Identification and Comparison of the Pollinators for the Purple-fringed Orchids *Platanthera psycodes* and *P. grandiflora*

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Abstract

The pollination ecologies of the two purple-fringed orchids, *Platanthera psycodes* and *P. grandiflora*, are compared to test the prediction that, despite an extraordinary similarity in appearance, internal differences in the position and shape of fertile structures contributes to a distinct difference in their respective pollination ecologies. Field observations and experiments conducted during the 2003 flowering season in Great Smoky Mountains National Park revealed a number of effective pollen vectors not previously documented for *P. psycodes*. In contrast, the single small population of *P. grandiflora* experienced complete pollination failure during the 2003 flowering season. Preliminary results suggest that while *Bombus* spp. effectively pollinated *P. psycodes*, visitation by *Bombus* actually disrupted pollination among *P. grandiflora.*

University of Tennessee, January 17, 2006

Introduction

Among the many species of terrestrial orchids that exist in eastern North America, two species appear so similar in vegetative and floral morphology that they have been a source of confusion and debate, even the among the world's foremost botanists, until as recently as the mid 1970's (Stoutamire, 1974). The small-flowered purple-fringed orchid, *Platanthera psycodes* (L.) Lindley, and the large-flowered purple-fringed orchid, *P. grandiflora* (Bigel.) Lindley, have alternately been considered either as two distinct species (Britton and Brown 1913, Fernald 1950), or else as mere varieties of a single species (Gleason 1963, Gleason and Cronquist 1963). In 1974, Warren P. Stoutamire published the results of a study that clearly demonstrated the specific status of each.

Although they appear identical to the untrained eye, *Platanthera psycodes* and *P. grandiflora* are distinguished by distinct differences in column shape and size as well as corresponding differences in nectary features that combine to result in a difference in the dynamics of pollen transfer (Stoutamire 1974). Typical of orchids, each delivers pollen to the vector in a discrete packet called a pollinium. For members of the genus *Platanthera*, the pollinium is attached by a stalk to an adhesive disk called a viscidium, and the entire structure is referred to as a pollinarium (Figs. 1-B, 2-B). The viscidium functions as the means of attachment to the pollen vector, effectively gluing the entire pollinarium to the insect. The functional difference between *P. psycodes* and *P. grandiflora* lies in the point of attachment upon the insect. Whereas visitors to *P. psycodes* consistently withdraw the pollinarium attached to the proboscis, visitors to *P. grandiflora* withdraw the pollinarium attached to the lateral surface of the compound eye. This functional difference is the result of differences in the shape, orientation, and relative positions of the nectary opening and the pollinaria (Stoutamire, 1974).

Platanthera psycodes possesses a nectary opening that is shaped rather like an hourglass oriented horizontally (Fig. 1-A). *P. grandiflora* possesses a nectary opening that is round to slightly oval along a vertical axis (Fig. 2-A). My observations at close range confirmed that the constriction in the center of the opening to *P. psycodes* influences the visitor to insert its proboscis into one of the two lateral expansions, which are positioned just below each viscidium (Fig. 1-A). The surface of the viscidial disk is oriented downward and slightly medially, corresponding precisely to the line of entry of the visitor's proboscis. As a result, the pollinaria of *P. psycodes* are consistently placed on the proboscis of pollen vectors.

Alternatively, the round nectary opening of *Platanthera grandiflora* allows for a centered approach by the visitor. With approximately 4.5 mm of space between the forwardly positioned viscidia, the anterior portion of the head of most visitors may be inserted between them to gain deeper access to the nectary. This action places the lateral surface of the visitor's eyes in a position adjacent to the viscidia. The surface of the viscidial disk is oriented medially and slightly downward as well as forward (Fig. 2-A). This orientation mirrors the surface of the compound eye of most visitors, resulting in attachment of the pollinaria on the lateral surface of the eye.

Beginning with the earliest studies of orchid pollination by Darwin in Europe (1862) and Gray in North America (1862, 1863), evidence rapidly mounted to show the intimate and often precise relationship between floral morphology and pollinator identity. In some cases, individual orchid species are obligately specialized for a single pollinator species. In other cases, pollination is less tightly constrained but still limited to a small assembly of pollinators referred to as a pollination syndrome (van der Pijl 1966, Faegri and van der Pijl 1979). Although pollination syndromes may overlap from one species of orchid to another, it is also possible for

the pollination syndromes of two closely related orchid species to be entirely dissimilar (Smith and Snow 1976, Catling and Catling 1991). Robertson and Wyatt (1990) found that even small differences in floral morphology between two populations of the same species of orchid (*Platanthera ciliaris*) corresponded with a distinct difference in pollination syndromes. Moreover, they found compelling evidence that these morphological differences are an evolutionarily adaptive response to locally available pollinators.

It is well known that members of Orchidaceae hybridize easily when artificially crossed. Horticulturists routinely obtain fertile offspring from not only interspecific crosses, but from intergeneric crosses as well. In experimental crosses conducted by Stoutamire (1974) between *Platanthera psycodes* and *P. grandiflora*, capsules developed and contained seeds with apparently normal embryos. Although these seeds did not germinate, this cannot be taken as proof of incompatibility since many other factors contribute to inconsistent germination rates for orchid seeds (Stoutamire 1974). Fernald (1950, p 474) states that the two species do hybridize, but does not elaborate on the evidence for this statement. Although many populations of *P. psycodes* and *P. grandiflora* are both spatially and temporally isolated from each other, there are also a number of reports of populations of the two species blooming in close proximity on or near the same dates (Stoutamire 1974). Since the two species do produce apparently normal embryos when crossed, yet very little evidence exists to suggest that hybrids occur under natural conditions, it is likely that little genetic exchange occurs. Moreover, since there is the ecological potential for genetic exchange, it appears that mechanical and behavioral barriers are in place (Stoutamire 1974).

Based on this information my prediction is that, while there may be some overlap of pollinators that function effectively for both orchid species, the overall assemblage of effective pollen vectors (pollination syndrome) for *Platanthera psycodes* is composed of a different set of species than the overall assemblage of effective pollen vectors for *P. grandiflora*, and that this in turn results in dissimilar pollination ecologies

There has been very little study of the pollination ecologies of either species, and Stoutamire's work in the 1970's seems to be the first serious treatment of the reproductive biology of the two. Although he made significant discoveries regarding the distinctive morphology of their respective fertile structures, and compiled the first collection of observations regarding pollinators and the mechanics of pollen transfer, the identification of the full pollination syndrome for both *Platanthera psycodes* and *P. grandiflora* remains incomplete.

The primary objective of this investigation is to more comprehensively identify effective pollinators for these two species, with special interest in those vectors functioning in Great Smoky Mountains National Park. In turn, the data gathered for each species will be compared in order to test the prediction that the differences in column shape and size along with differences in nectary features result in different pollination ecologies.

Materials and Methods

Study sites –Park records indicate several populations of *P. psycodes* distributed at various sites throughout the Great Smoky Mountains National Park (GRSM). In contrast, only one very small, localized population of *P. grandiflora* is known to exist in GRSM. Due to the

rare status of *P. grandiflora* within the Park, officials have requested that the precise location of this population be withheld from publication.

From a large population of *P. psycodes* surrounding the parking lot at Clingman's Dome, I selected a patch of approximately 70 plants occupying an area of 84 m^2 on the south side of the parking lot on a southward facing slope of moderate grade. The site is at 35°33'22" N; 83°29'42" W, and the elevation is approximately 1,920 m.

As stated above, the precise location for the population of *P. grandiflora* must remain confidential. The habitat is a meadow at approximately 1,664 m elevation that is rather level and receives full sunlight throughout the day. Among the approximately 15 plants at the site, many appeared to be the victims of herbivory, and a few appeared to suffer damage from a late frost. Only four plants were in bloom during the 2003 season.

Visitor observations – Insect visitors to *P. psycodes* were observed for a total of 48.5 hrs. over the course of 12 days in the field. Observations were made at various and overlapping periods of the day in order to obtain a composite view of as many hours in a twenty-four-hour day as possible. The only period wherein no observations were made was between the hours of 1:00 am and 8:00 am. Observations were made in a variety of weather conditions, from full sun to heavy downpour, in order to assess pollinator behavior patterns across the spectrum of conditions as well as to identify abiotic mechanisms of pollination such as rain-assisted autogamy. Weather conditions such as temperature, precipitation, cloud-cover, and wind were recorded at regular intervals to identify any weather related patterns of visitor activity. Sample specimens of each insect species observed to visit the plants were collected and killed using ethyl

acetate and now await identification and analysis. All visitors were treated as potential pollinators until extended observations and analysis demonstrate otherwise.

Field observations of visitor activity for *P. grandiflora* were limited by poor accessibility to the study site, the low number of flowering individuals, and poor weather conditions. Visitor activity for *P. grandiflora* was observed for a total of 9.25 hrs. over the course of two days. As above, weather conditions were recorded at regular intervals to identify weather related patterns of visitor activity, and sample specimens for each visiting species were collected.

Visitor effectiveness – Several strategies were employed to assess the effectiveness of each visiting species as a pollen vector. Captured visitors were examined for the presence of pollinaria attached to any part of the body. In addition, visitor interaction with flowers was directly observed at close range and photographed, with special attention to successful pollinaria removal.

To accurately identify the agents responsible for successful pollen transfer, I caged one individual plant from each species (*P. psycodes* and *P. grandiflora*) in order to control exposure to visitors. Exposure was limited to periods of exclusive observation during which each visitation was followed by a careful examination of floral parts for evidence of successful removal and/or deposition of pollen. Examinations were facilitated with the use of a 10x hand lens. Any affected flowers were tagged and the results of the visit were noted. This allowed me to precisely determine which species had visited the plant, whether or not it removed pollinaria, and whether or not it deposited pollen upon the stigmatic surface of any flowers.

General fertility – For *P. psycodes*, a basic understanding of general fertility and self-compatibility was sought through caging and manipulation of individual plants. Several treatments were employed. These include: 1) simple isolation of flowers from insect visitors. Subsequent analysis of fruit-set among these flowers would suggest whether or not there is unassisted or abiotic mechanisms of autogamy. 2) Simple emasculation (removal of pollinaria) from individual flowers to investigate the possibility of apomixis. 3) Emasculation plus self-fertilization to investigate self-compatibility. 4) Emasculation plus cross-pollination with another individual from the same patch. 5) Emasculation plus cross-pollination with an individual from a more distant patch within the same population. All cages were marked with aluminum tags for identification, and all treated flowers were marked with paper tags containing a code that indicates the date and type of treatment.

Due to the critically low numbers of flowering individuals of *P grandiflora*, no attempts were made to investigate reproduction by caging and experimental treatments. It seemed prudent to minimize interference with the reproductive potential of this population for this particular season.

Results

Visitor observations – Identification of all visitors to *Platanthera psycodes* has yet to be completed. A number of insect species were observed to visit flowers of *P. psycodes*; however, not all were observed to remove pollinaria. Those visitors confirmed to possess pollinaria, but not captured for specific identification include *Colias* spp., *Bombus* spp., an unidentified species of noctuid moth, and an unidentified species of beetle.

Visitors possessing pollinaria that were captured and identified include the clouded sulphur butterfly *Colias philodice*, the orange sulphur *Colias eurytheme*, the eastern tiger swallowtail *Papilio glaucus*, the black swallowtail *Papilio polyxenes*, the silver spotted skipper *Epargyreus clarus*, the drone fly *Eristalis tenax*, and an as yet unidentified species of *Bombus*.

In the case of *P. grandiflora*, results were for the most part disappointing due to poor weather and the small number of flowering individuals. The only insects observed to visit the flowers of *P. grandiflora* were unidentified species of *Bombus*. These bees did, in fact, withdraw from the flowers with pollinaria attached. However, in every case observed they immediately removed and discarded the pollinaria before visiting the next flower.

Visitor effectiveness – The results of controlled exposure of *Platanthera psycodes* to visitors showed that *Bombus* species effectively removed pollinaria and transferred pollen to stigmas. More casual observations strongly suggest that the butterfly *Colias eurytheme* is an effective and frequent pollen vector for *P. psycodes* within GRSM. Examinations of plants after uncontrolled visits by *C. eurytheme* revealed that pollinaria had been removed and fresh pollen had been deposited upon stigmas. In addition, examination of pollinaria on the proboscises of *Papilio polyxenes* and *Papilio glaucus* revealed that the pollen masses that comprise the pollinia had been ruptured, suggesting that they had come into contact with stigmatic surfaces.

As for *P. grandiflora*, in no case were the *Bombus* spp. observed to transfer pollen from one flower to another. Furthermore, repeated examinations with a 10 x hand lens revealed that no pollen had been deposited upon the stigmatic surface of any flower during the entire period of observations, and all pollinaria had been removed and discarded by the termination of observations.

Reproductive characteristics – Analysis of the results of hand-pollination treatments has yet to be completed. Initial observations indicate consistent fruit-set among all hand-pollinated flowers, including those that were selfed. Equally as consistent was the failure to set fruit among those flowers that were simply isolated from visitors as well as those that were simply emasculated. Future visits to the study site are necessary to confirm these initial observations.

Discussion

Previous observations by Stoutamire himself combined with reports he obtained from others resulted in a list of four species associated with *Platanthera psycodes*. These are the diurnal hawkmoths *Haemmorhagis thysbe* and *H. differmis*, the swallowtail butterfly *Papilio asterias polyxenes* (here *Papilio polyxenes*), and the skipper *Polites mystic* (Stoutamire, 1974). He stated that each of these had been observed to remove pollinaria from *P. psycodes* with the viscidium attached near the base of the proboscis.

Results of this study indicate that a number of Lepidoptera species not previously documented serve as effective pollinators for *P. psycodes* in GRSM. These include species predicted to be better suited to *P. grandiflora* based on general floral morphology. The most surprising discovery thus far is that species of *Bombus* function as effective pollen vectors for *P. psycodes* within GRSM. I have been unable to locate any literature that either documents or predicts pollination of *P. psycodes* by any member of the order Hymenoptera.

The only insects observed to visit *Platanthera grandiflora* during this study were bees of the genus *Bombus*. The precise species is yet to be determined. These were frequent visitors to nearby Asteraceae, but occasionally diverted their attention to *P. grandiflora* with apparently

catastrophic effects. Close observations under controlled field conditions revealed that visits by this group often resulted in the complete loss of individual pollinia from the pollination system, and in no case did a visit result in effective pollen transfer. Bees of this type did in fact remove pollinaria from *P. grandiflora* anthers, but rather than carry the pollinarium even to the very next flower, they discarded the pollinarium immediately upon withdrawal, leaving it to fall where it may. I observed dozens of pollinaria scattered about upon petals, leaves, and the foliage of other plants. Unfortunately, in the case of orchids bearing pollinaria, this results in a total loss of the investment of pollen from the half-anther. Over the course of two days, I witnessed entire racemes of *P. grandiflora* become completely depleted of pollinaria by these bees, effectively excluding the plant from sexual reproduction for the 2003 flowering season. Examination upon the termination of observations revealed that no stigmatic surface on any flower in this population contained pollen, and all pollinaria had already been removed and discarded by the bees.

Whereas *Bombus* spp. function as effective pollen vectors for *P. psycodes*, they not only are ineffective for *P. grandiflora*, but actually have a negative impact on successful pollination. This discovery stands out as significant. *P. psycodes* is considerably more abundant and much more widely distributed than is *P. grandiflora*, and this disparity is amplified in the Southern Appalachians. Additionally, populations of *P. grandiflora* seem to be declining at a much more rapid rate than *P. psycodes* throughout their respective ranges. Further investigation into the role that pollination ecology plays in the dissimilar abundance and ultimate survival of the two species may be warranted.

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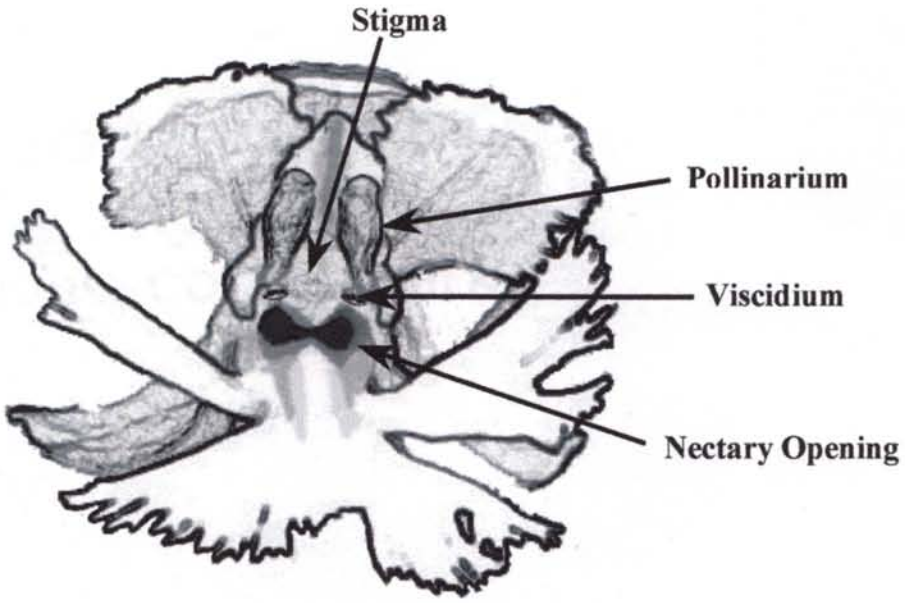
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Figure 1. *Platanthera psycodes* floral features. Drawing adapted from photograph of living specimen.

A. Flower



B. Pollinarium

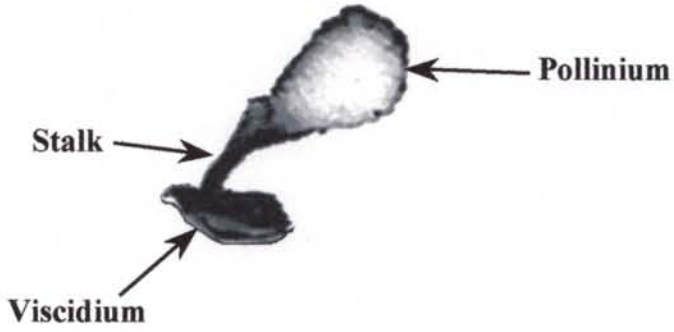


Figure 2. *Platanthera grandiflora* floral features. Drawing adapted from photograph of living specimen.

