ENHANCING POLLINATION OF THE ENDANGERED WESTERN PRAIRIE FRINGED ORCHID (*PLATANTHERA PRAECLARA*) BY SPHINX MOTHS (LEPIDOPTERA: SPHINGIDAE) IN TALL GRASS PRAIRIE IN SOUTHEASTERN MANITOBA AND AN EXAMINATION OF ORCHID NECTAR PRODUCTION

by

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A Thesis submitted to the Faculty of Graduate Studies

The University of Manitoba

In Partial Fulfillment of the requirements for the degree of

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES
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This is dedicated to the memory of my grandfather, Frank Schalla, for his keen interest in wildlife and countless stories about his adventures hunting, fishing and wildlife watching. His enthusiasm for nature captured my curiosity and developed my interests in wildlife that has guided my educational and career choices.

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ABSTRACT

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Enhancing pollination of the endangered western prairie fringed orchid (*Platanthera praeclara*) by sphinx moths (Lepidoptera: Sphingidae) in tall grass prairie in southeastern Manitoba and an examination of orchid nectar production.

Major professor: A. R. Westwood

The endangered western prairie fringed orchid, *Platanthera praeclara* (Sheviak and Bowles), is found in remnant tall grass prairie in southeastern Manitoba, and has a lower level of seed capsule development in comparison to more southern populations in the United States. Pollination of *P. praeclara* is limited to few select species of sphinx moths, Sphingidae, Lepidoptera, a group that is less abundant in comparison to other lepidopteran families. This study tested the hypothesis that the presence of ultraviolet light sources near orchids would attract more sphinx moths into orchid habitat and increase feeding activity of sphinx moth pollinators, thus increasing seed capsule production. The second part of this study measured orchid nectar quality and quantity during the bloom period and the possible link to pollination success. A significantly larger number of individual flowers and plants developed seed capsules in the ultraviolet light treatment plots (5.13 ± 0.42 % of available flowers; 35.12 ± 1.74 % of total plants) than the control plots (2.78 ± 0.42 % of available flowers; 21.76 ± 2.58 % of total plants). The intensity of the ultraviolet light treatment may have also had an effect on pollination success. Ultraviolet lights influenced seed capsule production by attracting sphinx moths into experimental plots. Results also indicated that ultraviolet light may be useful to manipulate seed capsule production for other research purposes. Nectar quality and quantity varied considerably during the study. The mean sugar concentration over the

sampling season was 23.9 ± 0.2 %; however, values ranged from a low of 13 % to high of 34 %. Nectar sugar concentration decreased by approximately 6 % as the flowering period progressed over the season.

CHAPTER 1

INTRODUCTION

The endangered western prairie fringed orchid, *Platanthera praeclara* Sheviak and Bowles, is found in remnant tall grass prairie in southeastern Manitoba. It also occurs in the United States including North Dakota, South Dakota, Minnesota, Nebraska and Iowa (Sheviak and Bowles 1986; Bray and Wilson 1992; U.S. Fish and Wildlife Service 1996). It is protected under the Manitoba Endangered Species Act and listed as an endangered species under the federal Species at Risk Act (Manitoba Conservation – Species at Risk 2006; Environment Canada – Species at Risk 2006). When in flower, these orchids will grow 38-85 cm tall (Sheviak and Bowles 1986). The inflorescence of creamy white flowers makes this species highly visible during its flowering period from late-June to mid-July in Manitoba. The flowers give off a sweet fragrance that becomes more intense in the late evening. The most striking visual characteristics of the flowers are the large, deeply fringed, tri-lobed lower petal and long, slender nectar spur (Sheviak and Bowles 1986). These floral characters limit pollination to a few select species of sphinx moths, Sphingidae, Lepidoptera (Cuthrell 1994, Westwood and Borkowsky 2004, Ralston *et al.* 2006).

Platanthera praeclara only occurs in wet sedge meadows within remnant tall grass prairie habitat in central North America, and loss of habitat is considered the leading cause for its' endangered status in Canada and the United States (Davis 1995, U.S. Fish and Wildlife Service 1996). Tall grass prairie is considered the most endangered ecosystem in North America (Samson and Knopf 1994, Hamilton 2005, Whiles and Charlton 2006) and in Canada the prairie is one of the most endangered natural regions (World Wildlife Fund 1989) with less than 0.5% of the original habitat remaining in Manitoba (Joyce and Morgan 1989). Westwood and Borkowsky (2004) have described the pollination process for *P. praeclara* in Canada. Westwood and Borkowsky (2004) also noted that there is a low level of seed capsule development in the Canadian population in comparison to more southern populations of *P. praeclara.*

This low level of seed capsule development may be related to the scarcity of pollinators (Westwood and Borkowsky 2004). Sphingidae are generally less abundant than many other lepidopteran families (Covell 1984, Duarte and Schlindwein 2005). The area surrounding *P. praeclara* habitat in Manitoba has become fragmented by agricultural land use ranging from tame pasture development to conversion to cropland, with considerable insecticide and herbicide usage. Westwood and Borkowsky (2004) suggested that nocturnal pollinators may be drawn to the light sources prevalent in the previously dark countryside, such as farm lights and intersection lights at highway junctions and away from the less inhabited areas that support populations of *P. praeclara.* Other factors that may influence pollinator visitation may include lack of larval host plants or competition from alternate nectar sources.

To test the hypothesis that low production of seed capsules in *P. praeclara* in Manitoba is dependent on sphinx moth density an experiment was designed to attract sphinx moth pollinators into orchid habitat to increase pollination success. This study tested the hypothesis that the presence of ultraviolet light sources near orchids will increase feeding activity of sphinx moth pollinators, thus increasing seed capsule production. Westwood and Borkowsky (2004) also noted that there exists no published data on nectar production in the western prairie fringed orchid or possible links between nectar quality and quantity, moth attraction and subsequent pollination success. There is evidence that the time frame available for pollination may be relatively short as sphinx moth pollinators are nearing the end of their flight period when the orchid is reaching the height of the flowering period (Westwood and Borkowsky 2004). This study also examined the role that orchid nectar quantity or quality may play in attracting sphinx moths during the important overlap period of orchid flowering and moth flight.

CHAPTER 2

LITERATURE REVIEW

Distribution of the Orchidaceae. The Orchidaceae is regarded as the second largest family of flowering plants; however, the estimated number of species remains inconsistent. Early estimates for the number of species ranged from 17000 (Willis 1973 in Dressler 1981) to over 24000 species (Hawkes 1961). Nilsson (1992) considered the possibility of 25000 species, while Dressler (1993) suggested that there are approximately 19500 species worldwide. More recently, estimates suggest 800 genera and 22000 to 35000 species (Romero-González *et al.* 2003). Dressler (1981) lists 153 known species representing 26 genera of orchids native to North America, however, Romero-González *et al.* (2003) includes 208 orchid species in 70 genera. Within the province of Manitoba, there are 36 known species of orchids from 14 genera (Ames *et al.* 2005). Three species, *Spiranthes magnicamporum* Sheviak, *Platanthera praeclara* Sheviak and Bowles, and *Cypripedium candidum* Muhl. Ex Willd. have been designated as endangered under the Manitoba Endangered Species Act (Manitoba Conservation – Species at Risk 2006).

Tropical regions possess thousands of orchid species compared to the hundreds of species found in temperate zones. Orchid abundance and diversity are dependent primarily on rainfall, with regions receiving annual rainfall of approximately 2.5 m or more having the greatest abundance and diversity (Dressler 1981). Orchids have evolved to fit particular niches within a habitat, growing in the ground (terrestrials), on rocks or cliffs (lithophytes) and perched upon trees or shrubs (epiphytes) (Dressler 1981). A few species are semi-aquatic and two genera of Australian orchids, *Cryptanthemis* and

Rhizanthella, are considered subterranean as only the flowers reach the surface of the ground (Hawkes 1961).

Orchid morphology relevant to pollination. Orchids are perennially flowering, herbaceous plants bearing a single seed-leaf during germination, thus belonging to the monocotyledon group of plants (Hawkes 1961). The orchid flower is composed of structures similar to those of other families of plants such as sepals, petals, anthers containing pollen, stigma, and ovary; however, through processes of reducing, enlarging, and/or fusing of these basic structures, orchids have evolved into a multitude of forms (Hawkes 1961, Dressler 1981, 1993). Nevertheless, a few generalizations may be made regarding the flowers of the Orchidaceae. Orchid flowers are bilaterally symmetrical and three fused carples form an inferior ovary (van der Pijl and Dodson 1966). In many orchid flowers, both the sepals and petals occur in sets of three with the sepals being of similar shape while the petals are not. Typically, one of the three petals, most often the median petal, is modified to form the labellum (often referred to as the lip), which may act as a landing platform for the pollinator (Hawkes 1961, van der Pijl and Dodson 1966). These structures are identified for an orchid and non-orchid flower in Figure 1.
Dressler (1981) identified and described three morphological features common to all orchids. Firstly, stamens are shifted to one side of the flower, secondly the stamens and pistol have united to form a compound structure called a column, and lastly the seeds are small and very abundant. Orchid seeds are often microscopic or dust-like as they lack an endosperm and are produced by the thousands (Bowles 1983, Light and MacConaill 2002, Lehnebach and Riveros 2003). The remaining characteristics, when present, reflect

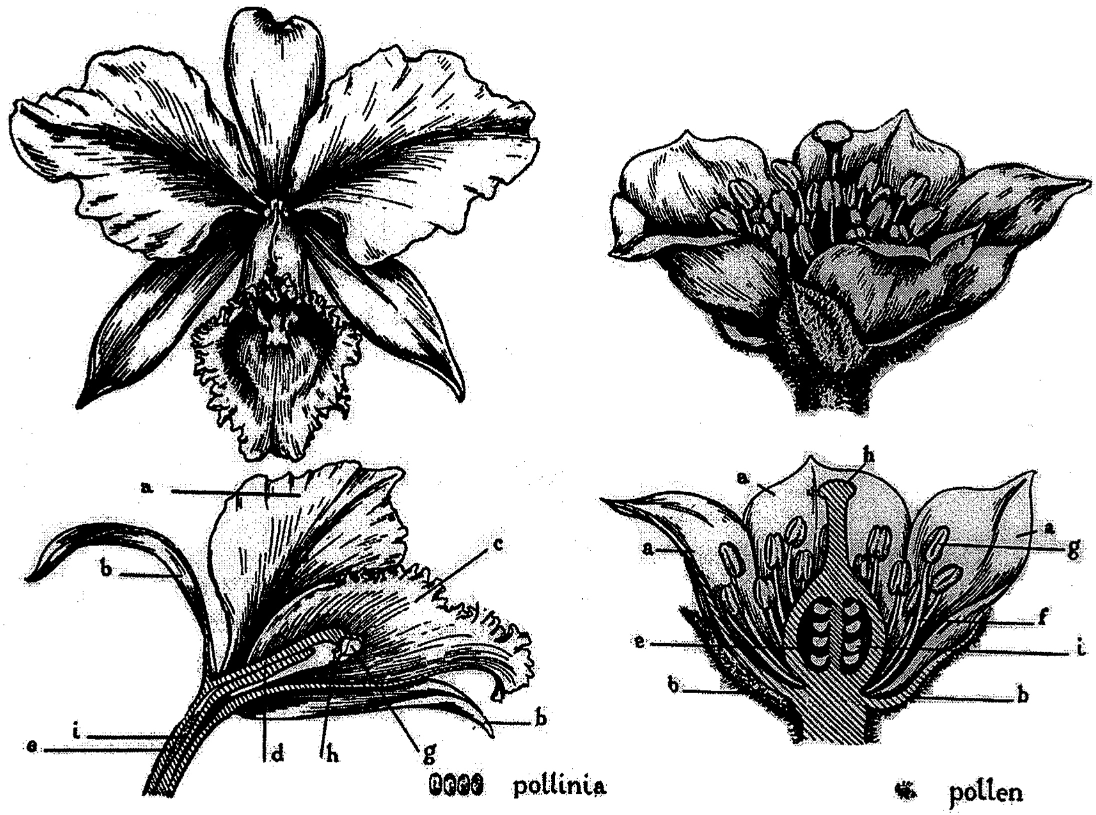

Figure 1. Comparison of a generalized orchid flower (left) with an uncomplicated flower (right). A, Petal; B, Sepal; C, Petal transformed into the labellum; D, Column; E, Ovary; F, Filament of a stamen; G, Anther; H, Stigma; I, Ovule. (from van der Pijl and Dodson 1966.)

modifications of the basic floral design found in most plants (Dressler 1981). The labellum is positioned opposite the column and the pollen is usually bound together in a few, large masses called pollinia (Dressler 1981). During orchid pollination the rostellum (the larger median lobe of the stigma) is involved with pollen transfer; providing an adhesive substance as in the *Vanilla* spp. or forming the viscidium structure of the pollinaria as in the *Platanthera* spp. In both instances, the rostellum secures the pollen to the pollinator and is a functionally important structure (Dressler 1981) (Figure 2). Finally, during their development, the flowers of most orchids twist in a process termed resupination (Dressler 1981). In early development, the bud is held such that, relative to the stem, the labellum is proximal while the stamen is distal. As the bud develops and matures, the ovary twists, such that when the flower opens the stamen becomes proximal to the stem and the labellum is positioned distally (Dressler 1981).

Among monandrous orchids (diandrous species, such as lady'slippers, have two anthers) the pollinarium is a specialized pollen bearing structure that becomes attached to the pollinator (Luer 1975, Johnson and Edwards 2000). The pollinarium consists of the pollinia (pollen masses) that are attached to a stipe, of varying length, the caudicles and the viscidium (Dressler 1993). The caudicles are highly variable among orchid species. They may be minute discs located between the stipe and the pollinia (Darwin 1904) or up to 20 mm in length as in *Cynorkis uniflora* Lindl. (Nilsson *et al.* 1992). The caudicles are important to the orientation of the pollinia after the pollinarium is attached to the pollinator (Darwin 1904, Johnson and Edwards 2000). The pollinarium has evolved via fusion of the stigma's median lobe and stamen(s) (Dressler 1983). The size and shape of

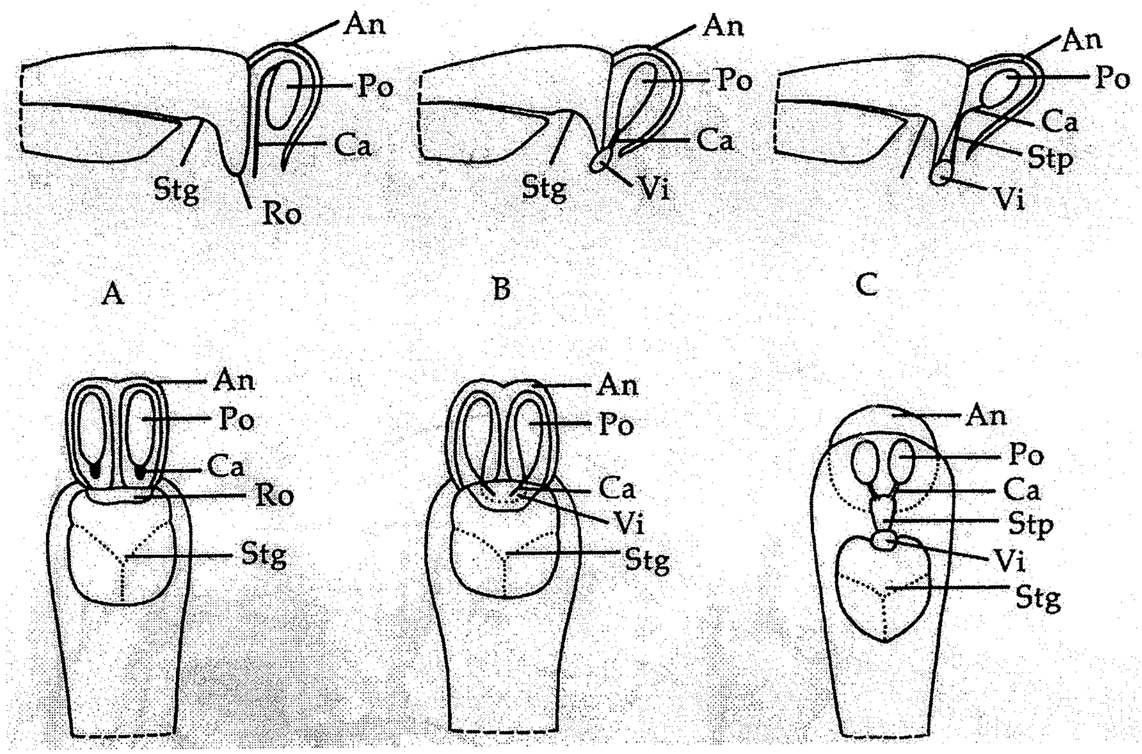

Figure 2. The relative position of the reproductive structures for three arrangements. Longitudinal sections above, ventral view below. A, Column with rostellum, but no viscidium; B, Column with viscidium; C, Column with viscidium and stipe. An, Anther; Ca, Caudicle; Po, Pollinium; Stg, Stigma; Stp, Stipe; Vi, Viscidium. (modified from Dressler 1981).

the pollinarium is unique among orchid species (Dressler 1981); pollinators can be associated with a particular orchid species through the identification of the pollinaria they carry (Ackerman 1983). As many orchids are autogamous or self-pollinating (van der Pijl and Dodson 1966), it would follow that this would be the most reliable method to guarantee pollination of a flower; however, several mechanisms will reduce the likelihood of this occurring. Firstly, the position of the pollinarium and stigma reduce the possibility of self-pollination. Relative to the stigma, the pollinaria are distally located at the end of the column within a protective pouch (Figure 3) (Dressler 1981). This spatial separation of the reproductive structures, i.e. pollinaria and stigma, is referred to as herkogamy. Webb and Lloyd (1986) suggested that herkogamy evolved to reduce self-pollination of a flower and self-pollination within a plant producing multiple flowers. The pollinarium is pulled from a protective pouch when the pollinator draws away from the flower and does not pass near the stigma. Secondly, the orientation of the pollinarium, immediately after removal from the pouch, does not align with the stigma and the pollinarium must undergo specific movements to do so. This movement, illustrated in Figure 4, is caused by differential drying of the stipe and/or caudicles, to change the orientation of the pollinia by as much as 90 degrees within a period of a few seconds or up to a few minutes (Darwin 1904, Dressler 1981, Johnson and Edwards 2000). Luyt and Johnson (2001) observed that after several minutes, movement of the pollinarium from *Mystacidium venosum* Harv. ex Rolfe flowers was completed and only then could contact be made with a specific notch in the stigma of subsequent flowers visited by the pollinator. Another important set of structures related to pollination are the flower nectaries. Nectaries are the nectar producing structures or glands in plants (Fægri

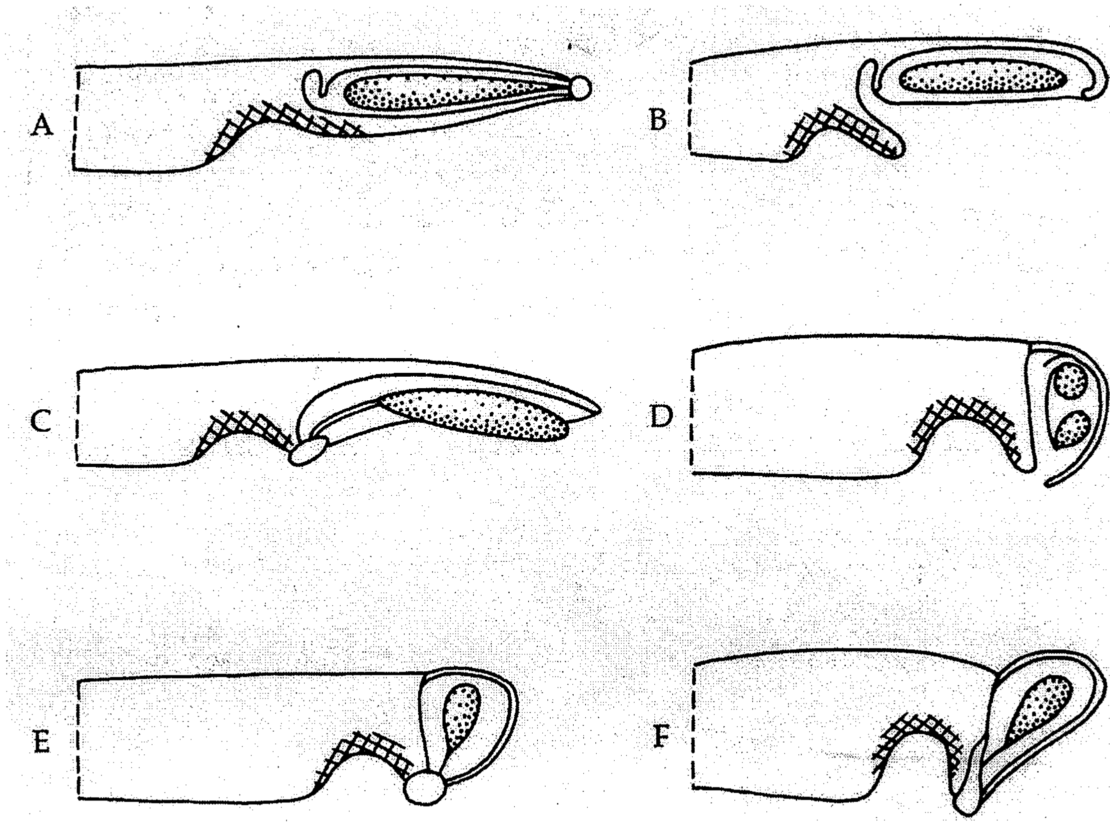

Figure 3. Diagrams showing relationships of pollinarium to stigma. Pollen, stippled. Stigma, cross-hatched. A, Spiranthoideae, with anther dorsal and rostellum subequal to pollinarium; B, Neottieae, pollinarium terminal, projecting beyond rostellum; C, Orchideae, with basal viscidia; D, Epidendroideae, with incumbent pollinarium; E, Vandoideae or advanced Epidendroideae, with viscidium, F, Vandoideae, with viscidium and stipe. (from Dressler 1981).

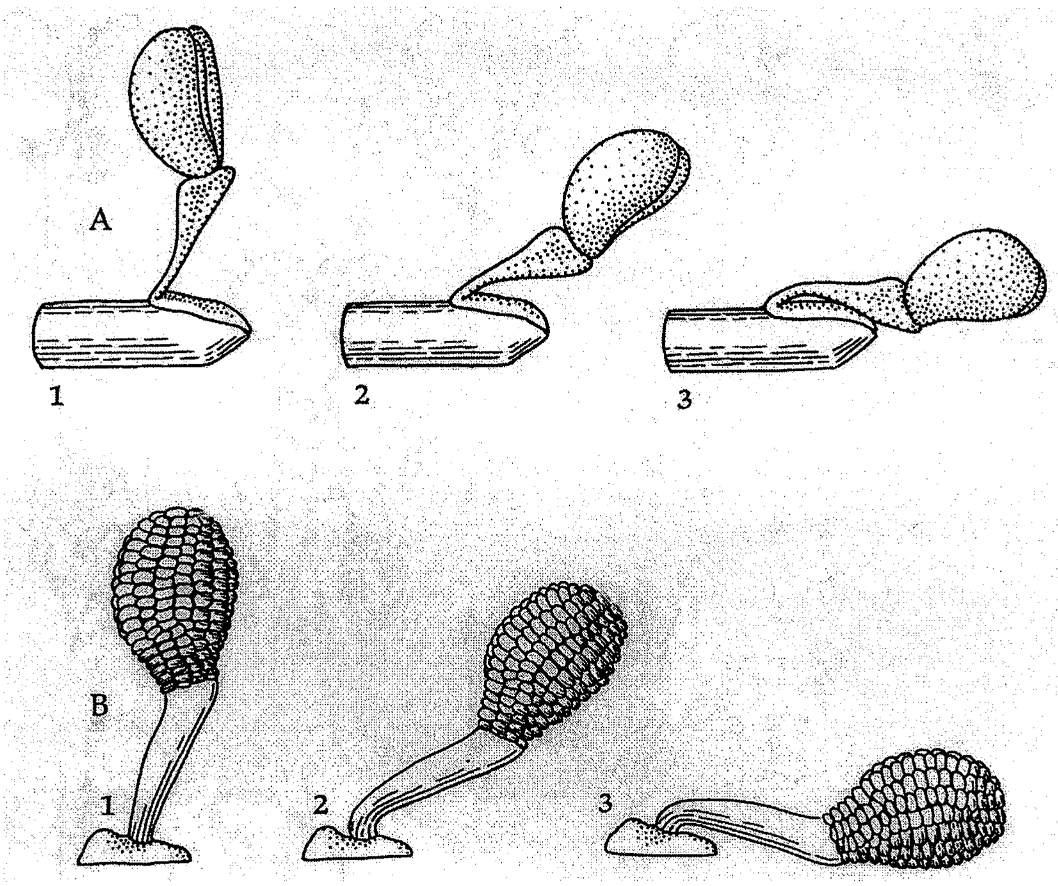

Figure 4. Movement in pollinaria. A, *Himantoglossum* spp.; B, *Rossioglossum* spp. (from Dressler 1981).