POLLINATION BIOLOGY IN SEVEN TAXA OF MICHIGAN ORCHIDACEAE AND A STUDY OF CYPRIPEDIUM CALCEOLUS IN MICHIGAN BASED ON LIVING PLANTS AND HERBARIUM SPECIMENS

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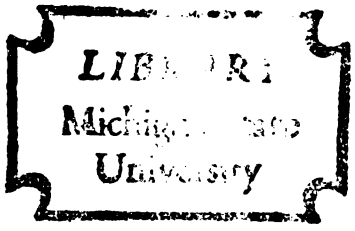

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ABSTRACT

POLLINATION BIOLOGY IN SEVEN
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AND
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IN MICHIGAN
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By

Cathey Jo Newhouse

Seven taxa of Michigan Orchidaceae (Pogonia ophioglossides, Calopogon tuberosus, Habenaria blephariglottis, Cypripedium acaule, Cypripedium reginae, Cypripedium calceolus var. pubescens, and Cypripedium calceolus var. parviflorum) were studied to determine the normal means of reproduction, the self-compatibility, and the pollinating agent. With the exception of Pogonia ophioglossoides, all species were found to be normally cross-pollinated, but to have no self-sterility barriers. Self-pollination is ruled out by mechanical factors preventing the transfer of pollen to the stigma. In addition, six of the species were found to be obviously capable of vegatative reproduction. Pogonia ophioglossoides exhibited a pattern indicating either some form of cleistogamy or a form of apomixis. Specific pollinating agents are given where observed.

A study was also carried out of the two varieties of Cypripedium calceolus in Michigan (var. pubescens and var. parviflorum) based on living plants and herbarium material. A classical

morphological analysis was used along with a statistical analysis of characters. In the area of study, at least, these two taxa were found to be behaving very much like two distinct species.

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By

Cathey Jo Newhouse

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GENERAL INTRODUCTION

Pursuing dual interests in the Orchidaceae and in pollination biology, I undertook a study of various aspects of reproductive biology in seven groups of Michigan orchids. In this study I attempted to determine the normal means of reproduction, the self-compatibility, and the pollinating agent for each taxon. I further studied Cypripedium calceolus in Michigan in order to find a method to readily identify infraspecific taxa on the basis of either living or herbarium material.

PART I. POLLINATION BIOLOGY IN SEVEN TAXA OF MICHIGAN ORCHIDACEAE

Introduction

Pollination biology of terrestrial orchids has long been debated and discussed, but relatively few people have studied this subject in detail. During the latter part of the nineteenth century several researchers made observations on different aspects of the pollination of some members of the Orchidaceae, but since that time, reports of such studies have been relatively few.

Calopogon tuberosus (L.) BSP.

Calopogon tuberosus (and not *Calopogon pulchellus*) is the correct name for this taxon (c.f. MacKenzie, 1935; Voss, 1966).

Calopogon flowers have long been a source of interest to naturalists. Because it is a widely distributed genus and is frequently encountered in bogs and marshes, it has been studied perhaps more than any other single group of terrestrial orchids in North America. Although it is not one of the groups studied by Charles Darwin (1862, 1877) or other early European investigators, it has been observed and studied by almost every one of the early researchers in North America.

Guignard (1886) offered a detailed description of the flower's structure but had a somewhat erroneous view of the manner in which pollination came about. It was his belief that the insect landed on the column and sucked from sweet juices found in a thickening of tissue

at the base of the lip. The pollinia, he thought, were attached to the insect's legs. Further, he thought that the insect, in struggling to get free of the flower, often self-pollinated the flower with the pollen on its legs.

By the following year, however, Charles Robertson (1887) was able to put forth a more accurate picture of the pollination mechanism in Calopogon. He observed that small bees, Andrenidae,

> "approaching the flower in front, light upon the crest [of hairs on the uppermost lip] when the labellum bends down suddenly so that the dorsal surface of the insect comes down upon the column. The broad, slightly upturned wings of the column keep the body from passing to either side, and so require it to slip off the end. In doing this the body strikes the stigma and is smeared with viscid matter. The pressure of the insect upon the stigma starts the anther from the pocket, so that the ends of the pollen masses are exposed. As the body slips off the end of the column the exposed ends of the pollinia strike the part which is smeared with viscid matter from the stigma, and the pollinia are drawn out and cemented to the exact spot which struck the stigma in the first place. When the insect visits another flower, the part to which the pollen is glued comes down upon the stigma.
> Cross fertilization results from the fact that the stigma is struck before the pollinia, from the startling action of the labellum, and from the fact that only two or three flowers are open [on one plant] at a time."

Robertson also reports that there is no nectar in the flowers.

This is a remarkably accurate description of the pollination mechanism. Since 1887 this same mechanism has been described by many other authors (Correll, 1950; Meeuse, 1961; van der Pijl and Dodson, 1966; Thien and Marcks, 1972; Thien, 1973; Dowden, 1975; and Luer, 1975). Thien (1973) adds that the entire sequence in the flower occurs within four seconds.

Figure 1a shows this flower with the crest of hairs on the lip. Figure 1b depicts the manner that the labellum would be bent forward by the weight of the insect.

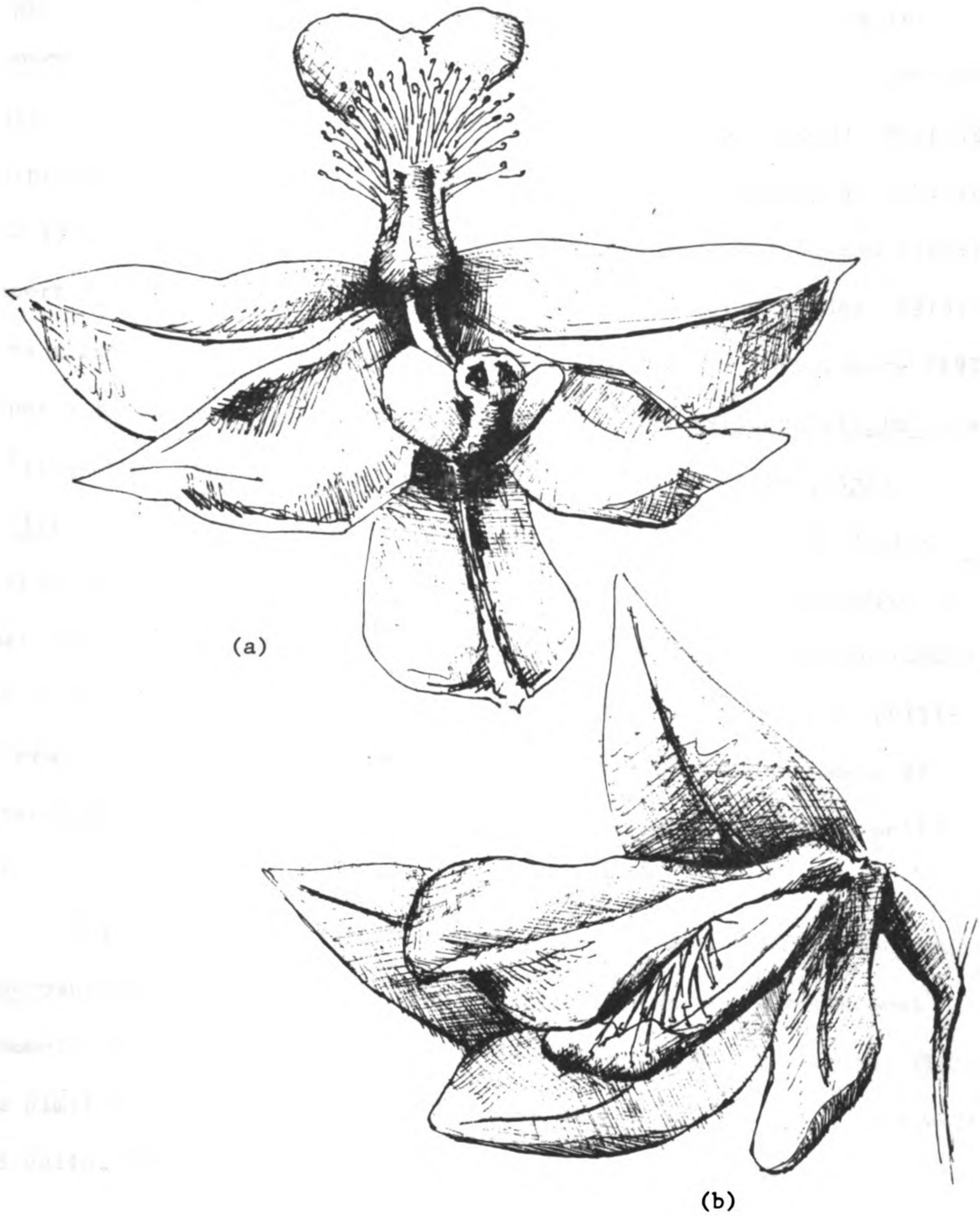

Figure 1. Calopogon tuberosus.

The pollinating agents reported for Calopogon have been many. Robertson (1887) reports bee species of Halictus (family Andrenidae), Augochlora (also Andrenidae), and Bombus (Apidae) pollinating the flowers (bending the lip). In addition, he reports other species of Halictus, and species of the families Vespidae (Hymenoptera), Syrphidae (Diptera), Papilionidae, and Hesperidae (both Lepidoptera) as visiting the flowers but not pollinating them. Van der Pijl and Dodson (1966) report Xylocopa micans (Hymenoptera) as a pollinator. Dowden (1975) also reports Xylocopa micans pollinating this plant. Stoutamire (1971) reports Bombus americanorum, B. grisecollis, and Xylocopa virginica as pollinators. Additional species of Bombus, the honey bee, Apis mellifera, the small orange skipper Thymelicus lineola, and Polites mystic (another skipper) are reported as pollinators of Calopogon by Luer (1975). Two species of Augochlora are reported by Meeuse (1961), and four species of Bombus are reported by Heinrich (1975) as pollinators. Thien and Marcks (1972) report both workers and queens of three Bombus species and of Megachile melanophea as Calopogon pollinators.

Thien and Marcks (1972) describe the similarities in Calopogon tuberosus and Pogonia ophioglossoides. These two species are most commonly found growing together and flowering at the same time. They are similar in color and in ultra-violet absorbance patterns (the white and yellow hairs on the lips of both species show very strong u-v absorbance patterns). Calopogon offers no food reward; Pogonia does have nectar. Presumably (according to Thien and Marcks) the insects in the area are searching for the nectar in Pogonia and happen to

pollinate Calopogon also. The area of the insect's body on which the pollen is deposited differs between the species, thus the two species are not hybridizing. Luer (1975) and Heinrich (1975) also refer to this phenomenon.

Pogonia ophioglossoides (L.) Ker.

S.H. Scudder (1862) was one of the first to study Pogonia ophioglossoides with regard to structure and mechanism of pollination. He gives an elaborate and accurate description of the flower's structure, then an equally elaborate description of the probable means by which an insect would effect fertilization. His visualization of this fertilization is as follows:

> "Flying to the flower intent upon its sweets, it [the insect] would alight upon the labellum, and, creeping in, would strike its head and back first against the protruding anther lid, only pressing it down more tightly, effecting nothing, and then against the stigmatic surface. The passage into the flower is narrow, allowing no room for anything but a very small insect to turn round in, so that no sooner does the insect draw itself backward, than the top of the back and of the head striking, as it almost infallibly must, against the front of the anther-lid (which at its upper portion projects forward somewhat, in order the more readily to catch the passing head), raises it more and more with its continued withdrawal, rolling the outer and under surface of the lid against the upper and front portion of the head of the insect, till it has passed, when the lid snaps back to its original position, leaving the pollen masses adhering to the upper portion of the front of the insect's head; -- or if only a portion of the pollen be removed, the lid, being closed again, is ready for the services of the next visitor. The insect flies to another flower, and, striking with the top of the head plump against the viscid stigmatic surface, leaves the pollen glued to it, and thus fertilization is ensured."

Scudder also reports that several aspects of the flower's structure aid in this mode of cross fertilization by insects. Examples of these aids

to fertilization that he details include anther structure, shape of the column, and the form and position of the beard on the labellum.

See Figure 2.

Of Pogonia ophioglossoides, Charles Darwin (1877) writes "Self-fertilisation seems to be effectually prevented; and the flowers on distinct plants must intercross..."

Baldwin (1884) gives an account of Pogonia ophioglossoides but it consists of merely a quote of Scudder's (1862) paper almost in its entirety.

In 1886 Guignard refers to Pogonia ophioglossoides. After detailing the structure, especially of the column, of the Arethuseae (containing Arethusa, Calopogon, and Pogonia), he describes the pollination mechanism. Again, much of this seems to be a recapitulation of Scudder's (1862) work, although Guignard does add

> "the insects must evidently be rather small to be able to pass under the column, and, further, must be winged, for entrance by creeping up the stem and sides is prevented by the petals and sepals, while, on the other hand, the lip spreads forward, carpeted with fringes, as a most convenient alighting place for winged visitors."

Niles reported in 1904 that "Self-fertilization seems impossible for the Rose-colored Pogonia... The plants must intercross." And in 1905, W.H. Gibson describes the structure of the rose pogonia and "the contrivance to protect this orchid from fertilising itself and to insure cross fertilisation." The method of pollination so described by Gibson (1905) resembles very closely that reported by Scudder (1862). In addition to the mechanism of pollination, Gibson also describes (in

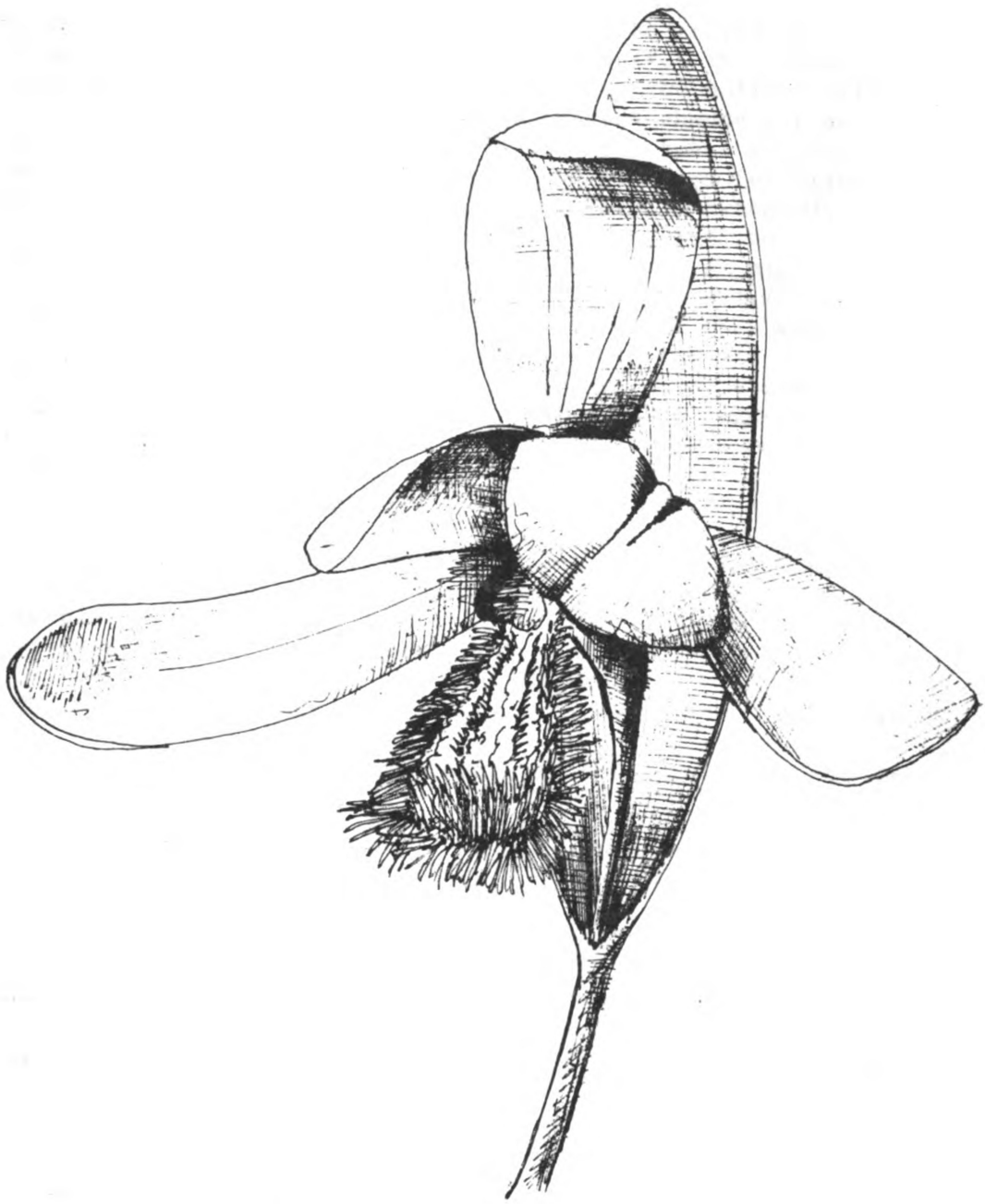

Figure 2. Pogonia ophioglossoides.

quite a teleological manner) some work done by Darwin[1]:

> "A British cousin [of Pogonia; the "British cousin" is not identified] that Darwin experimented with plainly shows by its actions that insect services could not be relied on... for it has become adapted to fertilise itself. The anther opens while the flower is in bud and pushes the pollen partly out, and then, before there is the slightest chance for an insect to come in, the pollen sends out long tubes toward the stigma that penetrate its tissue and begin to fertilise the ovules. Thus the welcome to the insects is extended, purely as a formal courtesy, it would appear, for the pollen mass is balanced in such a way as though it might fall at the slightest touch, exactly on its own stigma... [Darwin] covered four blossoms with a net while they were still in bud, and saw them open, and without the aid of any insect, produce capsules that looked as fine as any growing free. But on weighing the seeds of the capsules of the covered plants he found that they weighed more than one-third less than those from an equal number of capsules on uncovered plants. Moreover, under the microscope he discovered that the self-fertilised capsules had seven times as many bad seeds as those that were fertilised by the insects in the garden, proving that cross fertilisation did take place, but that the orchid had prepared itself for self-fertilisation in an emergency."

The odor or fragrance of Pogonia has been reported variously by many different authors. Guignard (1886) mentioned the "peculiar perfume" of the nectar; Baldwin (1884) describes an odor like that of violets, and in Gibson's 1905 book, both a smell "like sweet violets" and an "odour of red raspberries" are mentioned. A fragrance like raspberries has also been reported by Gibson (1901), Fuller (1933), Case (1964), and Luer (1975). Probably the most unusual reference to

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[1]Gibson gives no specific reference, only the name of Darwin. The work described (Darwin's) was not reported in The Various Contrivances by which Orchids are Fertilised by Insects (Second Edition, 1877), in On the Various Contrivances by which British and Foreign Orchids are Fertilised by Insects, and On the Good Effects of Inter-Crossing (first edition, 1862), nor in The Effects of Cross and Self Fertilisation in the Vegetable Kingdom (1895). Thus, I can only quote Gibson here.

this species' odor was that made by Henry David Thoreau[2] who likened it to a snake: "The adder's tongue arethusa [Pogonia ophioglossoides] smells exactly like a snake. How singular that in Nature, too, beauty and offensiveness should be thus combined." (Thoreau, 1884; also quoted in Correll, 1950 and Niles, 1904). Case states that one familiar with the odor of this plant can sometimes detect the presence of the plants before seeing them.

Oakes Ames (1948) observed that the tendency of the pollen of Pogonia ophioglossoides (as well as of Asiatic species of Pogonia) to germinate in situ is very common, and that pollen grains in dried material are often in masses held together by pollen tubes. Also along these lines, Thien and Marcks (1972) report that some plants of this species appear to be apomictic. In pollination experiments (performed by Thien and Marcks) it was found that Pogonia ophioglossoides flowers that were self-pollinated formed fruits that contained more than 90% fertile seeds (seeds with embryos).

Vegetative reproduction has been reported as an important means of propagation in Pogonia ophioglossoides. Stoutamire (1974) states "the formation of root buds is an efficient method of vegetative propagation in Pogonia ophioglossoides in eastern North America, where the wide-ranging root systems often develop numerous shoots..."; Correll (1950) reports that the species often forms large colonies because of the ease with which it spreads by means of stolons and

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[2]Thoreau made numerous references to the native orchids in his essays (notably "Summer", 1884). He frequently called the orchids by their scientific name and often described some aspect of the flowering in some detail.

rootshoots; Ames (1948) describes multiplication by means of root shoots in the species: "From the vertical rhizome root shoots extend widely in a nearly horizontal plane and eventually produce new plants"; and finally, Luer (1975) mentions the "frail, fibrous roots from which plantlets arise."

The manner in which the flower is pollinated by an insect has more recently been described by Thien and Marcks (1972) and Luer (1975). The mechanism described by these authors is essentially the same as that described by Scudder (1863). Luer (1975) indicates that the pollen is carried on the insect's back and Thien and Marcks (1972) report that the pollen of this species adheres to the head of the insect. The observations of the latter authors (Thien and Marcks) may have been more detailed and accurate since their study encompassed only three species and was strictly a study of floral biology, whereas Luer's book was a large one, encompassing all aspects of all species of orchids in North America.

Reports of insect visitors to Pogonia ophioglossoides have been few. Thien and Marcks (1972) report queens and workers of three Bombus species (B. ternarius, B. terricola, and B. vagans) visiting and pollinating this species.

Habenaria blephariglottis (Willd.) Hook

From an early time observers of the larger Habenaria (including H. blephariglottis) flowers realized that the nectar, which is contained in a very long spur, could only be reached by long-tongued insects such as some members of the order Lepidoptera.

In 1886 Guignard described the structure of Habenaria blephariglottis (along with other species of the genus) and speculated on the pollination: "The nectar can evidently be obtained by long-tongued Lepidoptera; the pendant lip shows that they must be hawk moths which feed without alighting." He also referred to Professor Gray's Botanical Textbook[3] in which a moth, Sphinx drupiferanum, is pictured carrying two pollinia of Habenaria orbiculata, a related large-flowered Habenaria.

Smith (1863) observed Habenaria psycodes (another of the "fringed" Habenaria's related to Habenaria blephariglottis). He gave a more detailed account of the actual pollination of these flowers. His observations indicate that moths (he reports two species of moths, one species of butterfly) suck the nectar while "poised on the wing." He states: "As the moth withdrew its proboscis from each flower, I could plainly see the pollinium pulled from the anther cell." There were many pollinia lined up along the probosces of the insects that he captured. In fact these insects "had their probosces so encumbered with the pollinia, that is was impossible for them to be coiled up between their palpi."

The insect would start at the bottom of the inflorescence and proceed spirally upward sucking nectar from each flower (Smith, 1863). Darwin reported a route similar to this one through an inflorescence of Spiranthes. Darwin further described a functional protandry within

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[3] I examined many of "Professor Gray's Botanical Textbooks" (different editions, different titles, etc.). The particular picture referred to by Guignard was not found.

the inflorescence; the very youngest flowers release their pollinia and only the older flowers (in which the pollinia have already been removed and the stigma has not yet been fertilized) have the stigma in a proper position to receive the pollinia (Darwin, 1877). It is not known for certain whether this functional protandry exists in Habenaria.

Another aspect of the pollination of Habenaria described by Darwin (1877) and Guignard (1886) and mentioned by Smith (1863), Gray (1862), and Baldwin (1884) is the movement of pollinia. At the base of the pollinia are viscid glands or disks. These disks adhere to some part of the insect's head so that the pollinia are in an erect or vertical position, which is approximately the position they occupied within the flower. If the pollinia remained in this position they would strike the front of the anther sacs and not effect pollination. In approximately one third to one half minute (roughly the time it takes the insect to get to another inflorescence) the caudicles or stalks of the pollinia bend forward bringing the pollinia into a proper position to contact the stigma of the next flower instead of the anther cells. This slight delay in the movement of the pollinia effectively prevents self-pollination (Guignard, 1886; Gray, 1862; Darwin, 1887; and others). Guignard (1886) and others say that the prompt movement of the pollinia when drawn out of the anther cells corresponds to the rapid flight of the moth darting from flower to flower; Smith (1863), however, does not agree. He states: "the shortness of the time occupied in the depression of the pollinia [in Habenaria psycodes], and the time that the insects remained at one plant, would seem to indicate that the upper flowers on the spike, at least, were fertilized by pollen from the same spike."

Gibson describes the structure of the flower in the genus and in the species (1901, 1905). Further, he (1905) details the pollination mechanism for the genus. He describes a moth[4] hovering in front of the flower; the space between the two pollen disks is exactly adjusted to the diameter of the insect's head. As the insect sips the nectar the sticky disks are brought into contact with the moth's eyes, to which they adhere. After a movement of the pollinia they are in a position (still on the moth's eyes) to contact the stigma of the next flower.

Gibson is the only author to report pollinia of Habenaria adhering to the moth's eyes. Dowden (1975), Smith (1863) and others report the pollinia adhering to the proboscis.

Grant (1951), van der Pijl and Dodson (1966), and other pollination biologists have described a syndrome of characters for flowers pollinated by moths. These characters are in agreement with the characters that Habenaria blephariglottis exhibits: nectar abundant, but deeply hidden in long, narrow tubes; white or cream colored flowers; landing place curved backward or turned upward, often deeply dissected; colored nectar guides absent, replaced by guidance through flower form (e.g., fringe points indicating center of flower) and so forth. In addition, a characteristic "moth flower" feature is the presence of a strong, sweet odor at dusk or dark, or, as Brantjes (1973) puts it

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[4]Gibson (1905) state that the pollinator must be one of the smaller sphinx moths. The shape of the flower and the spur "clearly shuts out" the bees, butterflies, and smaller moths. Larger sphinx moths could sip the nectar and not effect pollination.

"the heavy, sweet fragrance, [is] mostly emitted with a pronounced nocturnal periodicity." The correlation of the floral characters with the moth-flower syndrome has led many authors to speculate that it was moth pollinated even before moths were actually observed as pollinators, or to use this correlation as additional "proof" of the pollinator relationship (Gibson, 1905 and 1901; van der Pijl and Dodson, 1966; Guignard, 1886; Faegri and van der Pijl, 1966; Knuth, 1909; and others).

Some of the specific pollinators that have been reported for Habenaria blephariglottis and related species are as follows: clear-wing moth (Hemaris thysbe) reported by Dowden (1975) for H. psycodes; a sphinx moth (Sphinx drupiferanum) reported by Guignard (1886) and Sphinx sp. by Gibson (1905); small hawk-moths, mountain silver-spot butterfly (Speyeria atlantis), and a skipper (Polites mystic) all reported in Luer (1975); and two species of Sesia (moth) and Papilio asterias (butterfly) reported by Smith (1863).

Case (1964) describes a kind of vegetative propagation for Habenaria:

> "On one of the roots each season is produced a bud that will become next season's plant. Adjacent to the existing plant and during its first year, this bud develops a new set of roots or tubers. At the end of the season the old plant degenerates leaving the newly formed bud and roots to propagate the plant."

Cypripedium

In the subfamily Cypripedioideae the flowers contain two fertile anthers rather than one, and these are located near the base of the column rather than near its apex (Guignard, 1886; Case, 1964; Luer, 1975; others). In addition, most orchids outside the Cypripedioideae

have a viscid stigma that is more or less concave and pollen that is united into dry masses. However, Webster (1898) states that, at least in the British Cypripedium species, the reverse of this is true. The stigma may be slightly convex and is non-viscid, and the pollen grains are "coated by and immersed in viscid fluid, which is so glutinous that it can be drawn out into short threads" (Webster, 1898; also Darwin, 1862 and 1877). Asa Gray (1862) disagrees somewhat with Darwin's (1862) description of the Cypripedium pollen: "In none of the North American species is the pollen 'so glutinous that it can be drawn out into threads'" (Gray, 1862), but he does agree that the stigma is not "smeared with glutinous matter, as in ordinary orchids." Instead, Gray says, the stigma is closely beset with minute, rigid, sharp-pointed papillae, all directed forward; these papillae, then, function to comb the pollen off the insect (Gray, 1862; Baldwin, 1884).

When observers first studied Cypripedium flowers it was concluded that self-pollination was impossible or at least unlikely in these flowers. This conclusion was reached because of the spatial separation of the anthers and the stigma (the anthers are located toward the base of the column, the stigma toward its apex) (Gray, 1862; Guignard, 1886; Stoutamire, 1967; Darwin, 1887; others). Other factors preventing self-fertilization (besides the spatial separation) have been described by Guignard (1886) ("The pollen is too glutinous to become detached spontaneously from the anthers, and moreover the stigmatic surface is directed downward as if to prevent anything falling on it.") and by Stoutamire (1967) (the one-way mechanism used by the insect usually prevents the pollen from being carried backward to the stigma.)

In 1862 (first edition[5]) Charles Darwin reported what he supposed to be the mechanism an insect would use in pollinating Cypripedium flowers. He had never observed an insect's behavior in connection with these flowers, however. In this initial report Darwin presumed that the only convenient passages that existed for the insect were "directly over and close outside the two lateral anthers." Darwin's experiments with a bristle which he used to pollinate the flowers led him to conclude

> "If an insect were thus to act, and it could hardly act in any other way, it would infallibly get its proboscis smeared with the glutinous pollen, as I found to occur with a bristle thus inserted. When the bristle smeared with pollen was pushed in by the little notch outside the anther, some of the glutinous pollen was generally left on the slightly convex stigmatic surface. The proboscis of an insect would effect this latter operation better than a bristle, owing to its flexibility and power of movement. Thus an insect would place either the flower's own pollen on to the stigma, or, flying away, would carry the pollen to another flower. Which of these two contingencies commonly occurs, will depend on whether the insect first inserts its proboscis directly over the anther, or outside by the little notch."

Also in 1862 Gray reported on the fertilization of Cypripedium. He says that the insect may fertilize the flower "in the way that Mr. Darwin supposes", and later, that an insect may enter by one of the lateral openings at the base of the labellum, but he was confident that the insects ordinarily enter the pouch

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[5]The first edition of Darwin's book was published in 1862 under the title On the Various Contrivances by Which British and Foreign Orchids are Fertilised by Insects, and On the Good Effects of Intercrossing. Darwin's 1877 book is the second edition of this work and has the title The Various Contrivances by Which Orchids are Fertilised by Insects.

> "by the front entrance, crawl under the ample face of the stigma as they feed, where they cannot well avoid rubbing their heads or backs against the stigma, and passing on, make their exit by one of the lateral openings which now become visible to them, almost inevitably carrying off pollen on their head or shoulders as they escape, which pollen they would convey to the stigma of the next flower."

In the second edition (1877) of Darwin's book, he retracted his original idea on Cypripedium pollination and agreed with that suggested by Gray. Since that time many people have observed and experimentally proved (by introducing an insect into the flower and watching its actions) that, indeed, the typical mechanism of pollination in this group has the insect entering the labellum by way of the larger, dorsal opening and exiting (after passing under the stigma) from beneath one or the other of the anthers (Gibson, 1901 and 1905; Guignard, 1886 and 1887; Dowden, 1975; Baldwin, 1884; Niles, 1904; and others).

The following section gives a more detailed account of the pollination sequence. The stimuli that attract the insect to the flower are not known for certain, but probably color and form are important initially, with odor becoming more important as the insect gets closer to the flower (Stoutamire, 1967; others). The insect first lands on the labellum and enters through the large dorsal opening (or in the case of C. acaule through the anterior slit) of the labellum. When inside the pouch of the labellum, the insect attempts to exit by the same way it entered but because of the turned-in margin of the opening and the very smooth inner surface (or in some cases, the downward-pointing hairs) near the opening, it is prevented from doing so. There are many trichomes and often contrasting colored lines along the

floor of the labellum. Some differences of opinion have arisen as to whether there is a food source in or among these hairs; this will be the topic of a later part of this discussion. At any rate, the hairs do help to orient the insect toward the base of the labellum and the exit, and to offer "traction" for the insect. The escape or exit route through the narrow base of the labellum forces the insect to first crawl under the stigma, where any pollen on the insect's dorsal surface is scraped off. After passing the stigma, the visitor can leave the flower through one of the openings on either side of the base of the column, but in so doing, it must force its way under one of the anthers where a new load of pollen is picked up. Variations on this theme occur in the different species. For example, the dimensions of the entrance and exit vary with different species, and thus are selective as to pollinator size. In addition, in the Cypripedium calceolus group there are several clear areas in the tissue near the base of the labellum; these presumably act as "light windows" and make use of the insect's positive phototropic response to help to draw it toward the base of the labellum and the proper exit. Also, it has been reported that a large and vigorous visitor is able to chew through the tissue and escape through this hole. Reports of various dead insects found inside the labellum also abound. Numerous authors have reported all or portions of this mechanism: Darwin, 1877; Faegri and van der Pijl, 1966; Meeuse, 1961; van der Pijl and Dodson, 1966; Fox, 1898; Guignard, 1886 and 1887; Stoutamire, 1967; Baldwin, 1884; Gray, 1862; Webster, 1898; Niles, 1904; Correll, 1950; Dowden, 1975; Luer, 1975; Gibson, 1901 and 1905; Rafill, 1913; Smith, 1863; Müller, 1883; Knuth, 1909; Dodson, 1966; others.

The presence of nectar secreted by or among the hairs lining the bottom of the lip has been reported and denied many times. Most of the earlier authors (Darwin, 1862 and 1877; Smith, 1863; Gray, 1862; Guignard, 1887; Müller, 1883; Gibson, 1901 and 1905; Baldwin, 1884; Knuth, 1909; and even Dowden, 1975) refer to the abundant nectar among the hairs, to droplets of nectar on the tips of the hairs, or to insects licking or grawing the nectar secreting hairs. Faegri and van der Pijl (1966) and to some extent van der Pijl and Dodson (1966) and Stoutamire (1967) deny the presence of any food in Cypripedium. Faegri and van der Pijl indicate that the gnawing or licking of the hairs reported by some is perhaps due to "emergency reactions" of the trapped insects. Other authors (e.g. Luer, 1975; Correll, 1950) say that there is probably no true nectar produced, but that viscid drops of fluid adhering to the hairs may have some function in luring the pollinators.

Stoutamire (1967) reports "the local species of Lady's slipper all produce odors which may orient approaching visitors." He also indicates that the main sources of odor are the lateral petals and sepals with the labellum producing a weaker odor and the central column producing practically no odor. Reports of odor in the various species have varied. If an odor is reported for C. acaule and C. reginae it is usually described as "sweet" (Luer, 1975; Stoutamire, 1967; Dowden, 1975; others). In C. calceolus (two varieties) reports range from both varieties having a strong spicy odor with no clear odor difference between plants of the two varieties (Stoutamire, 1967) to var. parviflorum being "sweetly fragrant" and var. pubescens mostly lacking a scent (Luer, 1975; many others). Gibson (1905) reports C. hirsutum

(= C. calceolus var. pubescens) with a "heavy oily odour".

Figure 3 shows a longitudinal section of a Cypripedium flower. Note the in-rolled margin of the opening and the hairs and colored markings on the inside floor of the pouch.

Specific pollinators will be discussed for each species, but some general reports for the genus will be mentioned here. Stoutamire (1967) states "The floral characters developed in most species of Cypripedium are those most attractive to bees, although flies and other insects also visit the flowers but do not function as efficient pollinators." According to Luer (1975), lady's slipper flowers are often pollinated by bees of the genus Andrena; and van der Pijl and Dodson (1966) say "the genus Cypripedium appears to be pollinated by several species of Andrena or Megachile."

Cypripedium acaule Ait.

Pollinators reported for Cypripedium acaule include Bombus vagans (Stoutamire, 1967), probably Andrena or Megachile (Dowden, 1975), and Bombus sp. (queen foraging before workers emerge) (Luer, 1975). In addition Stoutamire (1967) reports "numerous flies, several small bees, and one crab spider" as visitors but not effective pollinators.

Cypripedium reginae Walt.

The list of reported pollinators of this species includes Megachile melanophaea (Guignard, 1886 and 1887; Stoutamire, 1967; van der Pijl and Dodson, 1966; Dodson, 1966). Incidentally, the specific epithet of this insect's name was spelled "melanophaea", "melanophora", and "melanophea" in different references; I assume all are referring

Figure 3. Cypripedium, longitudinal section of flower.

to the same species. Also reported have been Megachile centuncularis (Guignard, 1886; Stoutamire, 1967), Anthophora terminalis (Guignard, 1886), an un-identified medium-sized black bee (Stoutamire, 1967), an un-identified small "humble-bee" (Rafill, 1913), minute flower beetles or Anthobium convexum (Guignard, 1886; Smith, 1863; Baldwin, 1884), and another beetle (Trichius affinis) (Guignard, 1886). In addition, Guignard (1886) reported several Lepidoptera visiting the flowers and entering the labellum but not normally pollinating the flower -- these are Pamphila cernes, Pamphila mystic, and Eudamus tityrus, and several smaller moths.

Cypripedium calceolus var. pubescens (Willd.) Correll

and

Cypripedium calceolus var. parviflorum (Salisb.) Fernald

Reports of pollinators for Cypripedium calceolus[6] have been several. Herman Müller (1883) listed visitors to the flowers and indicated the manner in which pollination occurs. The pollinators he reports are females of five species of the genus Andrena (A. nigroaenea, A. fulvicrus, A. albicans, A. atriceps, and A. pratensis). In addition, he listed several insects that visited the flowers but did not pollinate:

> "Andrena parvulao and several flies (Empis punctata, Cheilosia sp., Anthomyia sp., and Spilogaster semicinerea) were often found dead in the labellum. Small beetles (Meligethes) are often able to creep freely out of the labellum, but sometimes they are held fast by the sticky pollen and remain to perish."

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[6]Either no variety was indicated or, more often, the European C. calceolus (today recognized as var. calceolus) was the taxon in question.

Many authors later quoted or referred to Müller's report of five species of Andrena pollinating Cypripedium calceolus (Baldwin, 1884; van der Pijl and Dodson, 1966; Dodson, 1966; Stoutamire, 1967; others). Darwin (1877) reported this orchid to be pollinated by five species of the bee genus Andrena but did not list the species. Knuth (1909) also reported C. calceolus being pollinated by "small bees of the genus Andrena." (An interesting fact is that in several references the genus name Andrena was spelled three ways. I assume all are referring to the same genus of medium-sized solitary bees of the family Andrenidae.)

Guignard (1886) reported a dead Buprestid beetle (Antaxia inornata) in the lip of a C. pubescens (= C. calceolus var. pubescens) flower. He also found a dead Andrena nivalis in one of these flowers, and reported an Osmia vicina (a bee) captured live, smeared with pollen, on the lip of a flower. He writes in another place, of a "dipterous fly" (un-identified) imprisoned in the lip. Further, he refers to a "yellow spider that had possession of the lip and had spun some threads on it... This spider is frequently found in the flowers of this lady's slipper, and so must get in them sufficient prey to repay it."

Similar reports of yellow spiders in or on the flowers of this group have been given by various authors, including Stoutamire (1967) who said: "Flowers are visited frequently enough by insects to make it worthwhile for yellow crab spiders to exploit the situation."

Robertson (1928) reported visits to C. calceolus by two bees now included in the genus Ceratina.

Van der Pijl and Dodson (1966) repeat the reports of visitors to C. calceolus and C. parviflorum (= C. calceolus var. parviflorum) given

by several of the authors discussed above. In addition, they list a bee of the genus Zaodontomerus supposedly reported by Guignard in 1886 as a pollinator on C. parviflorum. I could find no reference to such a bee in Guignard's papers of 1886 or 1887.

Stoutamire (1967) reports collecting the following bees in flowers of C. calceolus: Ceratina species, Evylaeus, Lasioglossum, Agopostemon, Apis, Osmia, Halictus, and Dialictus. However, he states

> "My only observation of a complete pollination event in this species involved a population of the taxon pubescens... A male Ceratina calcarata approached the flower, quickly entered, explored the labellum floor for approximately 1 minute, and then forced its way out of the exit, carrying a pollen smear on its thorax."

Referring to C. calceolus (perhaps as well as other species in the genus) van der Pijl and Dodson (1966) state that "some myophilous [fly-pollinated] traits are found in Cypripedium". The examples they give of these traits are the brown sepals, hairs, light windows, and a trap-type labellum. In this regard, it is interesting to note that most of the pollinators reported for these orchids have been bees, not flies, and that most authors say that Cypripedium is bee-pollinated.

A summary of the pollinators reported for each species is given in Figure 4.

Figure 4. Insect visitors to flowers studied.

PLANT SPECIES	INSECT VISITOR	COLLECTED OR OBSERVED BY	DATE	REMARKS
CALOPOGON TUBEROSUS				
	Hymenoptera			
	Bombus separatus	Robertson	1887[7]	bending lip, not removing pollinia
	Bombus americanorum	Stoutamire	1971	pollinator
	Bombus grisecollis	Stoutamire	1971	pollinator
	Bombus ternarius	Thien & Marcks	1972	both queens and workers pollinated
	Bombus terricola	Thien & Marcks	1972	both queens and workers pollinated
	Bombus vagans	Thien & Marcks	1972	queen pollinated, worker did not
	Bombus (4 sp.)	Heinrich	1975	"unconditioned" pollinator
	Augochlora festiva	Robertson	1887	bending lip, removing pollinia
	Augochlora sumptuosa	Robertson	1887	with pollinia on 1st abd. segment
	Augochlora n. sp.	Robertson	1887	bending lip, removing pollinia
	Augochlora (2 sp.)	Meeuse	1961	pollinators
	Augochlorella sp.	Luer	1975	too small to bend lip
	Augochlorella striata	Newhouse	1975	pollinator
	Xylocopa micans	Dodson	1966	pollinator
	Xylocopa micans	Dowden	1975	pollinator
	Xylocopa virginica	Stoutamire	1967	pollinator
	Halictus spp.	Robertson	1887	bending lip and removing pollinia; 1 sp. not bending lip
	Apis mellifera	Luer	1975	effective pollinator

[7] Robertson's 1887 paper dealt with Calopogon parviflorus Lindl. (= C. barbatus), a species quite similar to C. tuberosus.

Figure 4, continued.

PLANT SPECIES	INSECT VISITOR	COLLECTED OR OBSERVED BY	DATE	REMARKS
	Megachile melanophaea	Thien & Marcks	1972	pollinator, queens worker did not poll.
	Ceratina sp.	Newhouse	1975	pollinator, 3 individuals captured
	Odynerus histrio	Robertson	1887	bending lip, no pollinia
	Diptera			
	Mesograpta marginata	Robertson	1887	not bending the lip
	Lepidoptera			
	Papilio philenor	Robertson	1887	not lighting
	Pamphila sp.	Robertson	1887	lighting on column
	Thymelicus lineola	Luer	1975	occasional pollinator
	Polites mystic	Luer	1975	frequent inefficient pollinator
	Orthopters			
	Tettigoniidae (immature)	Newhouse	1975	probably a predator or pollen forager
POGONIA OPHIOGLOSSOIDES				
	Hymenoptera			
	Bombus ternarius	Thien & Marcks	1972	both queens and workers pollinate
	Bombus terricola	Thien & Marcks	1972	queen pollinates
	Dialictus sp.	Newhouse	1975	pollinating
HABENARIA BLEPHARIGLOTTIS				
	Lepidoptera			
	Sphinx drupiferarum	Guignard (Gray)	1886	*H. orbiculata* pollinia on proboscis
	Sphinx sp.	Gibson	1905	*Habenaria* in general
	Sesia thysbe	Smith	1863	*H. psycodes*
	Sesia diffinis	Smith	1863	*H. psycodes*
	Hemaris thysbe	Dowden	1975	*H. psycodes*
	Papilio asterias	Smith	1863	*H. psycodes*, loaded with pollinia
	Polites mystic	Luer	1975	carried off pollinia, approached fl. from side

Figure 4, continued.

PLANT SPECIES	INSECT VISITOR	COLLECTED OR OBSERVED BY	DATE	REMARKS
	Speyeria atlantis	Stoutamire	1971	pollinator (*H. blephariglottis*)
	small hawk moth	Luer	1975	*H. blephariglottis* pollinator
	moth sp?	Knuth	1909	*H. ciliaris*
	Coleoptera			
	Stethobaris tubulatus	Guignard	1886	occasionally pollinate *H. psycodes*
	Orthoptera			
	Phaneroptera curvicanda	Smith	1863	feeding on nectar, not pollinating *H. psycodes*
CYPRIPEDIUM ACAULE				
	Hymenoptera			
	Bombus vagans	Stoutamire	1967	captured in labellum with *Cypripedium* pollen on thorax
	Bombus sp.	Luer	1975	pollinating (queen)
	Andrena sp.	Dowden	1975	probable pollinator
	Megachile sp.	Dowden	1975	probable pollinator
	several small bees	Stoutamire	1967	visitor, not effective pollinator
	Augochlorella striata	Stoutamire	1967	dead in trap put over flower, no pollen
	Anthophora furcata	Stoutamire	1967	dead in trap put over flower, no pollen
	Diptera			
	Rhingia nasica	Stoutamire	1967	dead in trap put over flower, no pollen
	Lepidoptera			
	Tetracis lorata	Stoutamire	1967	resting on labellum, not pollinating
	Class Arachnida, Araneida			
	crab spider	Stoutamire	1967	visitor, not effective pollinator

Figure 4, continued.

PLANT SPECIES	INSECT VISITOR	COLLECTED OR OBSERVED BY	DATE	REMARKS
CYPRIPEDIUM REGINAE				
	Hymenoptera			
	Megachile melanophaea	Guignard	1886	caught in lip, pollinator
	Megachile centuncularis	Guignard	1886	caught in lip, pollinator
	Bombus sp.	Rafill	1913	caught with thorax smeared with pollen
	Anthophora terminalis	Guignard	1886	caught in lip, pollinator
	Augochlorella striata	Newhouse	1975	pollinator
	Lepidoptera			
	Adopaea lineola	Newhouse	1975	visiting, probably only accidentally pollinates
	Pamphila cernes	Guignard	1886	entered labellum, unlikely pollinator
	Pamphila mystic	Guignard	1886	entered labellum, unlikely pollinator
	Eudamus tityrus	Guignard	1886	entered labellum, unlikely pollinator
	Diptera			
	Sericomyia chrysotoxoides	Newhouse	1975	pollinating
	Coleoptera			
	Anthobium convexum	Smith	1863	swarming over flower, occasionally pollinating
	Trichius affinis	Guignard	1886	caught in lip, unlikely pollinator
CYPRIPEDIUM CALCEOLUS (VAR. PUBESCENS & VAR. PARVIFLORUM)				
	Hymenopters			
	Andrena nigroaena	Müller	1883	pollinator
	Andrena fulvicrus	Müller	1883	pollinator
	Andrena albicans	Müller	1883	pollinator
	Andrena atriceps	Müller	1883	pollinator
	Andrena pratensis	Müller	1883	pollinator
	Andrena sp.	Knuth	1909	pollinator

Figure 4, continued.

PLANT SPECIES	INSECT VISITOR	COLLECTED OR OBSERVED BY	DATE	REMARKS
	Andrena parvula	Müller	1883	dead in lip, not pollinating
	Andrena nivalis	Guignard	1886	dead in lip, probably not pollinating
	Osmia vicina	Guignard	1886	captured on lip, thorax smeared with pollen
	Osmia sp.	Stoutamire	1967	dead in var. pubescens
	Ceratina calcarata	Stoutamire	1967	captured in flower, pollen on thorax
	Ceratina dupla	Stoutamire	1967	killed by crab spider
	Ceratina (2 sp.)	Robertson	1928	pollinators
	Apis mellifera	Stoutamire	1967	living in pubescens pollen smear
	Lasioglossum coriaceum	Stoutamire	1967	dead in pubescens heavy pollen smear
	Lasioglossum forbesii	Stoutamire	1967	dead in labellum
	Lasioglossum pilosum	Stoutamire	1967	living in pubescens, no pollen
	Agopostemon sp.	Stoutamire	1967	living, thoracic pollen smears
	Zaodontomerus sp.	van der Pijl & Dodson	1966	pollinator
	Evylaeus pectoralis	Stoutamire	1967	captured in pubescens, no pollen
	Halictus rubicundus	Stoutamire	1967	dead in pubescens, no pollen
	Dialictus sp.	Stoutamire	1967	living in pubescens, no pollen
Coleoptera				
	Antaxia inornata	Guignard	1886	dead in flower, probably not pollinating
Diptera				
	Empis punctata	Müller	1883	dead in lip, not pollinating

Figure 4, continued.

PLANT SPECIES	INSECT VISITOR	COLLECTED OR OBSERVED BY	DATE	REMARKS
	Cheilosia sp.	Müller	1883	dead in lip, not pollinating
	Anthomyia sp.	Müller	1883	dead in lip, not pollinating
	Spilogaster semicinerea	Müller	1883	dead in lip, not pollinating
	Eristalis dimidiatus	Stoutamire	1967	dead under stigma of *pubescens*, pollen on head and thorax
	Zodion fulvifrons	Stoutamire	1967	dead in *pubescens*, no pollen
	Mesograpta marginata	Newhouse	1975	probably an occasional pollinator
Class Arachnida, Araneida				
	yellow crab spider	Guignard	1886	a predator insect, not a pollinator
	yellow crab spider	Stoutamire	1967	a predator insect, not a pollinator
	yellow crab spider (probably *Misumena vatia*)	Newhouse	1975	a predator insect, not a pollinator

Methods and Materials

In order to perform crossing experiments on plants growing in their natural habitat, it is necessary to have a means to completely exclude insects from the plants, while not excluding the normal light and water. To fulfill these requirements, screened enclosures for the plants were devised. The fine-mesh screen was stapled securely to a wooden stake to form a cylinder. A top was formed for the cage by inserting the vertical wires of the cylinder (a few of the top horizontal wires were first removed) into the mesh of another piece of screen laid on top of the cylinder; these vertical wires were then bent down on top of the second piece of screen to form a tightly fitting, secure closing. The wooden stake, which protruded four to six inches below the cage, was driven into the ground near the plant so that the bottom of the screen was in complete contact with the ground. The cage was thus securely placed so as not to be blown by wind, etc.

With this system, an enclosure was made to "fit" the plant (slightly taller and larger in diameter than the plant); the enclosure was securely placed, effectively keeping out insects yet still providing for free air circulation and a full amount of sunlight and water. See Figure 5.

There are essentially five types of preliminary experiments and observations that I performed. These are 1.) enclosing plants with intact, unpollinated flowers (it was made certain that the pollen mass was intact and that there was no pollen present on the stigma before these flowers were used) to see if self-pollination, or autogamy, normally occurs; 2.) enclosing plants with unpollinated flowers from

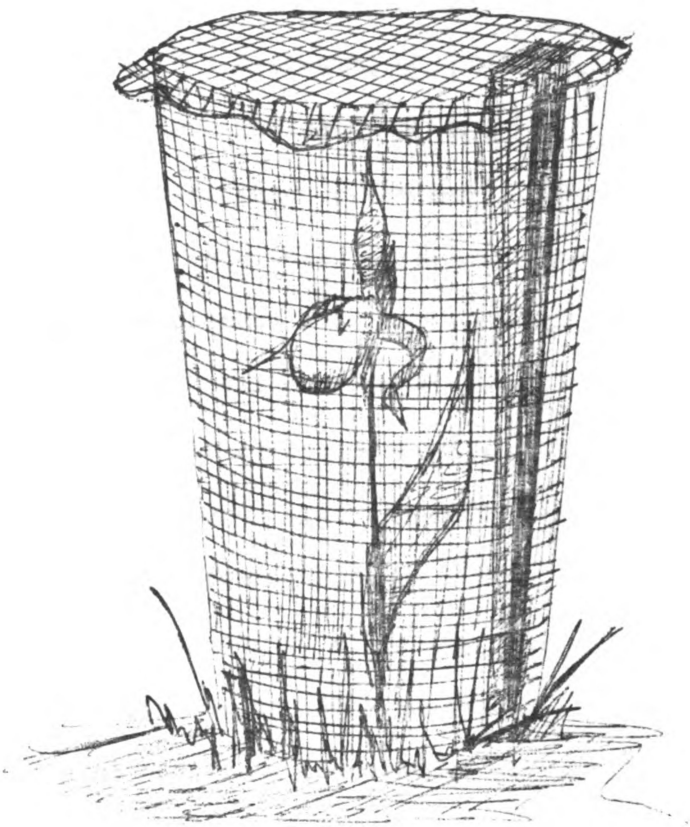

Figure 5. Apparatus used in insect exclusion experiments.

which all pollen[8] was removed to check for possible apomixis or cleistogamy; 3.) enclosing plants with flowers which I had artificially self-pollinated by placing a pollen mass on the stigmatic surface to see if the plants were self-sterile; 4.) enclosing plants with flowers from which I had removed pollen and had pollinated with pollen from a different flower of the same taxon to use as a "control" group of cross pollinated plants; 5.) observing root systems of the plants to determine if the plants are reproducing vegetatively. In addition crosses between taxa were performed in the yellow Cypripedium group.

After fruits were fully formed, but before they had dehisced, each enclosure was checked for the presence of fruits. Fruits from un-enclosed plants were collected as an overall control or basis of comparison for the fruits collected from within the enclosures. The experimental fruits were later examined and compared to the control fruits as to size of fruit, size of seeds, approximate number of seeds, appearance (including color) of seeds, and appearance and size of embryo, using a dissecting (30X) microscope. A seed was considered fertile or developed if it contained a developed embryo. Germination of the seeds was not attempted because of the mycorrhizal associations normally required in this family and because of the long development period of the plants.

Also, an arbitrary area of approximately $2m^2$ was chosen near the experimental plants in which all un-enclosed plants of the species

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[8]The whole anther (or anthers in the case of Cypripedium) was removed.

in question were counted and the number of plants with and without fruits were recorded. This provided a rough approximation of percentage of plants normally forming fruits in the population as a whole.

After the normal mode of pollination was determined for each group, observations were made to establish the particular agent or agents responsible for pollination and to verify the actual mode of pollination in each case. A standard insect net and killing jar were used to capture the pollinators. Later, the insects were pinned in a pinning box to preserve them for identification. Dr. R.L. Fischer of Michigan State University made determinations of the insects collected.

The insect exclusion experiments with the variously pollinated flowers were performed in the period of June through August of 1974 and repeated in June through August of 1975. The observations of pollinators and methods used by these pollinators were carried out in 1975.

The areas of study are as follows: (See Figure 6)

1. Calopogon tuberosus: southeastern Clinton County, Michigan (T5N, R1W, Sec 26), locality E on the map.
2. Pogonia ophioglossoides: southeastern Clinton County, Michigan (T5N, R1W, Sec 26), locality E on the map.
3. Habenaria blephariglottis: west central Barry County, Michigan (T3N, R9W, Sec 30), locality F on the map.
4. Cypripedium acaule: central Montmorency County, Michigan (T30N, R2E, Sec 12), locality C on the map and northwestern Ingham County, Michigan (T4N, R2W, Sec 35), locality D on the map.
5. Cypripedium reginae: northeastern Presque Isle County, Michigan (T34N, R6E, Sec 16), locality A on the map.
6. and 7. Cypripedium calceolus var. pubescens and var. parviflorum: northeastern Presque Isle County, Michigan (T34N, R6E, Sec 16), locality A on the map and north central Presque Isle County, Michigan (T35N, R5E, Sec 16), locality B on the map.

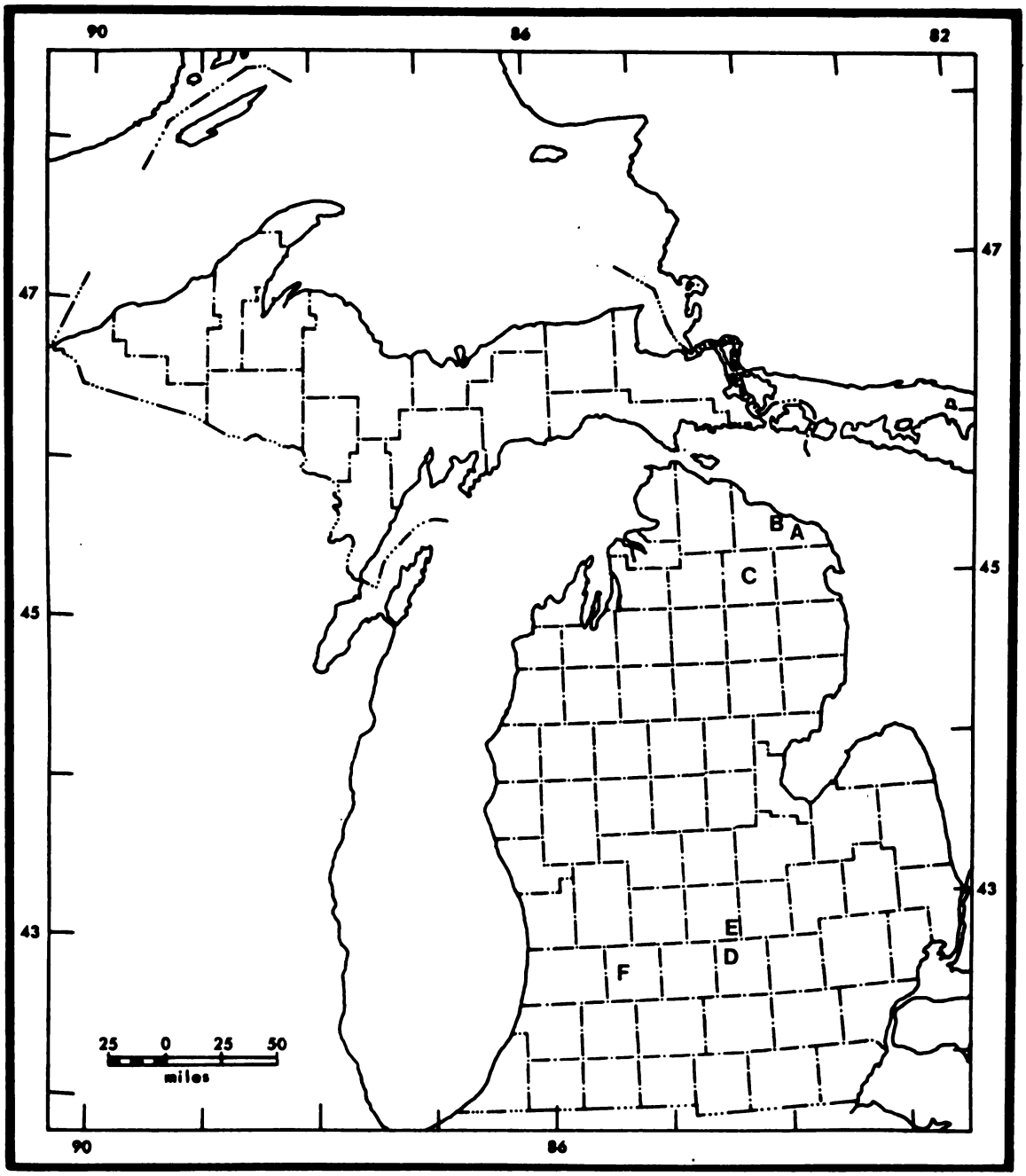

Figure 6. Map of Michigan showing locations of study areas. Species studied in each locality are as follows: Locality A - Cypripedium reginae, Cypripedium calceolus var. pubescens, and Cypripedium calceolus var parviflorum; locality B - Cypripedium calceolus var pubescens and Cypripedium calceolus var. parviflorum; locality C - Cypripedium acaule; locality D - Cypripedium acaule; locality E - Calopogon tuberosus and Pogonia ophioglossoides; locality F - Habenaria blephariglottis.

Results

Calopogon tuberosus (L.) BSP.

In category one (those plants that are enclosed with flowers intact and unpollinated) a total of 63 flowers on 16 plants[9] were used. When fruit set was examined later, not one of the 63 flowers had even begun to form fruit.

Of the five flowers on three plants in category two (those plants with unpollinated flowers from which the anthers were removed) no fruits were found later.

The six plants that were enclosed with artifically self-pollinated flowers (category three) included 13 flowers. Every one of the 13 flowers formed fruit that was indistinguishable from un-enclosed, normally pollinated fruits in size of fruit; size, color, and appearance of seeds and embryos; and approximate number of seeds per fruit.

In category four (plants that were pollinated with pollen of another member of the same species then enclosed) all seven flowers used set fruit that was also indistinguishable from un-enclosed fruit.

Un-enclosed fruits were very abundant; in an area ca. two meters square in the vicinity of the experimental plants, 17 fruits on seven plants were counted.

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[9] *Calopogon tuberosus* is a species with several flowers per inflorescence. When the number of flowers per number of plants is given, it is referring to the number of flowers used in the experiment. If a flower was already pollinated, had pollen masses removed, or was in bud or past anthesis, it was removed from the inflorescence and not considered in further experimental work.

When the root system was examined, it was found that plants growing close to each other were not apparently connected, although some plants had a pair of corms below ground rather than the usual one, and small buds were noted at the base of some corms.

Females of Ceratina (family Apidae) and Augochlorella striata (family Halictidae) were repeatedly seen acting as pollinators on the flowers of this species. In addition, many flowers were observed with part or all of the lip chewed off, and several others had holes elsewhere in the flower. An immature member of the family Tettigoniidae (Orthoptera) was observed repeatedly on the flowers, although this insect has apparently no pollination function. Bumble bees (Bombus, family Apidae) were seen to briefly hover in front of the flower but never to land on the flower and pollinate it.

Pogonia ophioglossoides (L.) Ker.

This species showed a pattern less straight-forward than in the other groups examined. Category one plants (enclosed with intact, unpollinated flowers) were seven in number, each with one flower. Of these seven, three showed no signs of setting fruit. The remaining four started to form fruits, but the development was arrested at some point; these fruits were smaller than the fruits of normal[10] or cross-pollinated fruits and were dark brown in color ("normal" fruits were a dark green color at the time of collection). The seed development within these smaller fruits was also considerably different: very few

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[10]The term "normal" fruits is referring to fruits that are indistinguishable in size, color, number of seeds, and appearance of seeds and embryo from those of the control or un-enclosed fruits.

seeds in the capsule had developed and hundreds of tiny, undeveloped ovules were visible in the capsule.

In the second category (unpollinated flowers with pollen removed) eight plants, each with one flower, were enclosed. Here there were five fruits that were normal in size and appearance characters described above, and three fruits with the smaller size, brown color, and few seeds also described above.

Category three consisted of nine self-pollinated flowers. Every one of these formed fruit indistinguishable from cross-pollinated fruits.

The three enclosed flowers that were cross-pollinated (category four) all formed normal fruits, as would be expected.

This species also had a high percentage of fruit set among unenclosed plants. A count in an area about one meter square revealed all of the eight plants visible bearing fruits.

Plants of this species that were growing close together had a connection between their root systems. More isolated plants were often seen to have a bud forming at the end of a long lateral root, which in turn grows from the plant's vertical rhizome.

A member of the bee genus Dialictus (family Halictidae) was observed pollinating this species.

Habenaria blephariglottis (Willd.) Hook.

There were 43 Habenaria blephariglottis flowers[11] on six plants enclosed intact and unpollinated (category one). None of the 43 flowers

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[11] Habenaria blephariglottis has several flowers per inflorescence. Number of flowers reported are those used in the experiment. Flowers that had pollen on the stigma, had pollen masses removed, were past anthesis, or in bud, were removed from the inflorescence.

formed fruits. Likewise, the 12 flowers on two plants in category two (unpollinated, pollen removed) formed no fruits. Twenty-nine flowers on seven plants formed category three (self-pollinated). In this category 23 flowers set apparently normal fruit, and six flowers formed no traces of fruit. In category four (cross-pollinated) nine flowers on two plants were used and all formed normal fruits.

This species showed a relatively low fruit set outside the enclosures. Only two of the five plants near the experimental plants were observed with fruits and these had only one and two fruits respectively.

An examination of the root system showed no evidence of interconnected roots or of vegetative reproduction.

No pollinators were observed in this species.

Cypripedium acaule Ait.

Eight plants (single flower per plant) were enclosed with intact, unpollinated flowers. None of these formed fruit of any sort. The two plants in category two (unpollinated, pollen removed) also showed no signs of fruit formation. The category three plants (four selfed flowers) formed fruit that was indistinguishable from control or cross-pollinated fruits. Two plants were enclosed after being cross-pollinated (category four); as expected, these formed normal fruits.

This species was studied in the northern part of Michigan (Montmorency County) and in the southern part of the state (Ingham County). Root systems were examined in each area. In Montmorency County the root systems of plants growing in proximity to each other

were connected by thin white rhizomes, and there were additional rhizomes with buds developing on the ends. In Ingham County, the plants examined appeared to be completely free from one another, and fewer rhizomes with shoot buds of the type described above were seen.

No pollinator was seen for this species in 1975. However, the weather during the flowering period of these plants was cool and overcast; no active insects of any kind were observed at this time. Later, when fruit set was examined it was found that very few un-enclosed plants (fewer than 10%) of this species formed fruits in 1975. Several flowers were seen to have medium-sized holes chewed in front or side of the lip.

Cypripedium reginae Walt.

For Cypripedium reginae, seven plants were enclosed with intact, unpollinated flowers (category one). Not one of the seven formed fruit. In category two (unpollinated, pollen removed) two flowers were used; these did not set fruit. Five flowers were selfed (category three); all five set fruit that was apparently normal. As would be expected, the two plants that were artifically cross-pollinated also set normal fruit.

Fruit formation in un-enclosed plants was relatively high. Of the nine plants counted in the immediate vicinity of the experimental plants, seven formed fruit.

Root systems were examined; plants growing near each other had roots interconnected by thin rhizomes, and vegetative buds were apparent on the rhizomes.

Several types of insects were seen visiting this species. Those that were captured include flies of the species Sericomyia chrysotoxoides (family Syrphidae), Augochlorella striata (a bee of the family Halictidae) and the European skipper, Adopaea lineola. Sericomyia and Augochlorella were probably the most effective of the pollinators observed.

Cypripedium calceolus L. var. pubescens (Willd.) Correll

Seven flowers were used in category one (intact, unpollinated) for this group. None of the seven formed fruits. Nor did the two flowers used in category two (pollen removed from unpollinated flowers). The three flowers that were selfed (category three) did set fruit, all apparently normal. Two flowers were pollinated with pollen from another member of the same variety (category four), and these both formed normal fruits. In addition, two flowers were pollinated with pollen from C. calceolus var. parviflorum. These two formed fruits indistinguishable (even in size) from those of plants cross-pollinated with pollen of var. pubescens.

Fruit formation outside the enclosures was relatively low. Only three of the 15 plants counted in the experimental area had fruits.

Upon examination of root systems in this variety, it appeared that close-growing plants do have interconnected rhizomes, and buds were visible on the rhizome ends. More widely spaced plants (the most typical case for this variety) had buds apparent on the ends of rhizomes but were not interconnected.

A syrphid fly of the species Mesograpta marginata was observed and captured while trapped in the labellum of the flower (presumably,

before it was able to escape and pollinate the flower). A yellow spider (probably Misumena vatia) was observed frequently on different flowers of this variety, but was not observed to have any pollination function.

Cypripedium calceolus L. var. parviflorum (Salisb.) Fernald

Category one (enclosed, not pollinated) of the experiments in this group made use of three flowers, none of which formed fruit. Category two (unpollinated, pollen removed) consisted of two flowers; neither set fruit. There were three flowers used for category three (selfed flowers). Two of these set normal fruits and one formed no fruit at all. The three plants in category four (artifically crossed with pollen from another member of variety parviflorum) all formed normal fruits. There were three more experimental plants used; these were pollinated with pollen of var. pubescens. All three formed fruits that were indistinguishable from other fruits of var. parviflorum or from those of var. pubescens.

The root systems that were examined were interconnected by rhizomes; furthermore, the members of this variety were observed very frequently growing in clumps of four to twelve or more plants all of which were interconnected by rhizomes. Vegetative buds were visible on the ends of additional rhizomes.

Pollinators were not observed for this group, but the weather was overcast and cool during the anthesis of this group; no insect activity of any kind was observed during this period. When fruit set was studied later, no fruits were found outside the screen enclosures;

the only fruits to be found were those on plants which had been artifically pollinated.

Discussion

Calopogon tuberosus (L.) BSP.

This species follows a pattern that seems to be found in many of the Orchidaceae: the plants do not self-pollinate but they are not self-sterile, i.e., they are prevented from self-pollinating by mechanical barriers but not by physiological or genetic barriers; thus they are herkogamous. Thien & Marcks (1972) reported that this species "appears to be self-incompatible." My observations do not support this.

The fact that the flowers that were enclosed intact and unpollinated formed no fruit indicates that the plants are not self-pollinating or autogamous. The emasculated flowers forming no fruit rules out cleistogamy or parthenogenesis as a normal means of seed formation; and the formation of normal fruits in the artifically self-pollinated flowers rules out any sort of physiological self-sterility. By examining the relative positions of the anther and stigma it can be seen that it would be impossible for the pollen to reach the stigma un-aided.

Fruits are very commonly formed in this species.

Several authors (van der Pijl and Dodson, 1966; Heinrich, 1975; Robertson, 1887; Dowden, 1975; Luer, 1975; Thien and Marcks, 1972; and others) indicate that there is no food reward for the insect in this group of orchids. Indeed, no nectar is detectable in the flower. Ackerman (1975) states that pollinators are more likely to make repeated visits to flowers offering a food source. Further, he indicates

that a low percentage of capsule set has been reported for orchids employing deception to lure pollinators. This does not seem to be borne out in Calopogon tuberosus, at least not in the area of Michigan where I studied this species. I observed a very high percentage of fruit set. Baldwin (1884) also reported that this species "rarely fails to perfect seed vessels." However, Thien & Marcks (1972) report a low (16%) capsule set for this species.

"The primary attraction mechanism appears to be the large masses of pink-red flowers" (Thien & Marcks, 1972). Furthermore, Heinrich (1975), Luer (1975), and Dowden (1975) indicate that the reason Calopogon gets pollinated frequently is that it grows with, and flowers at the same time as, species that do offer a nectar source (Pogonia ophioglossoides among others). Further, Calopogon and Pogonia flowers also have a very similar color and even a similar ultraviolet absorption pattern, especially on the lip (Thien and Marcks, 1972). It is purely accidental that the bee pollinates Calopogon as it is searching for Pogonia and the nectar (the insect carries the pollen masses of the two species on different parts of its body so that there is little likelihood that they would be crossed (Thien and Marcks, 1972). This may be the case, but the fact that non-pollinating insects visit Calopogon and are attracted directly to the crest of "psuedo-stamens" or hairs on the lip, and the fact that many Calopogon flowers show evidence of insects' chewing may indicate that there is a food source present in the flower, perhaps in or among the hairs or pseudo-stamens. In this regard, Heinrich (1975) attempted to extract nectar from flowers with capillary tubes and failed to obtain any. Van der Pijl and Dodson

(1966) indicate that the "hairs and 'glands' attracting bees to the vertical lip of Calopogon are perhaps edible, but appear to decieve the pollinator by their similarity to massed stamens." Robertson (1887) stated

> "There is no nectar. If there were any real source of attraction about the crest, small insects which are not heavy enough to depress the labellum would be the only ones to enjoy it undisturbed."

Yet, two pages later he reports several bee species visiting the flower and "not bending the lip".

It is interesting to note in this connection that both Verne Grant (1951) and van der Pijl and Dodson (1966) indicate that "bee flowers" have a sweet fragrance and nectar present. Reports of pollinators of Calopogon are almost entirely of various bees (Robertson, 1887; Dowden, 1975; van der Pijl and Dodson, 1966; Meeuse, 1961; Heinrich, 1975; Luer, 1975; Thien and Marcks, 1972; personal observations).

There must be a powerful attractant present in the flower (because of the high frequency of fruit formation). If this attractant is entirely deceit, it has been very efficiently evolved in this species of orchid.

In addition to a high percentage of fruit formation, the plants must be reproducing vegetatively to some extent as well. The pair of corms and additional buds observed suggest this. The second corm is probably for the next season's growth (Case, 1964) but the bud-like structures are probably additional corms (thus additional plants) being formed. Case (1964) states "Calopogon multiplies by offset corms as well as by seed..." Other authors (Carlson, 1943; Baldwin, 1884)

describe the formation of the first and additional corms and the development of one or more vegetative buds on these corms.

My observations support the idea (reported by numerous authors including Meeuse, 1961; Robertson, 1887; Baldwin, 1884; Dowden, 1975; and many others) that the uppermost lip is, or at least contains, the attractant, and that this lip acts like an elevator lowering the insect down onto the column; in sliding off the column, the insect slides past the stigma and then the anther, thereby effecting cross-pollination. I observed further that a small insect can land on the lip and not have the weight to tip the labellum forward, thus not pollinating the flower. It is probable that the flowers that were unpollinated but had parts of the flower chewed were visited and chewed by such small insects that are ineffective as pollinators. The small grasshopper (Tettigoniidae) observed may be an example of this, although this insect may be a predator on other insects, waiting "in ambush" for pollinators.

The bumble bees observed seemed to hover briefly in front of the flower but never to land on it. Perhaps these insects are searching for nectar and are attracted to the color pattern of the flower (including the u-v pattern described by Thien and Marcks in 1972) but are quick enough to notice its absence so that they don't land on the flower and pollinate it. Or, more likely, the particular bumble bees observed had been "conditioned" by previously visiting a flower of Calopogon. Heinrich (1975) states that the non-rewarding flowers are visited by unconditioned pollinators, and that bumble bees are conditioned rapidly.

Pogonia ophioglossoides (L.) Ker.

Pogonia ophioglossoides is a species for which the pollination biology is somewhat difficult to explain. The fact that apparently unpollinated[12] intact flowers and apparently unpollinated plants with anthers removed from which insects were excluded still were able to form fruit suggests different possibilities. One is that the plants are apomictic. The second possibility that comes to mind is that they exhibit a form of cleistogamy, i.e., the pollen tube grows from the anther through the column to the ovary to fertilize the ovules without having to be transferred to the stigma by some other source. The reports by Oakes Ames (1948) that pollen germinating in situ is a very common occurrence in Pogonia ophioglossoides, by Thien and Marcks (1972) that some members of this species appear to be apomictic, and by Gibson (1905) that a "British cousin" of this species self-pollinates while in bud, lend much support to these ideas.

The fact that the fruits formed by the flowers that were not cross- or self-pollinated (i.e. the categories one and two in the experiments) were smaller, were of a different color, and had very few seeds developed indicates that this ability for apomixis or cleistogamy is not the typical or the only method of fruiting. Rather, this method of reproduction may act as an "insurance policy", assuring at least some seed set if no pollinator is available. Darwin's studies (reported in Gibson, 1905) gave similar results (see introduction of part I of this paper).

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[12]Apparently unpollinated: no pollen had been transferred to stigma and the anther and pollen mass inside were intact.

Apparently, vegetative reproduction is also a very important means of multiplying in this species. As discussed in the introduction, Stoutamire (1974) reports "the formation of root buds is an efficient method of vegetative propagation in Pogonia ophioglossoides in Eastern North America, where the wide-ranging root systems often develop numerous shoots..." and Correll (1950) states "the species often forms large colonies because of the ease with which it spreads by means of stolons or rootshoots"

The pollinator observed, a species of the bee genus Dialictus, may not be the only effective pollinator of this species. Queens and workers of the genus Bombus are reported pollinating P. ophioglossoides by Heinrich (1975) and Thien and Marcks (1972). The Dialictus species observed is much smaller in size than Bombus, so the mechanism used to pollinate the flower may be different with the Bombus pollinators.

The method used by the insects I observed was to first land upon the lip. The fleshy ridges and hairs on the lip guided the insect toward the base of the lip and the nectar. In backing out, the dorsal thorax of the insect came in contact first with the stigma (depositing any pollen it may have been carrying, thus effecting cross pollination) and then the anther, picking up additional pollen.

Several authors (Scudder, 1862; Luer, 1975; Baldwin, 1884; Guignard, 1886; and Gibson, 1905) report that the anther cap is situated in such a way that the insect actually closes it more tightly as it enters the flower; it is only when the insect begins to back out that the edge of the anther cap is lifted allowing pollen to stick to the dorsal part of the insect (the pollen is probably attached to the

dorsal, posterior surface of the insect's head (Thien and Marcks, 1972)). My observations support this explanation, but the pollen seemed to be left on the thorax of the insect rather than on its head. Furthermore, the bees I observed (Dialictus sp.) seemed about the right size to accomplish the pollination effectively. The Bombus pollinators would presumably be too large to completely enter the flower as the smaller bee did. This larger size probably accounts for the pollen being placed on the head of the insect instead of on the thorax as I observed.

Habenaria blephariglottis (Willd.) Hook.

No fruits being formed by the enclosed, intact flowers supports the idea that this species is normally not self-pollinating at least in Barry County, Michigan. Observations of the column and relative positions of the anther and the stigma also supports this; it would be very difficult, if not impossible, for the pollen masses to reach the stigma un-aided. The fact that the category two flowers (unpollinated, pollen removed) also formed no fruits shows that this species presumably is not normally cleistogamous or apomictic.

Most (about 80%) but not all of the flowers artifically selfed formed fruits that were apparently perfectly normal. This seems to indicate that the species is probably not self-sterile. However, some factor is present which prevents a few of the self-pollinated flowers from forming fruits. Perhaps there is functional protandry in these flowers, that is, perhaps the stigma is not receptive (or not sticky enough to hold the pollen mass until the pollen begins germinating) when the flower first opens.

This species is presumably pollinated by a nocturnal moth. The characteristics of the flower agree with those described by Grant (1951), Brantjes (1973), and van der Pijl and Dodson (1966) for the syndrome of moth-pollinated flowers, i.e., long, narrow nectar spur containing abundant nectar, white color, "landing platform" not horizontal and modified into a deeply dissected "showplace" or attracting mechanism, etc. I detected no strong odor, but this may be obvious only in the evening and at night if the flower is nocturnally pollinated; this, then, would also correspond with the "sphingophilous syndrome". Various moth species (including sphinx moths and small hawk moths) are reported as pollinators of this and related species of Habenaria (Platanthera in some literature). (Guignard, 1886; van der Pijl and Dodson, 1966; Smith, 1863; Luer, 1975). The mechanism of pollination used by the moth is described briefly in the introduction of this paper.

The plants were relatively few and widely scattered and no evidence of vegetative reproduction was noted. Existing plants are apparently able to overwinter by means of the underground rhizomes, but production of new plants is apparently very low whether vegetative or from seed. Case (1964) describes a form of vegetative reproduction but this seems to be more a means of overwintering than of producing additional plants. His description of the process is as follows: "On one of the roots each season is produced a bud that will become next season's plant. Adjacent to the existing plant, and during its first year, this bud develops a new set of roots or tubers. At the end of the season, the old plant degenerates, leaving the newly formed bud and roots to propagate the plant."

Cypripedium acaule Ait.

This species followed a pattern of experimental results very close to that described for Calopogon tuberosus. The plants do not self-pollinate, but there are no self-incompatibility barriers other than mechanical or spatial ones.

In northern Michigan this species seems to be freely vegetatively reproducing, while in Ingham County in southern Michigan, it showed no evidence of vegetative reproduction. It is possible that effective pollinators are more scarce further north, creating a selective pressure for other means of reproduction.

The lack of pollinators observed (observations were made in late May in Ingham County and in early June in Montmorency County) and the low degree of fruit formation suggest that 1975 was a poor year for pollination of this species. During the peak flowering periods of these plants very cool and overcast weather prevailed, and practically no insect activity of any sort was observed.

A very low percentage of fruit formation is apparently not uncommon for this species.

> "Although the Pink Moccasin-flower may be found in large colonies producing as many as one hundred flowers in a space less than seventy-five feet square, it is seldom that more than a dozen or so capsules will be produced in the colony. This low percentage of capsule formation and consequent loss of reproduction may be due to several factors, such as the lack of pollinating agents and too long a period between pollination and fecundation, during which time accidents may take place." (Correll, 1950).

Further, Case and Luer report that frost often prevents more capsules from ripening. In Michigan, Case reports, "the species blooms at a time when late but hard frosts will sometimes occur." His and Luer's

observations show that frost will cut down most scapes, even though the leaves seem little affected. Following a frost-free blooming season, hundreds of seed pods ripen in the larger colonies (Case, 1964; Luer, 1975).

Although I observed no insect visits to the flowers, there must have been some, since some plants did form fruits. Also, the flowers that had holes chewed in the front of the lip indicate that some insects were attracted to the flowers and entered the pouch, but couldn't (or didn't) find the exit at the base of the column. Instead of effecting pollination by leaving through this exit (at the base of the column), the insect chewed its way through the tissue of the labellum. These plants did not set fruit. This phenomenon (holes chewed in the labellum) was observed somewhat frequently. The chewing of holes in the labellum by the insect has been reported in the literature by various authors in several species of Cypripedium. Guignard (1887) captured bees and released them in flowers of Cypripedium reginae in order to watch the insect to determine how it traveled through the flower and how it pollinated the flower. Guignard reports "If introduced into a flower of small size, from which it could not force its way as before, the insect had a very quick method of regaining its liberty; it immediately began to bite and tear away the walls of its prison with its two powerful jaws or mandibles, and very soon enlarged the opening or cut a new hole."

Although I could not study the insect's actual mode of pollinating this species, I did study the flowers to determine the presumed pathway taken by the pollinator. First of all, there is no

horizontal dorsal opening to the pouch as in most species of Cypripedium. Instead, there is a vertical opening in the very front of the flower. The darker colored venation of the tissues around this opening probably serves as a series of guide lines or "nectar guides" for the insect, since they all converge around the opening. Inside the pouch, the contrasting venation is oriented toward the base of the flower; also a series of hairs in the bottom of the pouch probably helps to orient the insect toward the back or base of the flower and finally toward one of the exits on either side of the base of the column. As the insect moves to the base of the flower, it first contacts the comb-like tissue around the stigma, where any pollen is scraped off, then one of the anthers, where a new pollen load is picked up. The mechanism of pollination has been described by several authors (Niles, 1904; Correll, 1950; Dowden, 1975; Luer, 1975; and others) and agrees with the suggested mechanism described above.

Cypripedium reginae Walt.

This species is another that follows the pattern common to many of the terrestrial Orchidaceae in its breeding system. It is not an autogamous species, but it shows no self-sterility. Self-pollination is prevented only by mechanical or spatial factors preventing the pollen from reaching the stigma in the same flower.

Vegetative reproduction is apparently very common in this species, even though fruit formation is also high.

The insect lands on the outside of the lip and is attracted down inside. Once inside, the insect attempts to exit by the same way it

went in, but because of the in-rolled margins of the opening and the slippery inner surface it cannot do so. From here the process is very similar to that described for Cypripedium acaule: the contrasting venation and hairs on the bottom of the lip serve to guide the insect toward the base of the flower and to the exit on either side of the base of the column.

The European skipper and probably other insects are attracted to the flower but cannot function efficiently in pollination because of their size and shape. A lepidopteran visitor (such as the European skipper) may possibly remove pollen on its proboscis if it was inserted down one of the openings at the base of the column. This behavior was not observed, nor has it been reported in the literature, barring Darwin's original report, which he later retracted. Various authors report that small beetles are very commonly attracted to this species and occasionally pollinate it (Dodson, 1966; Guignard, 1886; Smith, 1863; Baldwin, 1884; others). I observed no beetles on these flowers.

Most often this has been reported to be a bee-pollinated species, and floral characters agree with the basic bee-syndrome. It is interesting to note that the types of flowers visited by long-tongued flies (such as the family Syrphidae) are essentially the same types of flowers that are visited by bees (Grant, 1951). One of the main pollinators I observed was a syrphid fly.

Cypripedium calceolus var. pubescens (Willd.) Correll

and

Cypripedium calceolus var. parviflorum (Salisb.) Fernald

These groups also followed much the same pattern as that shown by Calopogon tuberosus, Cypripedium acaule, and Cypripedium reginae, that is, the plants are not normally self-pollinating but show no barriers to autogamy except mechanical ones. Furthermore, there appear to be no physiological barriers to crossing between varieties; the apparent barriers to such inter-variety crossing are in size of pollinator and flowering time. This aspect of the group's biology will be discussed further in Part II of this paper.

Again, the plants of both varieties are capable of vegetative reproduction and it occurs frequently in both groups.

The yellow crab spider observed on flowers of var. pubescens was apparently a predator on insects and had no function in pollination. This yellow spider on flowers of C. calceolus (var. pubescens) was also reported by Guignard in 1886 (he observed a dead Buprestid beetle in the pouch of the flower overpowered by the yellow spider), by Stoutamire (1967), and by a colleague of mine (Dr. Garrett Crow, personal communication, 1975) who was able to get a picture of this yellow spider capturing an insect as it was leaving the flower. Borror and DeLong (1971) indicate that crab spiders lie in ambush for their prey, often on flowers. Further, they state "One of the most common species in this group is the goldenrod spider, Misumena vatia (Clerck), which is white or yellow...; this species can change color (over a period of several days) depending on the color of the flower."

Also, some insects were attracted to the flower and actually entered the pouch without being effective pollinators. Some very small flies were examples of this.

The mechanism of pollination in this group is much the same as that described for C. reginae. An exception is the presence of "light windows" in the tissue at the back of the labellum. These light windows have been discussed by several authors (see introduction).

Conclusions

Most of the species of Orchidaceae studied in Michigan fall into the general pattern of being non-self-pollinating, but non-self-sterile. Self-pollination is normally prevented by spatial factors, i.e., the pollen cannot physically reach the stigma of the same flower. The exception to this pattern of pollination biology was in *Pogonia ophioglossoides*. This species appears to be capable of apomixis or cleistogamy. In addition, vegetative reproduction by means of budding of rhizomes or underground organs of some type occurs in most species.

The general pattern that was shown from my studies very much supports the idea put forth by Knuth (1909): "Automatic self-pollination [in the orchids] only occurs as an exception, and is much more generally excluded by the relative positions of stigmas and anthers." However, these results clearly refute Darwin's (1877) contention (about orchids in general) that "pollen from their own flower is quite impotent and is even in some cases poisonous to the stigma."

PART II. A STUDY OF CYPRIPEDIUM CALCEOLUS IN MICHIGAN BASED ON LIVING PLANTS AND HERBARIUM SPECIMENS

Introduction

In the summer of 1974 when I began studying the pollination biology in Cypripedium calceolus I observed that there were two very distinct groups within the species. In both of my study areas in Presque Isle County, Michigan these two groups were growing sympatrically but they remained very distinctive. I realized that it was important to know exactly which taxon or taxa I would be studying, but noted that many of the common plant identification manuals differed widely as to their delimitations of the infraspecific categories in this species. For these reasons I undertook a project analyzing Cypripedium calceolus in Michigan.

A review of the literature shows that the yellow lady's slippers in North America have been a confused group taxonomically from as early as the beginning of the nineteenth century. Correll (1938) states:

> "Beginning in 1791 with Salisbury's first segregation of North American yellow Cypripedium from the Eurasian C. calceolus L., botanists have been confused as to the true status of our so-called species and varieties... Later when Willdenow (1804) established C. pubescens as being different from C. parviflorum...he opened the way for later botanists to invent numerous varietal and specific names for the yellow Cypripediums of North America..."

The yellow lady's slippers have been assigned and re-assigned taxonomic names and position. Practically every botanist who studied

the group has recognized species or varieties on the basis of different characters from those used before. In addition, botanists have been diametrically opposed as to their concept of which group contained which characters. An example of these opposing ideas is the "argument" that went on between P.A. Rydberg (who revised the Orchidaceae in the 1901 edition of Britton's Flora of the Northern States and Canada) and Oakes Ames along with other botanists (e.g., Gray and Hooker). Rydberg (1902) gives a "pale yellow lip flattened vertically" as a characteristic of C. pubescens and a "bright yellow lip flattened laterally" as a feature of C. parviflorum. Hooker and Gray (supported by Ames) thought the case was reversed. Rydberg argued that it was not reversed; and so it went.

Furthermore, one botanist's description of one species was occasionally accompanied by a sketch of what another botanist considered to be the other species. Also, it was often the case that one botanist would or would not recognize the species on the basis of just a few herbarium specimens (it is very difficult to see many of the apparently key characters on a dried, pressed plant) and with no or very little knowledge of living plants. Synonymy became a real problem in the group with so many botanists changing and rechanging the taxonomic status within the group. A list of synonyms of one form might fill two pages (see Correll, 1938). One botanist would say the forms appeared to intergrade, the next that they don't. One botanist would attribute a certain habitat or color or specific size range to one form, the next botanist would reverse the case and attribute the same habitat or color or size range to the other form. One species with

two varieties, two species -- back and forth. Correll (1938) has given an excellent, in-depth review of this long sequence of events; he concluded that all the North American yellow Cypripediums belonged to one polymorphic variety, Cypripedium calceolus var. pubescens.

A survey of some of the more recent opinions on the group's taxonomy follows. Several authors have continued to follow Correll (1938) in recognizing only one variety (Correll, 1950; Gleason, 1952; Gleason and Cronquist, 1963).

Fernald, in 1946, published the name Cypripedium calceolus var. parviflorum, thus recognizing a second variety of Cypripedium calceolus in North America. He reported differences in range and habitat and referred to the descriptions of the two groups given by Fuller (1933) (however, Fuller described the two groups as two species rather than varieties of a single species).

Besides Fernald himself (1950), other authors following his concept (or at least his nomenclature) of two varieties, pubescens and parviflorum of C. calceolus are Case (1964) and Luer (1975).

Additional recent concepts of the group fall into various schemes. Examples follow: Winterringer (1967) recognizes all yellow Cypripediums as Cypripedium parviflorum (apparently referring to C. calceolus var. parviflorum). He does not recognize the other group as occuring in Illinois. Voss (1972) recognizes the species then indicates that some authors refer the plants to one or two varieties. Smith (1966) states that two varieties of C. calceolus occur in Michigan, but that they intergrade so that it is frequently impossible to distinguish them.

To say that botanists disagree on whether the plants are of one or two varieties is a great simplification of the issue. This disagreement is hardly significant compared to the large amount of disagreement as to what characterizes the groups. The most conspicuous differences in various authors' concepts of the two groups are in range and distribution, color, flowering time, and specific ranges of measurements such as length of lip or height of plant. This section will be an inter-author comparison in some of these features.

Case (1964) treats var. pubescens as a widely distributed taxon, found in much of especially eastern North America and in most of Michigan, including the northernmost parts; var. parviflorum, on the other hand, he considers to occur only in a narrow band across the very southern portion of Michigan, southern Wisconsin and northern Illinois. Correll (1950), although he recognized only var. pubescens, indicated that var. parviflorum recognized by some is a relatively northern plant. Hultén (1958) indicated that both varieties cover Michigan in distribution, and that var. parviflorum has a somewhat wider range, stretching further north and considerably further west than var. pubescens. Fernald (1946, 1950) considered var. pubescens as a relatively southern plant reaching its northeastern limit in Maine and occurring mostly in the East and Midwest, appearing south from Georgia to Missouri, while he considered var. parviflorum to be a more northern plant (in contrast to the view of Case and Luer) reaching from Laborador to Northern British Columbia and south to the northern Midwest. Winterringer (1967) stated that C. parviflorum (= C. calceolus var. parviflorum) is more western in its range than the eastern C. pubescens (= C. calceolus var.

pubescens). Luer (1975) considers var. pubescens to have a wide range in eastern North America, covering Michigan, and continuing to western Canada, while var. parviflorum (according to Luer) has a more restricted range in the northeastern U.S., covering only the southern part of Michigan. Fuller (1933) gave a similar distribution for the two groups with var. parviflorum (his C. parviflorum) perhaps having a wider distribution especially in western North America, but he states that in Wisconsin, the parviflorum group is "apparently confined to the glaciated portions of the state" while the pubescens group is distributed throughout the state.

Comparative flowering time of the two groups is another point of difference among authors. Fernald (1950), Luer (1975), and Smith (1966) all indicate that var. pubescens tends to flower first, with some degree of overlap in times but most often with var. parviflorum flowering as pubescens fades. Case (1964) treated the two groups as flowering more or less at the same time. Fuller (1933) indicated a flowering time for var. pubescens that starts before and lasts longer than that of var. parviflorum.

Plant height is another point of some dissent among the various treatments of the group. There is usually overlap, but there is great variation in the exact placement of the "dividing line" between the groups. Often the heights given are somewhat arbitrary. Case (1964) indicated that var. pubescens is variable in height, being from 10 to 80 cm. tall, while var. parviflorum is between 15 and 35 cm. tall. Fernald (1950) described var. parviflorum with a height range of 15-55 cm. and var. pubescens with a range of 20-70 cm. Luer (1975) states

that var. pubescens is "up to 80 cm. tall" and var. parviflorum is "up to 35 cm." tall. Fuller (1933) gave 23-70 cm. as a range of height for var. pubescens and 19-40 cm. for var. parviflorum. Earlier descriptions were very arbitrary, e.g., in 1898 Fox describes C. hirsutum (= C. calceolus var. pubescens) as having a stem 17 cm. high and C. parviflorum (= C. calceolus var. parviflorum) with a stem 10-30 cm. high.

Lip length is still another character that is sometimes assigned arbitrarily and often varies from one description to another. Correll (1950) indicated that the lip of var. parviflorum "is arbitrarily considered to be 3 cm. or less long". Case (1964) gave an overlapping range of 1.5-6.5 cm. for var. pubescens and 2-3 cm. for var. parviflorum. Luer (1975) uses a similar but more restricted overlapping range: var. pubescens has a 2.2-6.5 cm. lip, and var. parviflorum has a 2-2.5 cm. lip length. Fernald (1950) indicated var. parviflorum has a 2-4 cm. lip and var. pubescens has a 3-5 cm. lip.

Other characters commonly used in descriptions vary somewhat with one or two authors but are generally agreed upon by most other authors. An example of this is the habitat. It is usually agreed that var. parviflorum occupies a wetter area than does var. pubescens, but the habitat described by some authors for var. pubescens may vary considerably. Also, most authors agree that var. parviflorum has lateral petals that are more strongly spirally twisted than those of var. pubescens, but Fuller (1933) suggested that var. pubescens has more strongly twisted lateral petals. Other such inter-author variations can be found in description of color, fragrance, number of leaves, size

of leaves, and other characters. See Figure 7 for a summary of this information.

Classical or Morphological approach

Because my studies on the reproductive biology took place in the summer when the plants were in flower, I thought it logical to carry on the Cypripedium calceolus study mainly during the remainder of the year.

I began making measurements on the herbarium specimens contained in MSC (Michigan State University herbarium). However, after making about 30 measurements on each of the 60-odd specimens in MSC, I realized I had a problem -- I could not separate the specimens into the two varieties, even with the aid of about 30 measurements! The two groups that seem perfectly distinct in the living state seemed to intergrade in their characters. After continuing the project into the summer and making measurements on living plants, I discovered that most of the very diagnostic features of the two groups are lost when the plant is pressed. For example, one of the most outstanding differences between the two taxa is in the color of the lateral petals, dorsal sepal, and markings inside the lip, with var. pubescens having lateral petals and sepals greenish in color streaked with brown-purple and a few light-purple markings inside the lip, and var. parviflorum having dark purple lateral petals and sepals and many dark markings inside the lip. When a plant is pressed (especially old specimens or those that may have been poorly dried) the very dark color seems to lighten and the light color to darken so color becomes practically useless as a

Figure 7. A comparison of author differences in Cypripedium calceolus characters. "PUB" is used for var. pubescens and "PARV" for var. parviflorum. In the column at the far right are the results of my own observations for comparison.

CHARACTERS	FULLER (1933)	CORRELL (1950)	FERNALD (1950)	CASE (1964)	LUER (1975)	OTHER	NEWHOUSE (CURRENT STUDY)
Range and distribution	the two have similar distributions, PARV wider distribution in the west but more restricted in Wisconsin	PUB: transcontinental PARV: more northern	PUB: relatively southern in east and midwest U.S. PARV: more northern, wider distribution	PUB: widely distributed, covers most of Michigan PARV: restricted only southernmost part of Michigan	PUB: wide range covers Michigan PARV: more restricted, only southern part of Michigan	HULTÉN (1958): PARV: wider range than PUB, both cover Michigan WINTERRINGER (1967): PUB: more eastern than western PARV	both varieties occuring in northern Michigan
Flowering time	PUB: starts flowering before and lasts longer than PARV		PUB: flowers first PARV: flowers as PUB fades	PUB and PARV flower about same time	PUB: flowers first PARV: flowers as PUB fades	SMITH (1966): PUB: flowers first with PARV flowering as PUB fades	PARV flowers first, few days of overlap, then PUB flowering
Height of plant	PUB: 23-70 cm. PARV: 19-40 cm.		PUB: 20-70 cm. PARV: 15-55 cm.	PUB: variable 10-80 cm. PARV: 15-35 cm.	PUB: up to 80 cm. PARV: up to 35 cm.	FOX (1898): PUB: 17 cm. FOX: PARV: 10-30 cm.	no significant average difference PUB: 21-47 cm. PARV: 18-39 cm.
Lip length		PARV: arbitrarily under 3 cm.	PUB: 3-5 cm. PARV: 2-2.5 cm.	PUB: 1.5-6.5 cm. PARV: 2-3 cm.	PUB: 2.2-6.5 cm. PARV: 2-2.5 cm.		PUB: 2.5-4.8 cm. PARV: 1.4-3 cm.
Degree of twist in lateral petals	PUB more strongly twisted than PARV		No difference in degree of twisting	PARV more strongly twisted than PUB	PARV more strongly twisted than PUB		PARV much more strongly twisted than PUB

character on herbarium specimens. In addition, on a pressed flower, it is not possible to see the markings on the inside of the lip. The degree of twisting of the lateral petals is also very characteristic (var. parviflorum has a much stronger twisting of the lateral petals than variety pubescens). It seems that some people think they should straighten out all flower parts when pressing. From a dried specimen it is difficult to tell whether the parts were straightened, had few twists to begin with, or even gained the appearance of more twists through wilting. Fragrance is yet another very distinctive feature -- it is very sweet and strong in var. parviflorum and absent or very weak in var. pubescens. Fragrance is hardly a useful character on herbarium specimens! It would seem that all the size measurements would still be measurable on a dried plant; most are, but a three-dimensional structure like the labellum can be pressed in so many ways that sometimes it is now known if length, height, or width is being measured. Most herbarium labels are not very specific in data such as moisture and degree of shade, both of which can be useful characters in the group. Pubescence is a character that remains measurable on a herbarium specimen, but it is often the case that a plant may be pressed and mounted so that a particular surface of a leaf where pubescence is usually measured most easily is not visible, or the leaves may conceal the particular area of the stem needed. So it is difficult to obtain consistent measurements on dried specimens of this character as well. I could give other examples, but those given adequately demonstrate the problem. Case (1964) stated the situation well: "I consider the variety [var. parviflorum] distinct, in some

populations so distinct that it could well be considered a separate species. Unfortunately, diagnostic features other than size become obscured when the plants are preserved."

In June, 1975, I studied populations of both varieties growing sympatrically in each of two different areas in Presque Isle County, Michigan. I made 21 measurements, some quantitative (sizes), and some qualitative (color, fragrance, and habitat), then calculated five ratios of combinations of some of these measurements (e.g., ratio of length to width of top leaf) for a total of 26 pieces of data on 30 plants for which I could readily determine the variety. A variable not used that would probably be useful (if an appropriate means of measuring it were devised) is the angle that the lateral petals form with the axis of the flower. It appears that the lateral petals in var. parviflorum are held much closer to horizontal than the more pendulous petals of var. pubescens.

For each variable measured (including ratios) I tested the null hypothesis of no difference in variable means between var. parviflorum and var. pubescens using Student's t-test. Seven of the variables showed no significant difference ($P > .05$) between the two varieties. The remaining 19 variables resulted in significant ($P < .05$), highly significant ($P < .01$), or very highly significant ($P < .001$) differences. The variables used and the degree of significance for each (only the 19 variables showing significance were used in the analysis) are shown in Figure 8.

Next, the measurements for each variable (within each variety) were tested for normal distribution because normal distribution of

Figure 8. The 26 variables measured in the Cypripedium calceolus study. The 19 variables showing a significant difference between varieties are marked with an asterisk. Also given are the degree of significance for each variable and special notes for some indicating how they were measured or coded.

VARIABLE	DEGREE OF SIGNIFICANCE OF DIFFERENCE	SPECIAL NOTES
*lip length	very highly significant	
*lip height	very highly significant	
*lip width	very highly significant	
*lateral petal length	very highly significant	
*lateral petal width	highly significant	
*number of twists in lateral petals	very highly significant	
*dorsal sepal length	significant	
*dorsal sepal width	highly significant	
*number of leaves	significant	
height of plant	not significant	measured to top of flower
length of top leaf	not significant	
width of top leaf	not significant	
*pubescence on flower	very highly significant	pubescence was estimated on a scale of 1-4 with 4 being the most pubescent. Hairs were glandular. Flower pub. was measured at the base or point of attachment of the perianth parts. Leaf pub. was measured from the middle of the top leaf, top surface. Stem pub. was measured midway between the top leaf and floral bract.
*pubescence on leaves	very highly significant	
*pubescence on stem	very highly significant	
*soil moisture	very highly significant	coded as follows: dry = 1, damp = 2, wet = 3
*degree of shade	very highly significant	sun = 1, mostly sun = 2, mostly shade = 3, shade = 4

Figure 8, continued.

VARIABLE	DEGREE OF SIGNIFICANCE OF DIFFERENCE	SPECIAL NOTES
*color of markings inside lip	very highly significant	the color characters were coded as follows: light = 1, medium dark = 2, dark = 3, very dark = 4
*color of lateral petals	very highly significant	
*fragrance	very highly significant	none = 0, faint = 1, strong = 2, very strong = 3
degree of scallop in lip outline	not significant	scalloped = 1, entire = 3
ratio length:width of lip	not significant	
ratio length:width of lateral petals	not significant	
*ratio length:width of dorsal sepal	highly significant	
*ratio length:width of top leaf	highly significant	
ratio height:width of lip	not significant	

variables is an assumption inherent in most statistical tests. The methods for determining normality using probability graph paper described by Dixon and Massey (1957) was used. It was found that every set of measurements showed a very close approximation of normality.

Figure 9 shows the means, observed ranges, and one standard deviation about the mean for the 19 variables used. Eleven of these variables are shown to have ranges that are somewhat overlapping between the two varieties. To quote from Davidson and Dunn (1966):

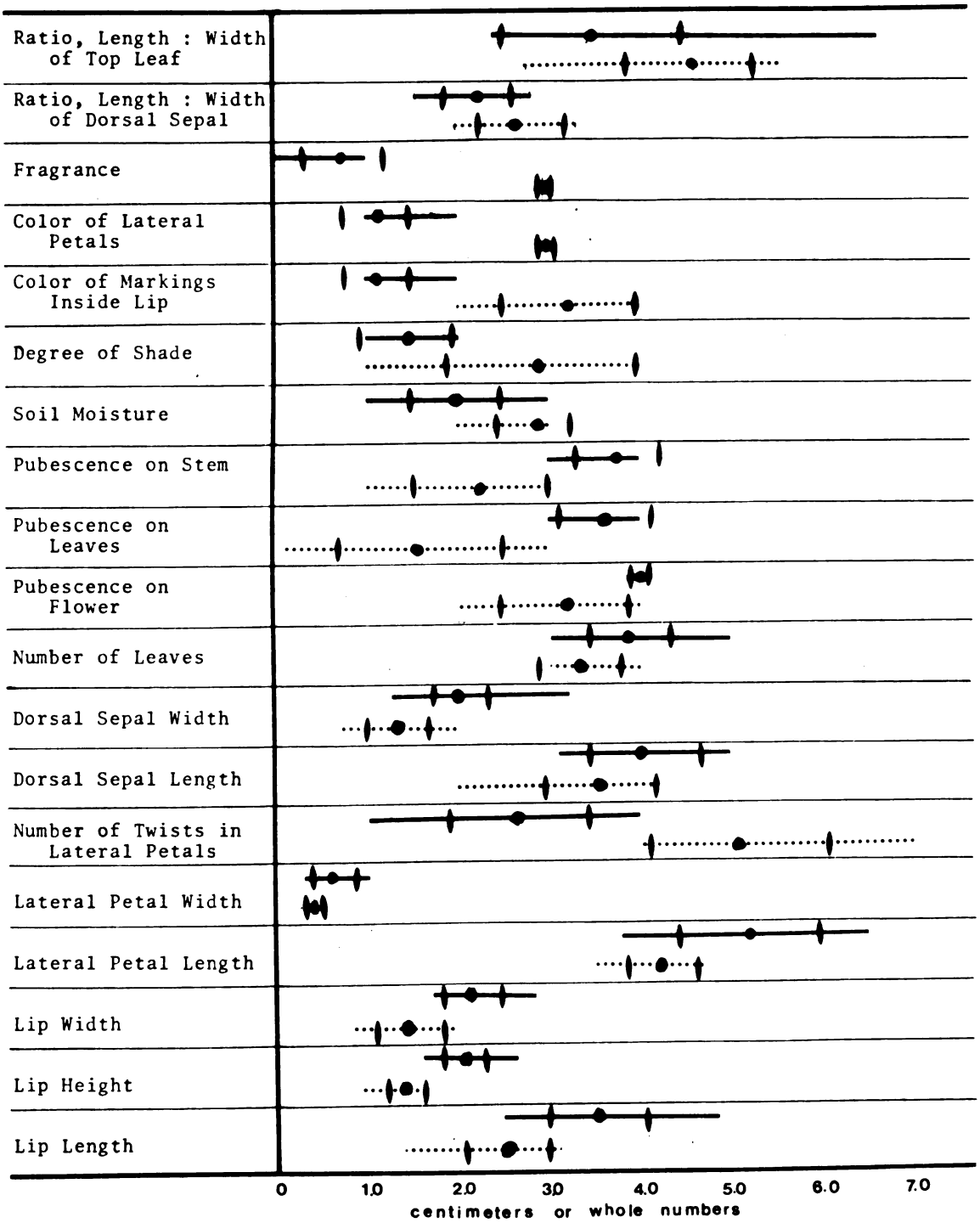
> "It should be obvious that no single character [of these eleven] would serve well in a description or key devised to discriminate between members of these taxa. However, if measurements within variables and populations are assumed to be normally distributed it should be apparent that any independent variable value would have a probability, p, of belonging to Γ1 [population 1 or variety 1] different from its probability of belonging to Γ2 [variety 2]."

Calculating the probability of a new or unknown specimen belonging to one taxon or the other is the subject of the next section of this paper.

It is interesting to note that the remaining eight variables, those that do <u>not</u> have overlapping ranges, in almost every case are those that are very difficult to measure on a dried herbarium specimen for the reasons explained previously. I cannot suggest an explanation for this observed phenomenon, but it does support the idea of the difficulty in using dried material!

Figure 9. Observed ranges, means, and standard deviations for each of the 19 variables used in the Cypripedium calceolus study. The means are represented by darkened circles, the observed ranges are represented by horizontal lines, and one standard deviation about the mean for each variable is indicated by vertical pointers. Only the variables that showed a significant difference between the two varieties and that were approximately normally distributed are given in this figure. The information for Cypripedium calceolus var. pubescens is indicated by (—●—) and that for Cypripedium calceolus var. parviflorum by (·●·).

Figure 9.


Ratio, Length : Width of Top Leaf
Ratio, Length : Width of Dorsal Sepal
Fragrance
Color of Lateral Petals
Color of Markings Inside Lip
Degree of Shade
Soil Moisture
Pubescence on Stem
Pubescence on Leaves
Pubescence on Flower
Number of Leaves
Dorsal Sepal Width
Dorsal Sepal Length
Number of Twists in Lateral Petals
Lateral Petal Width
Lateral Petal Length
Lip Width
Lip Height
Lip Length
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
centimeters or whole numbers

Statistical approach

As described above, Cypripedium calceolus in eastern North America can be readily divided into two[13] very distinctive groups on the basis of characters visible in living material. Unfortunately, many of the taxonomically useful characters are not measurable when using dried herbarium specimens. The useful characters that are observable on a dried specimen have ranges that are continuous within the taxa and overlap between taxa. Therefore, meaningful determinations of these taxa using herbarium material become extremely difficult.

A biometric or statistical approach to this type of problem was outlined in detail by Davidson and Dunn (1966). Some of the assumptions made in order to use this approach are as follows:

> "Two critical or sibling species (populations) have been suggested to exist by an investigator who can unfailingly assign certain individuals to one or the other taxon either by his intimate familiarity with perceptible patterns or by various special data (e.g. chromosomal, anatomical, and chemical information) at his disposal. All individual taxonomically useful characters known are continuous within taxa and overlap between taxa...
> Two or more populations (e.g. taxa)...which display a certain number of variables (characters) which can be adjudged homologous and measured in the same units between populations [exist]...

---

[13]For the purposes of this study, I am excluding the group of yellow lady's slippers recognized by some as Cypripedium calceolus var. planipetalum which occurs along the northern St. Lawrence River. Also excluded are the various hybrids between C. calceolus and C. candidum. The taxa I am dealing with have been treated variously but have been recently known as C. calceolus L. var. pubescens (Willd.) Correll and C. calceolus L. var. parviflorum (Salisb.) Fernald.

> It should be obvious that no single character would serve well in a description or key devised to discriminate between members of these taxa. However, if measurments within variables and populations are assumed to be normally distributed it should be apparent that any independent variable value would have a probability of belonging to population 1 different from its probability of belonging to population 2..." (Davidson and Dunn, 1966).

The following section will be a description of the Davidson and Dunn method and my application of it to the yellow lady's slippers.

First, two formal statistical restrictions are placed upon the characters used:

> "Restriction 1: Population distributions estimated by each within-sample set of measurements for each variable must be judged not different from normal at a predetermined significance level.
> Restriction 2: Population means estimated by each sample set of measurements for each variable must be different at a predetermined significance level." (Davidson and Dunn, 1966)

Twenty-six characters were used initially. Seven of these did not comply with restriction 2 so these were not used in further statistical analysis. For a list of variables used and a description of the methods used to check each variable against the two restrictions, the reader is referred to the "classical or morphological approach" section of this paper (Figure 8 and accompanying text). Therefore, it is assumed that two normal populations Γ1 and Γ2 (Γ1 = var. parviflorum in the sense of most recent classifications and Γ2 = var. pubescens) exist, each being r-variate (r is the number of variables used, in this case 19). From each of these populations random samples G1 and G2, can be drawn (G1 and G2 here are the samples of plants that I measured within each taxon). The size of each sample can be designated as G1n and G2n (G1n = 12 and G2n = 16). Davidson and Dunn denote the

variable measurements taken on each observation (an observation here is an individual plant) as $X_1,\ldots,X_r$ (r is the number of variables). The symbols $G1\bar{X}_j$ and $G2\bar{X}_j$ (j = 1,...,r) are used to denote the sample means and $G1s_j$ and $G2s_j$ (j = 1,...,r) to denote the sample standard deviations of the respective measurements. So that for each variable, X, there would be G1n (12) measurements with mean $G1\bar{X}$ and standard deviation G1s representing sample G1 from population Γ1 (var. parviflorum) and G2n (16) measurements with mean $G2\bar{X}$ and standard deviation G2s representing sample G2 from population Γ2 (var. pubescens).

The intervals $G1\bar{X}_j \pm 3(G1s_j)$ and $G2\bar{X}_j \pm 3(G2s_j)$ (where j = 1,...,19) (that is the intervals of three standard deviations about the mean for each of the nineteen variables measured) are considered next. For each variable the overall interval (the lowest of $G1\bar{X}_j - 3(G1s_j)$ and $G2\bar{X}_j - 3(G2s_j)$ to the highest of $G1\bar{X}_j + 3(G1s_j)$ and $G2\bar{X}_j + 3(G2s_j)$) is divided into a convenient number of intervals (I used 10-14 intervals) of equal length. Each of the interval limits are then transformed to standardized normal deviates (using the formula $Z = (Y-\bar{X})/s$ where Z = standard normal deviate, Y = the number in question, $\bar{X}$ = mean, and s = standard deviation) first using $G1\bar{X}_j$ and $G1s_j$ then $G2\bar{X}_j$ and $G2s_j$. The probability of occurence within each interval can then be determined for each sample G1 and G2 simply by reference to a table of areas under the normal curve. The table I used (Snedecor and Cochran, 1967) gives the areas from 0 to Z () so the appropriate addition or subtraction of areas was performed according to whether the standard deviates of

the interval limits were both positive, both negative, or one positive and one negative. The results of this can be seen in Table 1. In this table, the probabilities, p, with respect to $\Gamma 1$ (denoted $p(\Gamma 1)$) and to $\Gamma 2$ (denoted $p(\Gamma 2)$) that a measurement for variable 1 (lip length) will occur in the indicated intervals is shown. In addition, $1-p(\Gamma 1)$ and $1-p(\Gamma 2)$ are given. These figures (1-p) will be used later. Referring to this table it can be noted that if the distributions of variable 1 measurements are normal within both $\Gamma 1$ and $\Gamma 2$ and if sample means and sample standard deviations are valid estimates of the corresponding population parameters, then the probability of a value from $\Gamma 1$ in the exemplary interval 2.35-2.649 cm is .2598; likewise the probability of a value from $\Gamma 2$ in the same interval is .0380. To quote again from Davidson and Dunn (1966): "There is no theoretical or practical difficulty in transposing these concepts to suggest that an independent value in the interval [2.35-2.649 cm] from either $\Gamma 1$ or $\Gamma 2$ has a greater probability of belonging to $\Gamma 1$ than to $\Gamma 2$."

The probabilities with respect to $\Gamma 1$ and $\Gamma 2$ for the measurement intervals of variables 2-19 are found in Tables 2-19.

The above rationale for a case with one variable may be extended to a multivariate case by "suggesting that the probability of an independent multivariate normal observation, o, belonging to $\Gamma 1$ or to $\Gamma 2$ will be measured by combining probabilities associated with individual variables" (Davidson and Dunn, 1966). This combined probability is given by $CP(\Gamma 1) = 1 - \prod_{j=1}^{r} (1-p_j)$ where $CP(\Gamma 1)$ is the combined probability of an independent observation, o, with measurements $X_1,\ldots,X_r$ belonging to $\Gamma 1$, p_j is the probability of the observed measurements

Table 1. Probabilities with respect to $\Gamma 1$ and $\Gamma 2$ for the measurement intervals of variable 1 (lip length).

Measurement Intervals Lip Length (cm)	$p(\Gamma 1)$	$1-p(\Gamma 1)$	$p(\Gamma 2)$	$1-p(\Gamma 2)$
< 1.15	.0014	.9986	.0000	1.0000
1.15 - 1.449	.0085	.9915	.0000	1.0000
1.45 - 1.749	.0381	.9619	.0007	.9993
1.75 - 2.049	.1102	.8898	.0031	.9969
2.05 - 2.349	.2086	.7914	.0125	.9875
2.35 - 2.649	.2598	.7402	.0380	.9620
2.65 - 2.949	.2091	.7909	.0878	.9122
2.95 - 3.249	.1107	.8893	.1732	.8268
3.25 - 3.549	.0386	.9614	.2025	.7975
3.55 - 3.849	.0085	.9915	.2026	.7974
3.85 - 4.149	.0013	.9989	.1535	.8465
4.15 - 4.449	.0001	.9999	.0680	.9320
4.45 - 4.749	.0000	1.0000	.0382	.9618
4.75 - 5.049	.0000	1.0000	.0126	.9874
5.05 - 5.349	.0000	1.0000	.0031	.9969
$\geq$ 5.35	.0000	1.0000	.0007	.9993

Table 2. Probabilities with respect to $\Gamma 1$ and $\Gamma 2$ for the measurement interval of variable 2 (lip height).

Measurement Intervals Lip Height (cm)	$p(\Gamma 1)$	$1-p(\Gamma 1)$	$p(\Gamma 2)$	$1-p(\Gamma 2)$
< .810	.0013	.9987	.0000	1.0000
.810 - 1.009	.0201	.9799	.0002	.9998
1.010 - 1.209	.1334	.8666	.0018	.9982
1.210 - 1.409	.3418	.6582	.0134	.9866
1.410 - 1.609	.3435	.6575	.0591	.9409
1.610 - 1.809	.1343	.8657	.1599	.8401
1.810 - 2.009	.0205	.9795	.2655	.7345
2.010 - 2.209	.0013	.9987	.2629	.7371
2.210 - 2.409	.0000	1.0000	.1604	.8396
2.410 - 2.609	.0000	1.0000	.0594	.9406
2.610 - 2.809	.0000	1.0000	.0134	.9866
2.810 - 3.009	.0000	1.0000	.0018	.9982
$\geq$ 3.010				

Table 3. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 3 (lip width).

Measurement Intervals Lip Width (cm)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< .5	.0013	.9987	.0000	1.0000
.500 - .699	.0082	.9918	.0000	1.0000
.700 - .899	.0364	.9636	.0002	.9998
.900 - 1.099	.1012	.8988	.0013	.9987
1.100 - 1.299	.2038	.7962	.0074	.9926
1.300 - 1.499	.2574	.7426	.0291	.9709
1.500 - 1.699	.2131	.7869	.0800	.9200
1.700 - 1.899	.1160	.8840	.1579	.8421
1.900 - 2.099	.0415	.9585	.2212	.7788
2.100 - 2.299	.0097	.9903	.2114	.7886
2.300 - 2.499	.0014	.9986	.1580	.8420
2.500 - 2.699	.0002	.9998	.0804	.9196
2.700 - 2.899	.0000	1.0000	.0292	.9708
2.900 - 3.099	.0000	1.0000	.0075	.9925
3.100 - 3.299	.0000	1.0000	.0014	.9986
≥ 3.300	.0000	1.0000	.0002	.9998

Table 4. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 4 (lateral petal length).

Measurement Intervals Lateral Petal Length (cm)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 3.000	.0011	.9989	.0019	.9981
3.000 - 3.399	.0187	.9813	.0071	.9929
3.400 - 3.799	.1285	.8715	.0239	.9761
3.800 - 4.199	.3398	.6602	.0606	.9394
4.200 - 4.599	.3482	.6518	.1188	.8812
4.600 - 4.999	.1385	.8615	.1786	.8214
5.000 - 5.399	.0212	.9788	.2056	.7944
5.400 - 5.799	.0013	.9987	.1811	.8189
5.800 - 6.199	.0000	1.0000	.1223	.8777
6.200 - 6.599	.0000	1.0000	.0633	.9367
6.600 - 6.999	.0000	1.0000	.0250	.9750
7.000 - 7.399	.0000	1.0000	.0076	.9924
7.400 - 7.799	.0000	1.0000	.0017	.9983
≥ 7.800	.0000	1.0000	.0004	.9996

Table 5. Probabilities with respect to $\Gamma 1$ and $\Gamma 2$ for the measurement intervals of variable 5 (lateral petal width).

Measurement Intervals Lateral Petal Width (cm)	$p(\Gamma 1)$	$1-p(\Gamma 1)$	$p(\Gamma 2)$	$1-p(\Gamma 2)$
< .20	.0004	.9996	.0174	.9826
.20 - .29	.0295	.9705	.0362	.9638
.30 - .39	.3865	.6135	.0855	.9145
.40 - .49	.4332	.5668	.1498	.8502
.50 - .59	.0470	.9530	.1937	.8063
.60 - .69	.0004	.9996	.1852	.8148
.70 - .79	.0000	1.0000	.1283	.8717
.80 - .89	.0000	1.0000	.0683	.9317
.90 - .99	.0000	1.0000	.0262	.9738
1.00 - 1.09	.0000	1.0000	.0074	.9926
1.10 - 1.19	.0000	1.0000	.0016	.9984
$\geq$ 1.20	.0000	1.0000	.0003	.0007

Table 6. Probabilities with respect to $\Gamma 1$ and $\Gamma 2$ for the measurement intervals of variable 6 (number of spirals in lateral petals).

Measurement Intervals Number of Spirals in Lateral Petals (whole numbers)	$p(\Gamma 1)$	$1-p(\Gamma 1)$	$p(\Gamma 2)$	$1-p(\Gamma 2)$
< .20	.0000	1.0000	.0013	.9987
.20 - .799	.0000	1.0000	.0102	.9898
.80 - 1.399	.0001	.9999	.0518	.9482
1.40 - 1.999	.0009	.9991	.1533	.8467
2.00 - 2.599	.0054	.9946	.2677	.7323
2.60 - 3.199	.0231	.9769	.2746	.7254
3.20 - 3.799	.0697	.9303	.1663	.8337
3.80 - 4.399	.1476	.8524	.0591	.9409
4.40 - 4.999	.2204	.7796	.0125	.9875
5.00 - 5.599	.2307	.7693	.0015	.9985
5.60 - 6.199	.1701	.8299	.0001	.9999
6.20 - 6.799	.0884	.9116	.0000	1.0000
6.80 - 7.399	.0320	.9680	.0000	1.0000
7.40 - 7.999	.0082	.9918	.0000	1.0000
8.00 - 8.599	.0015	.9985	.0000	1.0000
$\geq$ 8.60	.0002	.9998	.0000	1.0000

Table 7. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 7 (dorsal sepal length).

Measurement Intervals Dorsal Sepal Length (cm)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 1.60	.0010	.9990	.0001	.9999
1.60 - 1.999	.0060	.9940	.0005	.9995
2.00 - 2.399	.0277	.9723	.0041	.9959
2.40 - 2.799	.0856	.9144	.0196	.9804
2.80 - 3.199	.1771	.8229	.0655	.9345
3.20 - 3.599	.2461	.7539	.1487	.8513
3.60 - 3.999	.2297	.7703	.2292	.7708
4.00 - 4.399	.1439	.8561	.2403	.7597
4.40 - 4.799	.0606	.9394	.1717	.8283
4.80 - 5.199	.0170	.9830	.0833	.9167
5.20 - 5.599	.0032	.9968	.0278	.9724
5.60 - 5.999	.0005	.9995	.0061	.9939
≥ 6.00	.0000	1.0000	.0010	.9990

Table 8. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 8 (dorsal sepal width).

Measurement Intervals Dorsal Sepal Width (cm)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< .300	.0008	.9992	.0001	.9999
.300 - .599	.0113	.9887	.0016	.9984
.600 - .899	.0782	.9218	.0106	.9894
.900 - 1.199	.2434	.7566	.0472	.9528
1.200 - 1.499	.3505	.6495	.1317	.8683
1.500 - 1.799	.2320	.7680	.2344	.7656
1.800 - 2.099	.0706	.9294	.2645	.7355
2.100 - 2.399	.0098	.9902	.1900	.8100
2.400 - 2.699	.0006	.9994	.0865	.9135
2.700 - 2.999	.0000	1.0000	.0251	.9749
3.000 - 3.299	.0000	1.0000	.0046	.9954
≥ 3.300	.0000	1.0000	.0006	.9994

Table 9. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 9 (number of leaves).

Measurement Intervals Number of Leaves (whole number)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 1.800	.0009	.9991	.0001	.9999
1.800 - 2.099	.0053	.9947	.0007	.9993
2.100 - 2.399	.0231	.9769	.0040	.9960
2.400 - 2.699	.0704	.9296	.0158	.9842
2.700 - 2.999	.1502	.8498	.0473	.9527
3.000 - 3.299	.2238	.7762	.1057	.8943
3.300 - 3.599	.2321	.7679	.1749	.8251
3.600 - 3.899	.1678	.8322	.2154	.7846
3.900 - 4.199	.0845	.9155	.1971	.8029
4.200 - 4.499	.0298	.9702	.1343	.8657
4.500 - 4.799	.0073	.9927	.0679	.9321
4.800 - 5.099	.0012	.9988	.0255	.9745
5.100 - 5.399	.0002	.9998	.0071	.9929
5.400 - 5.699	.0000	1.0000	.0015	.9985
≥ 5.700	.0000	1.0000	.0003	.9997

Table 10. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 10 (pubescence on flower).

Measurement Intervals Pubescence on Flower (whole number)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 1.000	.0013	.9987	.0000	1.0000
1.000 - 1.399	.0055	.9945	.0000	1.0000
1.400 - 1.799	.0213	.9787	.0000	1.0000
1.800 - 2.199	.0600	.9400	.0000	1.0000
2.200 - 2.599	.1250	.8750	.0000	1.0000
2.600 - 2.999	.1924	.8076	.0000	1.0000
3.000 - 3.399	.2187	.7813	.0000	1.0000
3.400 - 3.799	.1840	.8160	.0000	1.0000
3.800 - 4.199	.1143	.8857	1.0000	.0000
4.200 - 4.599	.0526	.9474	.0000	1.0000
4.600 - 4.999	.0178	.9822	.0000	1.0000
5.000 - 5.399	.0044	.9956	.0000	1.0000
≥ 5.400	.0009	.9991	.0000	1.0000

Table 11. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 11 (pubescence on leaves).

Measurement Intervals Pubescence on Leaves (whole numbers)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 1.00	.2598	.7402	.0000	1.0000
1.00 - 1.399	.1605	.8395	.0000	1.0000
1.40 - 1.799	.1753	.8247	.0001	.9999
1.80 - 2.199	.1579	.8421	.0020	.9980
2.20 - 2.599	.1166	.8834	.0175	.9825
2.60 - 2.999	.0711	.9289	.0837	.9163
3.00 - 3.399	.0358	.9642	.2183	.7817
3.40 - 3.799	.0148	.9852	.2995	.7005
3.80 - 4.199	.0050	.9950	.2394	.7606
4.20 - 4.599	.0014	.9986	.1008	.8992
4.60 - 4.999	.0003	.9997	.0231	.9769
5.00 - 5.399	.0001	.9999	.0029	.9971
≥ 5.40	.0000	1.0000	.0002	.9998

Table 12. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 12 (pubescence on stem).

Measurement Intervals Pubescence on Stem (whole numbers)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 0.00	.0014	.9986	.0000	1.0000
0.00 - .399	.0056	.9944	.0000	1.0000
.40 - .799	.0202	.9798	.0000	1.0000
.80 - 1.199	.0545	.9455	.0000	1.0000
1.20 - 1.599	.1123	.8877	.0000	1.0000
1.60 - 1.999	.1752	.8248	.0000	1.0000
2.00 - 2.399	.2086	.7914	.0013	.9987
2.40 - 2.799	.1878	.8122	.0154	.9846
2.80 - 3.199	.1290	.8710	.0920	.9080
3.20 - 3.599	.0670	.9330	.2584	.7416
3.60 - 3.999	.0265	.9735	.3429	.6571
4.00 - 4.399	.0080	.9920	.2148	.7852
4.40 - 4.799	.0018	.9982	.0634	.9366
4.80 - 5.199	.0004	.9996	.0088	.9912
≥ 5.20	.0000	1.0000	.0006	.9994

Table 13. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 13 (soil moisture).

Measurement Intervals Soil Moisture (whole numbers)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< .400	.0000	1.0000	.0010	.9990
.400 - .499	.0000	1.0000	.0049	.9951
.700 - .999	.0000	1.0000	.0203	.9797
1.000 - 1.233	.0000	1.0000	.0608	.9392
1.300 - 1.599	.0009	.9992	.1311	.8689
1.600 - 1.899	.0075	.9925	.2032	.7968
1.900 - 2.199	.0440	.9560	.2271	.7729
2.200 - 2.499	.1448	.8552	.1823	.8177
2.500 - 2.799	.2698	.7302	.1054	.8946
2.800 - 3.099	.2862	.7138	.0440	.9560
3.100 - 3.399	.1722	.8278	.0131	.9869
3.400 - 3.699	.0587	.9413	.0029	.9971
3.700 - 3.999	.0113	.9887	.0004	.9996
≥ 4.000	.0013	.9987	.0001	.9999

Table 14. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 14 (degree of shade).

Measurement Intervals Sun or Shade (whole numbers)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 0.00	.0035	.9965	.0025	.9975
0.000 - .499	.0092	.9908	.0305	.9695
.500 - .999	.0253	.9747	.1614	.8386
1.000 - 1.499	.0567	.9433	.3506	.6494
1.500 - 1.999	.1028	.8972	.3159	.6841
2.000 - 2.499	.1507	.8493	.1177	.8823
2.500 - 2.999	.1801	.8199	.0181	.9819
3.000 - 3.499	.1744	.8256	.0011	.9989
3.500 - 3.999	.1363	.8637	.0000	1.0000
4.000 - 4.499	.0870	.9130	.0000	1.0000
4.500 - 4.999	.0448	.9552	.0000	1.0000
5.000 - 5.499	.0186	.9814	.0000	1.0000
5.500 - 5.999	.0063	.9937	.0000	1.0000
6.000 - 6.499	.0018	.9982	.0000	1.0000
≥ 6.500	.0005	.9995	.0000	1.0000

Table 15. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 15 (color of markings inside lip).

Measurement Intervals Color of Markings Inside Lip (whole numbers)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 0.00	.0000	1.0000	.0005	.9995
0.000 - .399	.0001	.9999	.0164	.9836
.400 - .799	.0005	.9995	.1533	.8467
.800 - 1.199	.0027	.9973	.4145	.5855
1.200 - 1.599	.0110	.9890	.3303	.6697
1.600 - 1.999	.0342	.9658	.0771	.9229
2.000 - 2.399	.0807	.9193	.0051	.9949
2.400 - 2.799	.1451	.8549	.0001	.9999
2.800 - 3.199	.1976	.8024	.0000	1.0000
3.200 - 3.599	.2046	.7954	.0000	1.0000
3.600 - 3.999	.1610	.8390	.0000	1.0000
4.000 - 4.399	.0961	.9039	.0000	1.0000
4.400 - 4.799	.0436	.9564	.0000	1.0000
4.800 - 5.199	.0151	.9849	.0000	1.0000
5.200 - 5.599	.0039	.9961	.0000	1.0000
≥ 5.600	.0009	.9991	.0000	1.0000

Table 16. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 16 (color or lateral petals).

Measurement Intervals Color of Lateral Petals (whole numbers)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 0.00	.0000	1.0000	.0005	.9995
0.00 - .299	.0000	1.0000	.0074	.9926
.300 - .599	.0000	1.0000	.0540	.9460
.600 - .899	.0000	1.0000	.1919	.8081
.900 - 1.199	.0000	1.0000	.3304	.6696
1.200 - 1.499	.0000	1.0000	.2763	.7237
1.500 - 1.799	.0000	1.0000	.1123	.8877
1.800 - 2.099	.0000	1.0000	.0220	.9780
2.100 - 2.399	.0000	1.0000	.0021	.9979
2.400 - 2.699	.0000	1.0000	.0001	.9999
2.700 - 2.999	.0000	1.0000	.0000	1.0000
3.000 - 3.299	1.0000	.0000	.0000	1.0000
≥ 3.300	.0000	1.0000	.0000	1.0000

Table 17. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 17 (fragrance).

Measurement Intervals Fragrance (whole numbers)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 0.00	.0000	1.0000	.0467	.9533
0.000 - .299	.0000	1.0000	.1097	.8903
.300 - .599	.0000	1.000	.2108	.7892
.600 - .899	.0000	1.0000	.2620	.7380
.900 - 1.199	.0000	1.0000	.2107	.7893
1.200 - 1.499	.0000	1.0000	.1100	.8900
1.500 - 1.799	.0000	1.0000	.0372	.9628
1.800 - 2.099	.0000	1.0000	.0081	.9919
2.100 - 2.399	.0000	1.0000	.0012	.9988
2.400 - 2.699	.0000	1.0000	.0004	.9996
2.700 - 2.999	.0000	1.0000	.0000	1.0000
3.000 - 3.299	1.0000	.0000	.0000	1.0000
≥ 3.300	.0000	1.0000	.0000	1.0000

Table 18. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 18 (ratio of length to width of dorsal sepal).

Measurement Intervals Ratio length: width Dorsal Sepal (ratios)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 1.400	.0013	.9987	.0184	.9816
1.400 - 1.599	.0042	.9958	.0389	.9611
1.600 - 1.799	.0133	.9867	.0856	.9144
1.800 - 1.999	.0340	.9660	.1457	.8543
2.000 - 2.199	.0711	.9289	.1923	.8077
2.200 - 2.399	.1200	.8800	.1965	.8035
2.400 - 2.599	.1642	.8358	.1553	.8447
2.600 - 2.799	.1820	.8180	.0950	.9050
2.800 - 2.999	.1637	.8363	.0450	.9550
3.000 - 3.199	.1191	.8809	.0165	.9835
3.200 - 3.399	.0701	.9299	.0048	.9952
≥ 3.400	.0519	.9481	.0012	.9988

Table 19. Probabilities with respect to Γ1 and Γ2 for the measurement intervals of variable 19 (ratio of length to width of top leaf).

Measurement Intervals Ratio length: width Top Leaf (ratios)	p(Γ1)	1-p(Γ1)	p(Γ2)	1-p(Γ2)
< 0.00	.0000	1.0000	.0003	.9997
0.000 - .499	.0000	1.0000	.0011	.9989
.500 - .999	.0000	1.0000	.0052	.9948
1.000 - 1.499	.0000	1.0000	.0173	.9827
1.500 - 1.999	.0001	.9999	.0460	.9540
2.000 - 2.499	.0009	.9991	.0948	.9052
2.500 - 2.999	.0081	.9919	.1533	.8467
3.000 - 3.499	.0431	.9569	.1928	.8072
3.500 - 3.999	.1354	.8646	.1903	.8097
4.000 - 4.499	.2533	.7467	.1464	.8536
4.500 - 4.999	.2802	.7198	.0883	.9117
5.000 - 5.499	.1852	.8148	.0415	.9585
5.500 - 5.999	.0728	.9272	.0153	.9847
6.000 - 6.499	.0168	.9832	.0043	.9957
6.500 - 6.999	.0025	.9975	.0010	.9990
≥ 7.000	.0002	.9998	.0002	.9998

X_j belonging to $\Gamma 1$ and Π is the symbol indicating a multiplicative series in the manner that Σ indicates an additive series. Similarly, $CP(\Gamma 2) = 1 - \prod_{j=1}^{r} (1-p_j)$ is the combined probability of an independent observation belonging to $\Gamma 2$.

The following hypotheses associated with independent r-variate observations, o, can now be tested:

$$H = o \text{ belongs to } 1$$

$$H = o \text{ belongs to } 2$$

The hypothesis corresponding to the Γ for which $CP(\Gamma)$ is maximum is accepted.

In order to check the power or the probability of a correct decision involved in this method, a random sample was drawn from the observations made for which the taxa were known. A sample of 5 was taken from G1 and another sample of 5 from G2 using a random numbers table (Snedecor and Cochran, 1967) to choose the observations. I will call these samples G1.2 (meaning the second sample drawn from $\Gamma 1$) and G2.2. Table 20 gives the combined probabilities for each of these samples (each observation or plant in these samples was tested as an independent or unknown observation), the hypothesis chosen on the basis of this combined probability, and whether it was the correct decision. Exemplary measurement values and values for 1-p are given only for variables 1, 2, and 19 but all variables were used to figure combined probabilities. Note that the correct decision was made (the correct hypothesis was chosen) in every case. Results this conclusive actually weren't expected here because of the high degree of overlap of many of the character ranges; larger sample sizes might have given a better

Table 20. Exemplary variable measurements and probabilities, combined probabilities, and hypothesis chosen on the basis of the combined probabilities for 10 observations. Variable measurements are denoted $X_1, X_2, \ldots, X_{19}$; the individual probabilities (1-p) are denoted 1-p1, 1-p2, ..., 1-p19. Information for 5 observations from each of G1.2 and G2.2 is given. Two 1-p and CP values are associated with each observation; the first refers to Γ1 and the second to Γ2.

Sample[14]	Observation	X1	1-p1	X2	1-p2	... X19	1-p19	CP	Hypothesis Chosen	Correct ?
G1.2	01	1.4	.9915 1.0000	.9	.9799 .9998	5.30	.8148 .9585	.9551 .5217	H1	yes
	02	2.5	.7402 .9620	1.6	.6575 .9409	4.41	.7467 .8536	.9642 .9197	H1	yes
	03	2.8	.7909 .9122	1.4	.6582 .9866	4.53	.7198 .9117	.9877 .6221	H1	yes
	04	2.5	.7402 .9620	1.3	.6582 .9866	4.92	.7198 .9117	.9798 .6371	H1	yes
	05	2.3	.7914 .9875	1.6	.6575 .9409	4.50	.7198 .9117	.9752 .8871	H1	yes
G2.2	01	3.5	.9614 .7975	1.6	.6575 .9409	.304	.9569 .8072	.8171 .9696	H2	yes
	02	2.8	.7909 .9122	1.9	.9795 .7345	3.31	.9569 .8072	.6977 .9914	H2	yes
	03	2.5	.7402 .9720	1.8	.8657 .8401	4.12	.7467 .8536	.8889 .9825	H2	yes
	04	4.0	.9987 .8465	2.0	.9795 .7345	3.32	.9569 .8072	.6734 .9916	H2	yes
	05	3.4	.9614 .7975	2.2	1.0000 .8396	3.00	.9569 .8072	.4751 .9787	H2	yes

[14] Samples taken randomly from the original samples G1 or G2 rather than from Γ1 or Γ2 as a whole.

indication of power in this test. Even with a very large sample size I would still expect the power of this method to be relatively high. An additional way of checking the procedure is to examine the absolute combined probability difference, d ($d = |CP(\Gamma 1) - CP(\Gamma 2)|$ where $0 \leq d \leq 1$). The closer d is to one, the greater is the probability that a correct choice between hypotheses has been made; if $d = 0$ no decision is justified. An arbitrary lower limit can be set for d, below which the value is considered identical to $d = 0$. Such a limit set at 0.05 results in only one of the 10 observations justifying no decision. Therefore, even taking into consideration the very small d-values being equivalent to 0, the power in this procedure is still sufficiently high to justify its use. The d-values are given in Table 21 (column headed d_1).

As mentioned above, Davidson and Dunn extend this statistical method of identification into identifying independent or unknown observations as belonging to one or the other group. If the two groups of yellow lady's slippers in question are so readily distinguishable in the field, why should such a method be applied to these plants? The answer, I believe, lies in the difficulty in identifying herbarium specimens. If a dried specimen (which cannot be precisely identified by simple "eyeball" methods) is considered the unknown observation, then this approach could help the user identify (or at least probabilistically associate) the specimen as one variety or the other. I have already discussed the fact that when a plant of this group is dried it loses many of its diagnostic features. This may seem to immediately preclude the use of this type of method. But, I have

Table 21. A comparison of absolute combined probability differences when based on all 19 characters and when based on the nine characters that are likely to be measurable on herbarium specimens. The absolute combined probability difference is calculated as follows: $d = |CP(\Gamma 1) - CP(\Gamma 2)|$. There are two combined probabilities (CP) given in each category for each observation; the first refers to $\Gamma 1$ and the second to $\Gamma 2$.

Sample	Observation	CP1 (all 19 characters)	d1	CP2 (nine characters)	d2
G1.2	01	.9551 .5217	.4334	.7823 .2710	.5113
	02	.9642 .9197	.0445	.9147 .6371	.2776
	03	.9877 .6221	.3656	.9025 .5334	.3691
	04	.9798 .6371	.3427	.8578 .5520	.3058
	05	.9752 .8871	.0881	.9025 .5918	.3107
G2.2	01	.8171 .9696	.1525	.6864 .7511	.0647
	02	.6877 .9914	.2937	.5392 .7278	.1886
	03	.8889 .9825	.0936	.7278 .7346	.0068
	04	.6734 .9916	.3182	.1900 .8715	.6815
	05	.4751 .9787	.5036	.1900 .8113	.6213

several reasons for believing this approach can still be very useful in dealing with this group of plants. Reference to Figure 9 will remind the reader that the characters which remain measurable on a pressed specimen are those that have overlapping ranges of measurement. Also note that one of Davidson and Dunn's premises was that individual taxonomically useful characters are continuous within taxa and overlap in range between taxa. Furthermore, I used the same random samples and calculated the combined probability for each of the ten observations on the basis of only those characters that will usually be measurable on herbarium specimens.[15] The results were that once again the correct decision was made in every instance. If the d-values or the absolute combined probability differences (see Table 21) are again examined, the result is the same as before -- only one of the ten is less than the chosen lower limit for d, 0.05. Further, the majority of the d-values are higher when the CP's are calculated with only the nine characters that are usually measurable on herbarium specimens than when using all 19. Therefore, the probability of a correct decision is usually even greater using the second method of calculating CP! From these facts and this reasoning, it appears to be quite safe to apply this biometric method to the identification (within the yellow lady's slippers group) of herbarium specimens.

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[15]The characters that will be measurable on most herbarium specimens are: lip length, lip height, lateral petal length, lateral petal width, dorsal sepal length, dorsal sepal width, pubescence on the flower, pubescence on the stem, and the ratio of length: width of top leaf.

Conclusions

Case (1964) indicated that he considers the two varieties of Cypripedium calceolus distinct, "in some populations so distinct that [they] could well be considered separate species." At least in the areas of Presque Isle County in which I have observed these plants, they are behaving very much like distinct species. The fact that the two types occur together and retain their identity with no intermediate types being formed seems to strongly suggest two species. Furthermore, Luer states "The two varieties have maintained their genetic characteristics over many years in cultivation by Fred Case of Saginaw, Michigan" (Luer, 1975). Also, information from Dr. S.N. Stephenson of Michigan State University (personal communication, 1976) indicates the existence of an area in Ingham County, Michigan, where the two groups occur together in large numbers, and where no intermediate forms were apparent.

These facts, the measurements and observations that I have made (including the results of the statistical analysis), the largely separate flowering periods, and the apparent differences in pollinators have convinced me that, in fact, two species of yellow lady's slippers occur in North America. One fact that causes some problem in the two species idea is that when pollinated with pollen from the other taxon, the plant will set fruit that is apparently perfectly normal. That is, there is no barrier to crossing between the two, except size of flower, probable size of pollinator, and probable separation of flowering-time peaks. This is not actually too great a problem, when it is taken into consideration that hybridization occurs freely in the orchid family.

Hybrids are even extremely common between different genera, so it should not be too surprising to find that hybrids are physiologically possible between two closely related species!

The idea, which I alluded to in the previous paragraph, that the difference in pollinators may be an important factor in separating and keeping separate the two taxa has also been explored by other authors. Stoutamire (1967) developed the idea as follows: Since the two varieties tend to be associated with slightly different habitats, and since the insect visitors may be ecologically limited in their foraging and nesting habits, these populations of C. calceolus "may be preferentially visited by different species of Hymenopters... If this species [C. calceolus] is adapting to available pollinators, the semi-isolated populations, already adjusted to slightly different habitats, may be diverging further on the basis of the available visitors." Van der Pijl (1965) states this idea even more strongly: "Avoidance of chaotic hybridisation and the maintainance of the individualism of the species [of orchids in general] is due almost entirely to pollinator specificity, the more so as the genetic barriers to hybridisation with other species are weak here."

The suggestion that these two groups of Cypripedium calceolus are "diverging further" is also made by Luer (1975): He says it may be "plausible to assume that we have a variable population of orchids undergoing active speciation."

I plan to continue studies of pollinator relationships, to try to find other information which would shed light on the problem, and

to observe additional populations of the plants, in order that I may make a better judgement on the taxonomic status of the yellow Cypripedium group.

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