Hoffman & Brown 1984), and *P. fimbria* occurred in recently burnt vegetation in large numbers.

In all cases hand pollination increased fruit set compared with open pollinated flowers (Fig 1). Five species had between 80 to 100 % fruit set. Only *Pterostylis recurva* showed little difference between the hand pollination treatment and open pollination. The species that provided a food reward had a higher fruit set from open pollination than the other species, but *Pterostylis recurva*, which offers no nectar had almost equivalent results to those that do.

Discussion

A number of studies has compared Western Australian and southern South African systems and concluded that they have general similarities but

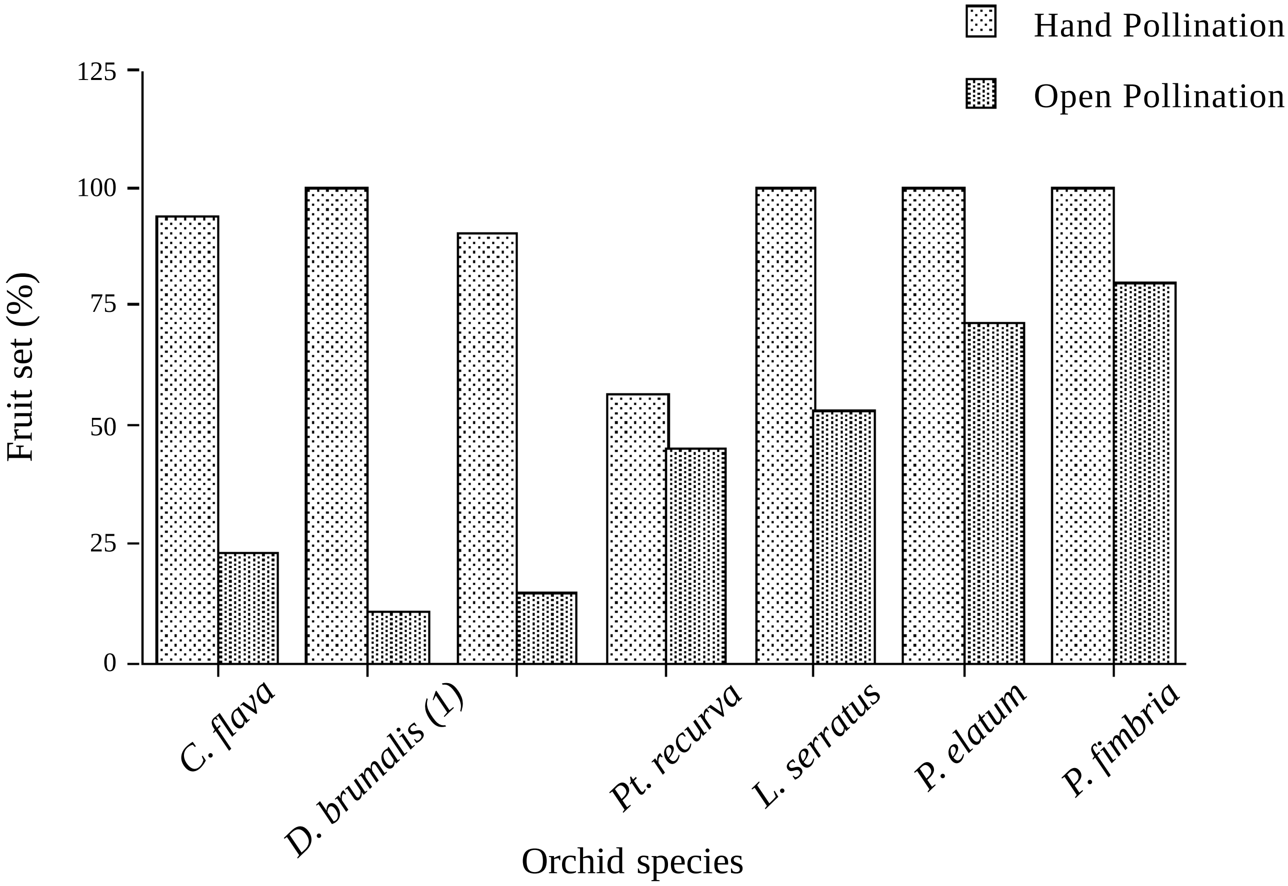

Figure 1. Fruit set of hand and open pollinated orchid species.

differences in some specific areas (Cowling *et al*. 1994). A South African study of the pollen limitation of Cape wildflowers by Johnson & Bond (1997) found that fruit set was frequently limited by pollen availability. The pollen limitation of fruit set in five of the six terrestrial orchids examined here agrees with Johnson & Bond's (1997) model. Therefore the Western Australian system appears to operate similarly to the South African system, at least in relation to orchids.

Our study also confirmed the generalisation from other orchid studies that species with pollinator rewards show higher fruit set than deceptive or mimic species (Johnson & Bond 1997; Neiland & Wilcocks 1998). However one species *P. recurva* which grew in a very shaded site showed little increase in fruit set with supplemental pollen. This may indicate resource limitation in the low light environment. Most *Pterostylis* species tend to grow in shaded sites (Ladd, personal observations) and may also show similar resource limitation of fruit set.

Caladenia flava is a general food mimic orchid that attracts foragers with dummy structures devoid of either nectar or pollen. The *C. flava* plants were in the same general area as *P. fimbria*, and the contrast between the reasonable natural capsule set of the nectar producer and the absence in the general mimic is striking (Fig 1). There were few yellow flowered species in the area at the time the orchids were flowering which could have acted as models for *C. flava*.

The genus *Diuris* shows elements of Batesian mimicry, as its species mimic papilionoid legumes such as *Daviesia* and *Oxylobium*, and the orchids are pollinated by bees that utilise pollen and nectar offered by these legumes (Adams & Lawson 1993; Dafni & Bernhardt 1988; Nilsson 1992). Pollination effectiveness should be greatest when a low density, non-rewarding species mimics the flowers of a model species offering copious rewards at a much higher density (Dafni 1984; Weins 1978). This was found in *Thelymitra antennifera* (Dafni & Calder 1987) and in *Orchis* (Dafni & Ivri 1981). The very low natural capsule development in the *Diuris* correlated with a complete lack of nearby flowering legume shrubs at the study sites in 1999. The low fruit production found in this investigation was similar to the very low pollinia deposition by relevant insects for *Diuris maculata*, another Western Australian species (Beardsell *et al*. 1986).

Many Australian terrestrial genera, such as *Prasophyllum,* are stimulated to flower in increased numbers after fire. However, Adams & Lawson (1993) could find no reports on the effects of fire on pollinator activity or pollination rates. In this study *Prasophyllum fimbria* was in an area that had been burnt in the previous summer, and was flowering abundantly, compared to the other species. The observation that large numbers of fruit were produced by the species after fire indicates that the nectar reward was a strong lure for insects to repopulate the area. However *Lyperanthus* does not require fire to induce flowering (Hoffman & Brown 1998), indicating that nectariferous species may also be successful in undisturbed vegetation.

Populations of plants are increasingly subjected to size reduction and fragmentation through human action. Some studies show plants in smaller populations generally receive fewer visits from pollinators and the availability of pollen may become limiting for fruit set (Briggs & Leigh 1996; Lamont *et al*. 1993). While there are examples where species populations are buffered against pollinator loss (Bond 1994), this may only be short term. Cunningham (2000) investigated the fruit set efficiency of *Acacia brachybotrya* and *Eremophila glabra* in linear strips of vegetation and those in bigger fragments. It was found that a depressed pollination in the linear strips of vegetation occurred, causing lower fruit set than for the same species in the larger reserves. Similarly in a study of an orchid (*Pterygodium catholicum*) the plants in a small remnant native vegetation patch showed no seed set while populations in a larger remnant did set low numbers of fruit (Donaldson *et al*. 2002). If the situation of depressed pollination occurs widely in orchids, particularly non-nectariferous species which are extremely pollen limited under natural conditions, the system will be heavily impacted and conservation biology of such species will need to consider how to improve pollination effectiveness.

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