Pollination of the Bearded Greenhoods (Orchidaceae) by Dagger Flies (Diptera: Empididae)

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Aquatic Photographics

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Abstract

Bearded Greenhoods are part of the large genus *Pterostylis,* which comprises many natural groups that are variously treated by different authors. The name *Plumatichilos* was proposed for the Bearded Greenhoods by D.L. Szlachetke (2001) and recognised by Jones & Clements (2002), whom proposed a break up of *Pterostylis* with 12 genera, but this was not widely accepted. Most *Pterostylis* members are pollinated by fungus gnats flies of the families Mycetophilidae and Sciaridae (Kuiter, 2016), many of which by sexually attracted males, but pollinators of the bearded greenhoods were apparently unknown, not photographed, and generally speculated on as being fungus gnats. Observations over many seasons were made by us on the populations of *P. plumosa* in the western regions of Victoria, but evidence of pollination was totally lacking. The first evidence came in the form of a digital image from a location near Chiltern, showing a male Empididae member, which had just emerged from a *P. plumosa* flower with pollinia attached to the thorax. It had another pair of eroded pollinia below it and pollination of the flower was confirmed when checked by the photographer a few weeks later. This pollinator was tentatively identified as *Empis* sp. Soon after, *Empis* flies were found at a few sites and were observed in the pollination process.

Discussion

Bearded Greenhoods have a typical winged column, shared by all members of the genus *Pterostylis*, but the labellum is unique in being free moving, has thin yellow hairs and a thick apical knob, which flutters in the wind. Two species are recognised in Victoria, *Pterostylis plumosa* and *P. tasmanica*, but both taxa appear to comprise complexes of localised forms. *P. plumosa* was said to be insect-pollinated and by fungus-gnats, probably because at the time they were recognised to be primary pollinators for the majority of *Pterostylis* taxa, and *P. tasmanica* self-pollinating (Jones *et al,* 1999). According to Bates (2012) the bearded greenhoods are also insect-pollinated or self-pollinating in South Australia. The insects were referred to as 'stocky flies' and it was thought that the females of some were wingless. The details given seem to suggest members of the Empididae family. The wingless females statement may be based on nuptial gifts (sometimes missing wings) carried by the males in flight thought to be copulating females, reminiscent to thynniid male wasps copulating with wingless females in flight. Physical pollination was not observed and Bates wrote: "The person who first records the whole pollination event will be very lucky indeed".

In our general research on orchid-insect relationships of terrestrial orchids in Victoria, pollinators and seed pods of the bearded greenhoods were looked for over many seasons. Seed-pods were found only on *Pterostylis tasmanica*, that were apparently set from self-pollination, and no insect was seen taking an interest in the flowers. No seed-pods were found with *P. plumosa,* but in 2016 a photographer from Chiltern noticed an insect moving around inside a flower. After about 5 minutes it emerged with fresh pollinia on the thorax and a pollinator was finally photographed (Fig. **1**), confirming the pollinating by an insect. The image was sent to the first author and knowing what to look for was very encouraging and provided enough reason to continue our efforts in locating pollinators. Observations were made on *P. plumosa* colonies in different parts of Victoria, but pollination appears to be lacking in most regions. Where pollinators were present the orchid attracted only a single member of the Empididae family and, from the way the males behaved, the orchids are certainly species-specific. If it attracts the same or a sibling member of the genus *Empis* in different regions is not known, but at this stage it appears to involve only a single taxon for *P. plumosa*. The

Fig. 1 Photograph by Neil Blair near Chiltern. The insect was discovered trapped inside the flower, moving about attempting to find its way out. It emerged about 5 minutes later. Below the fresh pollinia sits a well eroded pair, and much of the pollen was deposited on the stigma, as when checked a few weeks later a seed pod had formed.

Fig. 2 A male dagger-fly *Empis* sp. carrying a nuptial gift to *Pterostylis plumosa*. The orchid attracts males by deception, its flower emitting a scent which mimics the female's sex-pheromones. The scent is produced during conditions the pollinators are likely to be flying in search of females, and usually occur in good numbers when present.

orchid taxa being species-specific applies to at least a particular site, but it may involve a complex of close sibling-flies in different regions.

Pollinators

The dipteran insects attracted to the orchid were members of Empididae family, commonly known as dagger flies. Pollinator activities were mainly observed on warm days in the afternoon until sunset. Amazing courtship behaviours are known for some members of this fly-family in the northern hemisphere with some species going through elaborate rituals, in which the male offers captured prey as a nuptial gift to the female in order to be accepted as a mate (McAlpine *et al*, 1981). Nuptial gifts presented by the pollinators were usually relatively large Diptera members (Fig. **2**), most commonly fungus-gnats (Mycetophylidae), and crane-flies (Tipulidae), but also other Empididae flies and occasionally small moths (Lepidoptera) (Fig. **3**), and all of which were about the same or slightly larger than their own size (Fig. **4**). At one site, away from the orchids, many females were witnessed grabbing the gift from the males in flight while the male took hold of the female. A female would probably fly out to the individual making the best impression with its nuptial gift, perhaps related to size? The males flew with the females grasped in their legs back to the shrubs and held the female during copulation, whilst she held the nuptial gift to feed on (Fig **3**). As females of some species may …

Fig. 3 Copulating pair, female feeding on nuptial gift.

Fig. 4 Nuptial gifts comprise relatively large Diptera members, usually about the male's size or slightly larger.

not hunt and instead feed on nectar or pollen, the nuptial gift is thought to provide essential protein required for the development of their brood. The apparent lack of pollinators in many areas suggest that *Empis* flies are extirpated due to habitat loss, caused by land clearing, and ruin from chemical uses and unnatural fires. The so-called fuel-reduction fires are a misleading advocacy causing species-reduction and the ecology suffers greatly, as usually the fires are conducted too frequently and during the wrong time of the year. The frequent fires are particularly destructive and very detrimental to the ecology of remaining habitats, especially affecting the highly specialised orchids and their pollinators. These species continue to be driven to local extinction by the ruining or degrading of habitats. Fire disrupts insects life cycles and when conducted in Autumn, kills many *Pterostylis* spp. as they are in their developing stages with rosettes or flowers. An undetermined *P. tasmanica*-complex taxon was wiped out by an 'ecological' burn in Langwarrin, before the pollination method was determined. The few colonies of this Mornington Peninsula sand-belt taxon represented the last population, and several other orchid species were lost.

Empis fly species involved in pollination were found only well away from cleared regions in the larger forests of East Gippsland, Castlemaine and Chiltern. Environmental changes and habitat conditions in the majority of other sites may have caused Empididae insects to go locally extinct, or they have thrown the flowering times of the orchid and flying times of a potential pollinator completely out of tune.

Autogamy and Entomophily

The column design suggest potential self-pollination as pollen material could fall onto the stigma, which sits directly below the anther holding the pollinia (Fig. **5**). A small number of finishing flowers of *Pterostylis plumosa* were checked and they showed the pollinia shrivelling up, but nothing had fallen on the stigma. Seed-pods were only found at localities where the *Empis* flies were observed in action. The

reputed self-pollinating *Pterostylis tasmanica* were checked at Wilson's Promontory & French Island and pollinia crumbles in the aging flowers, dropping onto the stigma and autogamy occurred commonly at these sites. Other populations of *P. tasmanica* need to be checked to find out if autogamy is the norm, or only a back-up when the pollinators are not active. Pollination of *P. plumosa* with a fungus gnat was shown in a simple drawing (Jones & Clements, 2002, p 40), whilst a photograph (#64) captioned "showing partly emergent microdipteran", clearly showed a dead Empididae fly on a preserved *P. tasmanica*. The photograph #64, suggests that insect-pollination does occur in this taxon. Based on visible characters the insect in the image is also a member of Empididae, but smaller than the species involved with *P. plumosa*. As the *P. tasmanica* taxon is reported to be self-pollinating (Jones *et al*, 1999) the orchid that is shown in photograph #64 may be a particular localised form (locality was not stated) of a complex of similar siblings in which both insect and self-pollination may happen. Bates (2012) also reported that in South Australia insect pollination takes place with *P. tasmanica*.

Pollinating methods between localised populations of a particular species may vary in different regions or it is influenced by the availability of a local *Empis* pollinator. In the *Pterostylis plumosa* populations checked near Melbourne and western regions, the Grampians, Stawell and Ararat, where the orchids occur regularly in good numbers, no evidence of pollination was found.

Insect to orchid behaviour

Normally *Empis*-fly females emit sex-pheromones to attract the males with their nuptial gift, whilst orchids attract male insects by deception, emitting kairomones, which is a mimic scent of the female's sex-pheromones. The males would be looking for a female, and through insect eyes the hairy labellum with the apical knob fluttering in the slightest breeze appears to be very convincing for one to be present. It seems that the labellum acts as a visual cue to arouse the male, as females of some species have visual features such as hairy legs for this purpose (McAlpine *et al*, 1981). The hairy labellum of the bearded orchids may have evolved for holding the nuptial gift as well. In the pollination process males were observed flying to the flowers with their gift and when landing on the labellum tried pushing it in to the hairs (Fig **6**). The labellum lowered from the combined weight and revealed the flowers' entrance above. When a male moves forward it probes with its abdomen seeking copulation and, as it goes in to the hood, the weight shifts and as the labellum rises up it obscures the entrance. …

Fig. 5 One side of the galea was cut away to show column details. The wings are enclosed by the narrow tubular galea section. The stigma sits directly below them and the thorax of an entering insect would be directed on to it, depositing pollen when present. Coming out, the thorax brushes the viscidium, picking up some glue and collects fresh pollinia.

Fig. 6 Male *Empis* fly pushing nuptial gift in to the hairs.

Fig. 7 Individual with eroded pollinia on thorax.

One individual flew in with well-eroded pollinia on the thorax (Fig. **7**). Lacking fresh pollinia suggested that it had gone through a flower in which pollinia was removed by an earlier visitor. Most of the pollinia was probably deposited on the stigma, but the remaining bits would still be enough to pollinate another flower.

Males were seen landing on the labellum, but appear to be going through courtship routines first. They eagerly flew in with their gift, but usually were only hitting the apical knob a couple of times on route to bushes in close vicinity for a brief rest. This action was usually repeated a few times before actually landing on the labellum.

The *Empis* flies were most active in the afternoon on warm days on flowers close to the shrubs and the orchids may have lots of visitors on any given day when conditions are right. Several populations of *Pterostylis plumosa* were checked in different regions and on many different days. Pollinator action was limited to few sites, but when present the *Empis* males were flying in good numbers, which was usually close to a creek or wet gully. None were seen near cleared land or historically dry places as larvae are water-dependent. The Empididae are considered to be important with natural control of some pest insects. Adults of several genera prey on emerging or swarming mosquitoes and other flies (McAlpine *et al*, 1981), and many feed on other diptera larvae such as mosquitos and fungus-gnats.

Based on the wing venation the primary pollinator of *Pterostylis plumosa* belongs to the genus *Empis*, very similar to the northern hemisphere *E. livida**, but probably represents an undescribed species. A flower, which was photographed with a pollinator, was measured to determine the size of the insect, and was calculated to be 7 mm in body length (not including antennae). The numerous individuals seen were all about the same size.

Note

This report is the first publication on our research into the pollination of the bearded greenhoods. Results from future observations on the bearded greenhoods, that may add significantly to this paper, will be treated in another short-paper.

Acknowledgements

We like to thanks Neil Blair for providing the first photograph of a pollinator on *Pterostylis plumosa*. Dr. Dan Bickel from the Australian Museum confirmed our tentative identification of the *Empis* fly from photographs.

*http://www.metafysica.nl/nature/insect/nomos_77.html

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