ISSN 1554-1169

The Native Orchid Conference *Journal*

Native
Orchid
Conference, Inc.
Founded 2002

Volume 12(3)
Jul-Aug-Sept, 2015

Volume 12, Issue 3 Table of Contents ISSN 1554-1169

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The Native Orchid Conference Journal is published four times a year by the Native Orchid Conference, Inc., a non-profit [501(c)3] organization, as a service to its members. The organization is devoted to fostering the study, conservation, and enjoyment of orchids native to North America. Membership dues are: US individual: $30); US Family: $35; student: $15, and non-US Individual or Family: $40. Address inquiries about membership, back issues of this journal, and requests for copies of the bylaws to the editor at 343 John Wayne Trail Victoria, Texas 77905, or to noceditor@gmail.com

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The NOC *Journal* 12(3): 1-2. 2015. Ferry, R. J.: Your Editor's Notes –Re: Hal Horwitz (1940 – 2015)

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Your Editor's Notes –Re: Hal Horwitz (1940 – 2015)

It seems to this editor that one of the finest measures of an individual is that of wanting to help, to contribute, *to give of oneself* in constructive causes. It's not a case of "how much" in financial terms, but of the inner *attitude* of the giver. In late June your editor received a note from Hal. He wanted to place a thank you note in the *Journal*, and asked the editor to let him know how to submit it. The editor's text was brief, "Email your proposed thank you note to me and I'll see that it gets into the Journal." His reply on 27 June follows:

> "What I had in mind is more in line with purchasing an advertisement; I certainly don't want to ask for free space. In fact, it is a thank- note, but I surely don't want the Journal to be burdened with a load of requests for free space for messages, when the online discussion group is for those kinds of things. Please, please, set an advertising rate and I will send along a check."

Some serious personal health problems delayed your editor's reply until early July, "…you, of all people; after all these years of service, can't *purchase* space! Just write your thanks and it will *certainly* find space in the journal! If you don't care to do so, I can do something , but I think it'd be better coming from you.
Hal never replied. His time was too short! In early July, the following was posted:

"Dear NOC members and friends,

It is with much sadness that I inform you of the passing of Hal Horwitz on July 6, 2015.

Hal with his wife Helen were an integral part of the growth and promotion of the NOC in many ways. Hal tirelessly worked on our conference's quality by developing our Handbook for Conference Chairman, enlisting speakers, producing a multimedia program on orchids and their history, and displaying his wonderful photography. His skills with the camera produced true art.

We will miss Hal's joyful and vigorous greetings and his optimistic outlook on life, and offer our condolences to Helen, family and friends.

I wanted also to share Jay O'Neill's message to NAOCC, see below.

Phil Oyerly

"Dear colleagues, We are saddened by the news of the passing of our friend and colleague Hal Horwitz.

Hal and his wife, Helen, enjoyed a rich and active life, sharing their passion for orchids as they travelled from Florida to Newfoundland to Alaska capturing their beauty on film.

Hal was an early champion of NAOCC and supported our efforts in many meaningful ways. He was instrumental in creating opportunities for us to tell the NAOCC story and established many of the contacts that continue to support our conservation efforts on the web. Our initial Gallery featured his photography and his images form the banner on the opening page of the NAOCC site. The stunning Yellow Lady's Slipper, an image Hal was especially proud of, has become synonymous with the *Go Orchids* web site. Hal envisioned a field guide on native orchids and was working with us to develop the guide as a companion to *Go Or-*

chids. We intend to honor his dream and complete this mission. Hal freely shared his knowledge, taught many, and worked with many others to capture the essence of native plants, especially orchids. We will miss his passion for life, and extend our condolences to his family and friends." -Jay O'Neill/NAOCC

Your editor could have received no finer measurement of Hal's selflessness and deep concern for the welfare of the organization. His last note was one of wanting to say thanks, yet concerned that it be done by *giving*. Hal's last note to this editor illustrates why he will be missed, by members of this organization and others.

Others have expressed sentiments via the organization's chat line, but I prefer that another individual than your editor speak here; one who was closely acquainted with Hal over these all-too-few years. RJF

What I Learned From Hal Horwitz (1940 - 2015).

Ronald A. Coleman
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I first met Hal Horwitz at the initial orchid conference organized by the now defunct North American Native Orchid Alliance. We found out we were both interested in orchid photography. Turns out Hal and I had gotten into orchid photography for different reasons. Hal had long since been an accomplished nature photographer, specializing in wildflowers. He started photographing native orchids because they were a greater challenge. I was interested in the science of native orchids. An editor had told me "Your paper is fine; your photography is really bad." I learned orchid photography because I had to do so in order to document my papers for publication.

Hal and I developed a relationship, and over the years spent much time together photographing orchids. I visited him in Richmond. He visited us in Tucson. We met in Big Bend National Park multiple times. We met in the Michigan Upper Peninsula. We spent three weeks together in Newfoundland. We chased orchids in Pennsylvania. We got together early or stayed late for the orchid conferences each year. Most of my best wild orchid experiences were with Hal. We emailed often, sometimes going to great length on photography, equipment, or other aspects of orchidology. I learned a lot from Hal. He probably did not learn much from me. Here is just part of what I learned from Hal Horwitz.

Hal and I never understood photographers who would walk up, bend over, take a photo of a plant, and move on. Orchid hunters who went out with us knew we could be an hour or two with one plant. Before digital cameras I would shoot 3 rolls of film on one flower. Hal captured it perfectly: "You need to spend time to commune with the orchid."

I learned from Hal that "habitat enhancement" did not have to be destructive. We all have stories of Hal's size 14 feet taking out some of the plants he was trying to protect, and we will laugh about those for years, but for the most part when Hal finished photographing, it was hard to tell someone had been there. I saw how he searched for and removed leaves and rocks that would reflect more light than the flower. But the best thing I observed is that he used sticks and branches to hold grasses and other plants out of the way, rather that pulling them up. When he was finished, he removed the restraints and fluffed up everything back as it was. Then he smoothed out the dirt. I learned no impact photography from Hal.

A neat trick of his was aspect angle adjustment. Many of our wild orchids grow very close to the ground and vertical. Most photographers just get down on the ground with them. Hal used a tiny stake to adjust the angle of the flower spike, bending it 20 or 30 degrees from vertical. It makes for a much easier tripod set up, and the photographer need only crouch, not sprawl on the ground, wiping out other orchids in the process. After watching Hal, I bought a 6” aluminum stake with a hook on the end from a camping supply store and carry it with me on all orchid trips to adjust plant angles. I call it my Horwitz stake.

Hal was a pediatric dentist with skills which may have been helpful on orchids in the field. One year we were out looking for the recently described *Corallorhiza bentleyi*. Hal's sources had given him pretty good directions, and sure enough we soon found a plant with several buds and one flower that may or may not have been open. The flowers of *C. bentleyi* are very tiny; are prone to self pollination; and seldom open completely. On this flower I could see enough of the lip to know it was *C. bentleyi* so I was happy since I had never seen it before. Hal let me go first, so I photographed the habitat, the plant, and the semi-opened flower, very proud of the partial stripe or two of the lip that I could see. Hal then communed with the flower while I searched unsuccessfully for more plants. When he was finished Hal said "Ron, take a look at it now." The flower was wide open. I now have photographs of a *C. bentleyi* with an open flower! I have ever since been amazed that in the time Hal was taking his photos, the flower had been able to open up.

I learned one should handle all circumstances with aplomb. One of the orchids Hal came to Arizona to photograph was *Hexalectris colemanii*, which blooms in late May. Its blooming is very unpredictable: some years many plants bloom; some years only a few. The year Hal chose to visit we found only one plant with good blooms. It was off the canyon floor, about 30 feet up a 45 degree slope of loose dirt covered with oak duff. As Hal was communing with the orchid, his footing gave way, and Hal and his camera gear came sliding back down to the canyon floor. I rushed over and asked "Hal, are you OK?" He allowed that he was, as was the *H. colemanii* now in his hand.

Hal taught me that life takes courage. We had been getting updates on his condition. The last one from Hal was a message to his family and friends that he had decided to forgo additional chemo treatment so he could spend the time he had left with his family without the interruptions the frequent trips to the hospital caused. A few days later we got a message from Helen that he had been successful doing that. Perhaps the most important thing he taught me I learned in increments over the years. Hal and I are different: different personalities; different reasons we went into orchid photography; different views on aspects of orchid science; different political persuasions; we lived on different coasts. Had we met at a business meeting, or at the home of a common acquaintance, we would have chatted a few minutes and moved on. But we were friends for over 20 years. Hal taught me that orchids bridge gaps. We shall miss him dearly.

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Platanthera leucophaea (Eastern Prairie Fringed Orchid) - Pollinator.

Al Menk
acmenk@hotmail.com

All photos by Gary Hofing

A friend, Gary Hofing, wanted to photograph the pollinator of the endangered *Platanthera leucophaea.* In Michigan, there are just a couple sites where these orchids have survived with just a few plants each season.

Fig. 1. Hermit Sphinx Moth (*Lintneria eremitus*); with pollen of *P. leucophaea* on proboscis.
Photo Wednesday night, 15 July, 2015.

After confirming that the orchids were blooming, Gary made his first effort on a nearly cloudless night, when the moon was almost full. He arrived at the location after dark, around 2:30 am, staying until 6:30 am. But his attempt failed to see a pollinator visit the plants he was monitoring. I suggested that if he was to make a second attempt, he should arrive just before dusk as the fragrance released by the Eastern Prairie Fringed Orchid would be at the highest degree of aroma then to entice a pollinator.

Gary was persistent and for his second attempt he waited for a single plant to begin to bloom. The moon was past full and the sky was overcast with clouds. He arrived around 8:40 pm while it was still light enough for him to set up his equipment. He used a 300mm lens with a 2X tele-convertor so he would be situated far enough away from the orchid without disturbing the potential pollinator. He was also concerned about the insect repellent he was wearing interfering with the orchid's fragrant emittance and distracting any insects. For lighting, he set his camera flash with a diffuser so that it would not wash out the white flowers of the plant. Due to the darkness, he placed a small LED to shine dimly on the plant so he could see it. His hopes of success were marred by the possibility that the LED would attract insects that were not pollinators. In fact though, the LED did not seem to attract any insects.

Fig. 2. Hermit Sphinx Moth (*Lintneria eremitus*); with pollen of *P. leucophaea* on proboscis.
Photo Wednesday night, 15 July, 2015.

Being all set up, now it was time to sit patiently and pray that a pollinator would visit the target as the sun set at the edge of the horizon and darkness deepened into the evening. It was around 9:15 pm.

At about 9:45 pm, a moth flew into view and proceeded directly to the subject orchid that Gary was monitoring. The moth began visiting each flower; Gary was ready and started photographing as fast as his camera could release the shutter. On a few of the images, the flash could not recycle quickly enough to keep up with the shutter depression, but Gary was able to capture successfully 14 of 20 images of the pollinator. The moth was quick at work collecting nectar from each of the Eastern Prairie Fringed flowers and did not seem bothered by the flash from the camera. It spent nearly 30 seconds at the plant before flying off into the darkness of the night.

In reviewing Gary's images, notice the pollinia of the orchid stuck on the proboscis of the moth (yellow globes near the mouth area). As the moth visits other orchids, the pollinia can make contact with the next plant, thus causing pollination and ensuring future survival of the species.

So what was the pollinator? Aware that the moth needed to reach the deep throated nectar of the Eastern Prairie Fringed orchid, the moth would need to be sizeable. Gary's images confirmed that it was a hawk moth. In comparing images of hawk moths, Gary and I feel confident that it was *Lintneria eremitus,* the Hermit Sphinx Moth.

Fig. 3. Hermit Sphinx Moth (*Lintneria eremitus*); with pollen of *P. leucophaea* on proboscis. Photo Wednesday night, 15 July, 2015.

The Hermit Sphinx Moth has been documented as a pollinator of the Western Prairie Fringed Orchid, *Platanthera praeclara.* The moth is in the family *Sphingidae* and is found from the southern plains of Canada through the Midwest and Eastern United States. There is one generation per year, which just happens to concur with the same blooming time as *Platanthera leucophaea*.

I want to thank Gary Hofing for his contribution to the article and allowing permission to have it and his images shared in the NOC Journal, as well as for having the desire, patience and capability to attempt photographing a night time pollinator of the endangered *Platanthera leucophaea,* Eastern Prairie Fringed Orchid.

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Sex and the Single Orchid: Observations on Pollination in *Corallorhiza mertensiana* Bong. With Comparisons to *Corallorhiza maculata* Raf.

John H. Horner

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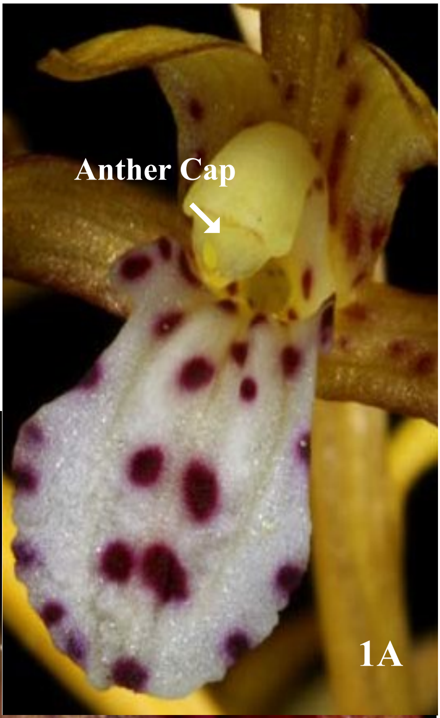
Introduction

Corallorhiza mertensiana is a species of coralroot orchid native to the west coast of North America from California to the Alaska Panhandle. The range extends inland in the northwestern United States as far as Montana and Wyoming. Also found in this region is *C. maculata,* a similar but much more widely distributed species (1). The morphologic similarity of the two species was long recognized; even to the point of one author in the 1960's proposing to reduce *C. mertensiana* to subspecies status under *C. maculata* (2). Although this was rejected, subsequent morphologic and phylogenetic studies by Freudenstein have shown that the two species are indeed closely related. (Freudenstein 1997, 2008, 1994a, 1994b, Barrett and Freudenstein 2008). *Corallorhiza mertensiana* and *C. maculata* clearly fall under the definition of sympatric species; they are closely related, their ranges overlap, with individual plants often occurring in close proximity, and yet they are able to maintain distinct identities. Consistent with this is the lack of any reported hybrids between the two species. This report describes observations of a single isolated plant of *C. mertensiana* in Glacier National Park, Montana in June 2014 that suggest that *C. mertensiana* and *C. maculata* have very different pollination strategies.

Pollination studies of all North American orchids, including the genus *Corallorhiza*, have been summarized by Argue (2012). His comprehensive review of the botanical literature located no studies of pollination in *C. mertensiana*. Thus, as an initial reference point, a comparison to other *Corallorhiza* species, with particular attention to its close relative *C. maculata*, is in order. In all *Corallorhiza* species the pollinia are borne at the end of the column behind an anther cap. The stigma is located immediately behind on the adaxial surface of the column. The pollinia are separated from the stigma by a flap of tissue known as the rostellum. In some species the rostellum is well developed and functions to minimize autogamy. In other species its development is minimal so as to facilitate autogamy. The pollinia are attached to the rostellum by an elastic stalk of tissue known as a stipe. The particular type of stipe found in *Corallorhiza* is sometimes referred to as a hamulus, which was defined by Rasmussen (1985) as a recurved pollinium stalk that is formed from the tip of the rostellum.

Pollination in *C. maculata* was studied by Catling (1983), who showed that the flowers of *C. maculata* commonly undergo autogamous pollination as the anther cap at the tip of the column decays, and the now exposed pollinia, which are still attached to the stipe, first rotate downward and then backward until they make contact with the stigmatic surface (Catling 1983, Argue 2012). Photos of stipe rotation in *C. maculata* are shown here in Figs. 1a, b, and c. Rotation sometimes starts even as the flower begins to open, or may be delayed to provide for the possibility of insect visitation and outcrossing. What is known about insect mediated pollinia transfer in *C. maculata* has been summarized by Argue (8). The only well documented reports indicate that small insects are pollinia vectors; in one case flies of the genus *Empis* and in another a small bee in the genus *Andrena*.

The column of *C. mertensiana* (Fig. 2) is superficially similar to *C. maculata* but two striking differences are notable. First, the column of *C. mertensiana* is less curved, projecting upward above the la-



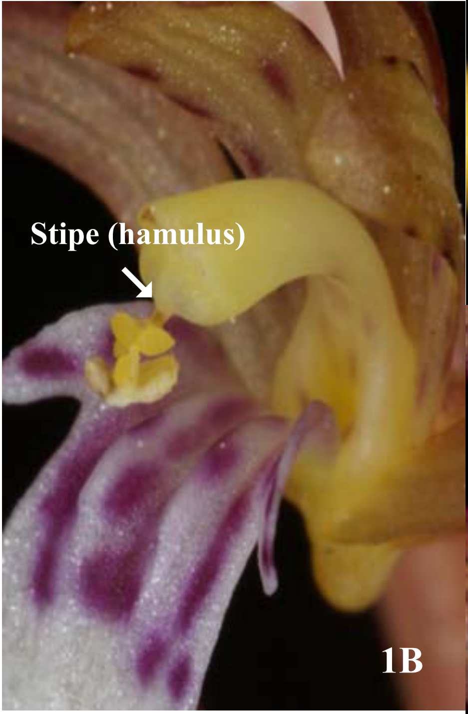


Fig. 1**.** Autogamous pollination in *C. maculata*: (1A) flower with anther cap intact; (1B) flower with decayed anther cap showing pollinia held on elastic stipe in the process of rotating downward, and (1C) flower with the rotation process completed showing pollinia now fused to the stigmatic surface.

bellum (Fig. 4). Second, a prominent rostellar beak is present in *C. mertensiana*. (Fig. 2) The beak projects forward, apparently serving to minimize autogamous pollination by preventing contact between the pollinia and the stigmatic surface immediately behind and below on the underside (adaxial surface) of the column. Two pairs of light yellow pollinia rest in a concave depression at the end of the column just behind the anther cap. They are connected to the rostellum by a short stipe (hamulus).

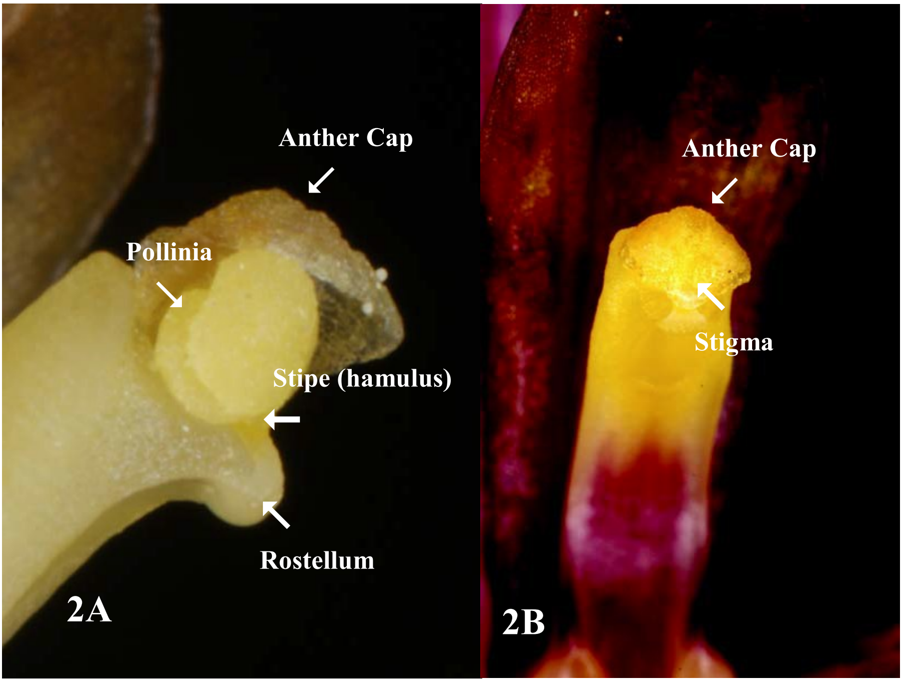


Fig. 2. Front (2A) and side (2B) views of *Corallorhiza mertensiana* flowers showing column.

Detailed descriptions of how deposited pollinia interact with the stigmatic surface to ultimately effect pollination have not been reported for *Corallorhiza*, but have been reported by Slater (1991) for *Dendrobium speciosum*, a distantly related epidendroid orchid native to Australia. Immediately after deposition, the pollinia begin to hydrate and dissolve into the stigmatic surface. After four days, the pollinia have largely dissolved and pollen tube growth is initiated. The process described for *Dendrobium speciosum* is similar to the changes observed in the current work for *C. maculata*.

Observations

A single isolated plant of *C. mertensiana* was observed in Glacier National Park near West Glacier, Montana, on three separate days; June 13, 15, and 21, 2014. The open flowers were photographed *in situ* using a Sony A390 DSLR, using a Minolta 1X-3X macro lens equipped with a Minolta R-1200 ring flash. On June 13, a single flower was open; by June 16 two flowers had opened; by June 21 seventeen flowers had opened, with five more in bud. On June 21, eight of the flowers had undergone either pollinia removal, deposition or both. Two flowers clearly had recently deposited pollinia on their stigmatic surfaces. Three more had round yellow drop-like masses on their stigmatic surfaces, which were interpreted as partially dissolved pollinia. A single flower exhibited pollinia attached to the margin of the labellum resulting from unproductive deposition. Autogamy by stipe rotation analogous to that commonly seen in *C. maculata* was not observed for any of the flowers. Attempts to locate other blooming specimens of *C. mertensiana* within 100 meters of the observed specimen were unsuccessful. Within this distance approximately twenty specimens of *C. maculata* were located. All were in bud except for a single plant with one flower open on June 21. The remaining plants in bud were tentatively identified as *C. maculata* based on the observation that the mentum on the underside of each bud was appressed to the underside of the ovary, while in the *C. mertensiana,* the mentum projects at a 90 degree angle from the ovary. It thus seems likely that all pollinia transfers involved only the single plant observed. Pollination events here are thus either autogamous or geitogamous events mediated by an external vector.

Pollination in orchids can be divided into two steps ; male success and female success. Male success is commonly defined as removal of the pollinia from a flower even though the pollinia may or may not subsequently be deposited on a stigma. Female success involves pollinia deposition on the stigma and subsequent fruit capsule development. Thus the first step in male success is pollinia removal and the first step in female success is pollinia deposition onto the stigma. For the purposes of this manuscript female reproductive success will be approximated by pollinia deposition, since travel and time constraints made it impossible to observe the plant at a later date when fruit capsule formation might have been observed. The efficiency of transfer was high; a total of eight pollinia were removed, with 5 successful stigmatic depositions. Table 1 summarizes male and female reproductive success for the 17 open flowers. Eight flowers had experienced removal of pollinia (male reproductive success; Figs. 3E, G, and H), while five had experienced pollinia deposition onto the stigma (Fig. 3A-E; female reproductive success).

Table 1. Summary of male and female reproductive success of flowers on a single *C. mertensiana* plant. Glacier NP, June 21, 2014.

Male Success	**Female Success**	**Number of Flowers**
+	+	**1**
+	**-**	**7**
-	+	**4**
-	**-**	**5**

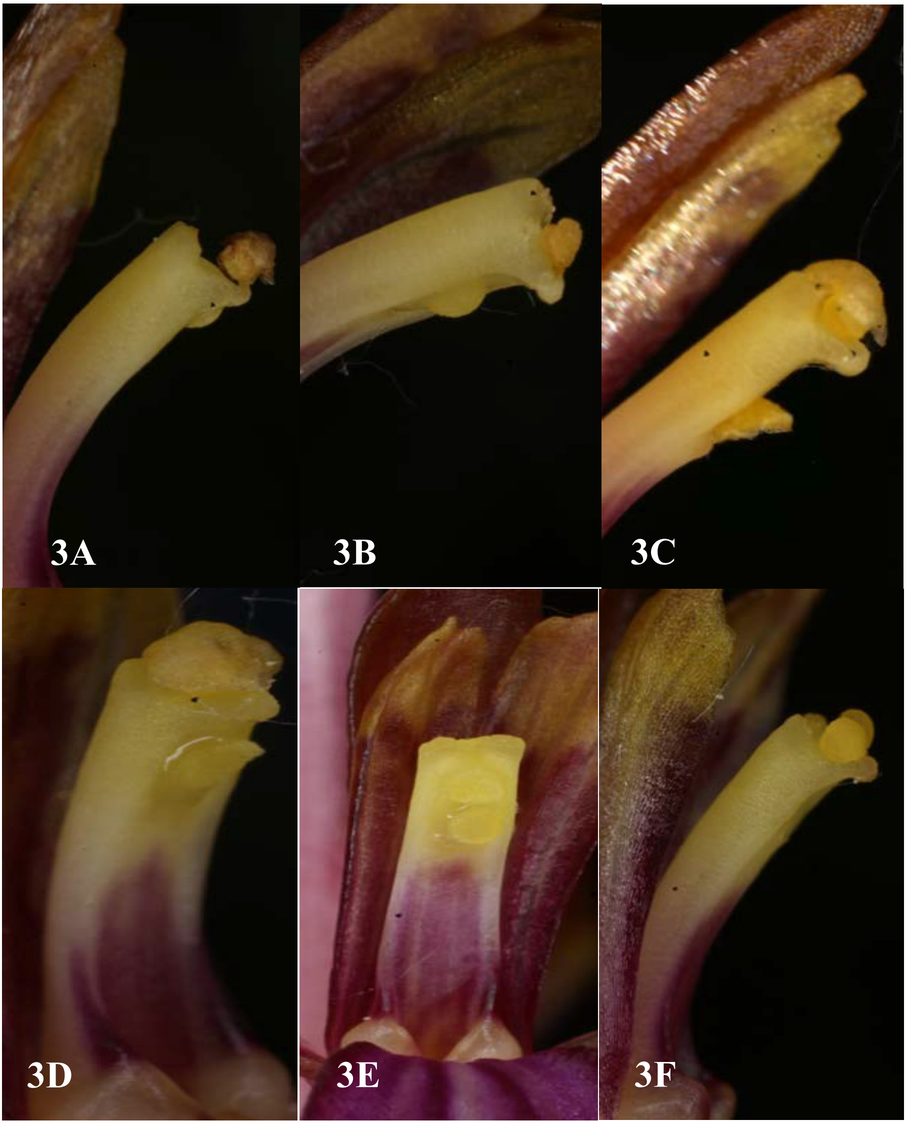


Fig. 3. Close-up photos of the columns of six *C. mertensiana* flowers. All photos taken on June 21, 2014 of flowers on a single plant. 3 A, B, C, D. Flowers showing deposited pollinia beginning to dissolve onto the stigmatic surface; with unremoved pollinia at end of the column. (male success +; female success -). 3E. Plant with pollinia removed from column end with pollinia deposition onto the stigma. (male+; female +). 3F. Flower with anther-cap removed leaving pollinia attached with no evidence of pollination. (male -; female -). 3G. Flower with pollinia removed leaving anther cap intact with no evidence of pollinia deposition on stigma. (male +; female -). 3H. Flower with anther cap and pollinia removed with no deposition on stigma. (male +; female -). Most significantly, four flowers had experienced pollinia deposition (female success) without male success. (Figs. 3A-3E); indicative of geitogamous pollination involving pollinia transfer between flowers by an external agent. Figs. 3A-3D all show flowers with pollinia in various stages of dissolution on the stigmatic surface, while the beak is present in *C. mertensiana*..

Fig. 4. Views of *C. mertensiana* (4A) and *C. maculata* (4B) *columns* with scale (1 division = 1 mm.

Discussion

The observations of the plant in Glacier National Park prompted the author to examine photos of *C. mertensiana* previously encountered in Olympic National Park (July 2000) and Crater Lake National Park (June 2006). A further search was made of the photo sharing website FlickR. These searches revealed one photo of a single flower undergoing autogamous pollination by stipe rotation. For comparison, the examination of *C. maculata* photos revealed numerous examples of stipe rotation. Despite being common in *C. maculata*, autogamy by stipe rotation appears to be rare in *C. mertensiana*. Thus it seems likely that pollination in *C. mertensiana* is dependent on an external vector to transfer pollinia between anther and stigma.

The nature of the pollen vector transferring the pollinia is a matter of speculation. The geometric constraints provided by floral geometry in *C. mertensiana* are illustrated in Figure 4A. The nearly straight column projects above the labellum. The distance between the surface of the labellum and the column tip is approximately 5 mm. In analogy to numerous other orchid species, the lip presumably acts as a landing platform for visiting insects. Searching for a reward, the insect then probes the mentum, which projects at nearly a right angle from the underside of the ovary, requiring the insect to tip forward, putting its dorsal surface in proximity of the pollinia. One would expect the pollinator to be a modest sized insect capable of spanning the 5 mm distance between the labellum and the column tip. Figure 4B shows the column of *C. maculata*, which arches over the labellum; the distance from the column tip to the surface below being only approximately 2 mm. This much smaller gap is consistent with reports of the small insects *Empis* and *Andrena* as pollinia vectors (Rasmussen, 1985).

How sympatric species are able to maintain their identities has been studied extensively in numerous species. The mechanisms of genetic isolation have been divided into pre-zygotic and post-zygotic. Pre-zygotic mechanisms function to prevent fertilization and can be temporal, mechanical, or genetic. Post-zygotic barriers commonly involve seed viability or hybrid sterility. For a discussion of pre- and post-zygotic isolating mechanisms and their relative importance in various species, the reader is referred to a review article by Widmer et al (2009).

The work here indicates a mechanical barrier operating through differences in flower morphology. Grant (1994) defined mechanical isolation as occurring when floral structure differs sufficiently so as to interfere with or prevent interspecific pol-lination. For a detailed analysis of how mechanical barriers function, readers are referred to a recent study by Case who demonstrated that sympatric populations of *Cypripediun parviflorum* var. *makisin* and var. *pubescens* were able to maintain their identities by pollinator selection mediated by floral size (Case & Bierbaum, 2013). The complete story of how *C. mertensiana* maintains its genetic identity in the presence of the more widespread *C. maculata* is a matter of speculation at this point. What is clear is that it is reproductively isolated, and that there is minimal gene flow between the two species. The differences in pollination strategy outlined in this manuscript appear to be a powerful pre-zygotic mechanism that helps *C. mertensiana* to maintain its identity. What is unclear and unaddressed is the presence of other pre-zygotic barriers, or the importance of any post-zygotic barriers to interspecific gene flow. Without evaluation of other potential barriers a caveat must be added that the differences in pollination strategy observed here may be a result rather than a cause.

Unknowns awaiting further study

Since the author lives at least 2000 km from the nearest populations of *C. mertensiana*, and the plant has never been cultivated due to its mycoheterotrophic nature, he is geographically constrained from carrying out any long term studies. Given the paucity of information in the botanical literature regarding pollination of *C. mertensiana*, the following studies would be of interest: 1) pollinator exclusion studies to verify the need of an external vector; 2) studies of fruit set to evaluate autogamy, geitogamy, and outcrossing; 3) investigations of the nature of reward present in the mentum; 4) identification of *C. mertensiana* pollinators; 5) cross pollination studies with *C. maculata* to evaluate inter-specific fruit set and embryo viability.

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Conservation of New England's Lady's Slippers

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All photos by Elaine Faletra.

If variety is the spice of life, life in all its variety is the spice of our planet. The scientific term for the variety of life on earth is biodiversity. To environmental biologists, biodiversity is *the* primary indicator of the health of any place on earth and our planet as a whole. Places of intense biodiversity are called hotspots, which are of particular interest to biologists because they can be used as a general measure of the world's biodiversity (Brooks et al., 2002). Hotspots covered about 12% of the world's land in 1950. By 2009, hotspots decreased to 1.4% of the planet's land surface (Swarts and Dixon, 2009). This rapid decline of hotspots is a sign that the world is facing what many scientists believe is the sixth mass extinction.

Conservation through habitat protection has been the most common approach in the fight against plant extinction. This approach hasn't worked (Swarts and Dixon, 2009). Restoring endangered species to the wild could be an alternative, but worldwide plant restoration efforts are rare,surprisingly, even rarer than animal restoration efforts. The objective of the research program at Crossroads Academy is to establish a model for worldwide plant restoration and prevention of biodiversity decline.

Why Use Orchids As A Model System To Study Plant Endangerment?

Because of their sensitivity to environmental changes, orchids are often considered a red flag for habitat and biodiversity decline. This gives orchids a valuable role as a model system for worldwide plant extinction. We hope to develop our system for rescuing native orchids of New England as a model that can be applied to worldwide plant biodiversity.

The most diverse family of flowering plants is the Orchidaceae with over 25,000 species appearing in a wide variety of biomes. Orchids are frequently found in biodiversity hotspots and are very sensitive to environmental changes because of their deep entanglement with the organisms in their habitats. An example of this entanglement is the seed of the orchid. In most fruits, like an apple, the bulk of the fruit is nutrient (endosperm), the seeds are small in comparison to the entire fruit, and they do not depend on external nutrients for germination. Orchids are peculiar in the plant world because their seeds have no endosperm. Endosperm is formed in most other flowering plants when one of the pollen nuclei fuses with the two central nuclei of an ovule. Without investing energy in making endosperm, orchids can produce thousands of seeds, each about the size of a dust particle. This comes with a catch; orchids depend on something else in their environment to provide them with nutrients. Orchid seeds have co-evolved with mycorrhizal fungi to supply them with food and to enable germination. Another example of the orchid's entanglement with its environment is its relationship with its pollinators. Many orchids have a single pollinator. If the specific pollinator is not present, the orchid will be unable to produce seeds. If the environment changes rapidly, the mycorrhiza or the pollinator might disappear and lead to the regional extinction of the orchid.

Some of the most spectacular orchids of New England belong to the *Cypripedium* genus, many of which are critically endangered. Our research is primarily focused on *Cypripedium reginae*, the showy lady's slipper. To save the showy lady's slipper, we need to know how to grow them efficiently.

Charles Darwin was one of the first people to attempt to grow lady's slippers. He never succeeded and for the next 150 years or so, showy lady's slippers remained difficult to grow from seed, usually taking about nine months to germinate with less than 20% of the seeds germinating. In the late 1990's, Dr. Peter Faletra and his students developed a procedure to germinate showy lady's slipper seeds in about three weeks. Their publication (Sokolski et. al., 1998) provided a method for drastically increased germination and developmental rates of the showy lady's slipper.

A few commercial concerns in the USA sell lady's slipper seedlings. These seedlings are not appropriate for restoration (repopulating to the wild) purposes because the plants may not originate from the area in which they will be planted. Keeping the genetic properties of a plant population from mixing with non-endemic plants of the same species is a common objective in repopulation attempts.

Fig. 1. A typical showy lady's slipper plant with two blossoms; one almost fully opened and the other to the rear not yet emerged.

The Showy Lady's Slipper *Cypripedium reginae* In The Wild

In the wild, it takes eight to ten years for the showy lady's slipper to reach maturity (Faletra et. al., 1997). It flowers between mid-June and early July and usually has two flowers on each plant that emerge within a few days of each other (Fig. 1). As figured, one blossom has just opened in mid-June with another unopened. The plant has large leaves alternating up its stem and the entire plant is pubescent (covered with hairs). There is a skin irritant at the end of the hair (Fig. 2).

The showy lady's slipper is a trap flower with a large vertical white sepal. It has two white side petals, a bold fuchsia pouch, and another white sepal behind the pouch that is actually two fused lateral sepals. The pouch is an evolved petal that traps pollinator insects (Dressler, 1981). The staminode is a pale yellow spotted structure in the center of the flower. It hides the anthers and the stigma and helps prevent self-pollination (Fig. 3). Each anther has a mass of brown sticky pollen. Directional hairs in the pouch guide the pollinator past the stigma then past the anthers to help ensure cross-pollination.

Fig. 2. Light microscope image of a showy lady's slipper hair or trichome. The upper bulbous end contains a skin irritant. The dense dark staining circular object in the end is most likely a nucleus.

The ovary is attached where the fused sepal and the petals meet. The mature seed capsule is about 2.5 cm long and 1 cm in diameter. If fertilization is successful, the capsule will dehisce and release its seeds from about late September to early October. The capsule contains about 200 thousand seeds that are nearly a sixteenth of an inch long. Only 1% of the seeds will germinate and less than 1% of those will reach maturity. It is most common for lady's slippers to reproduce asexually by rhizomes.

Why then do slipper orchids produce seeds? Since seeds can be transported for miles in the wind, it is probable that seeds are a backup plan in the case of local habitat decline. When lady's slipper orchids become established in a place, they can survive for over a hundred years.

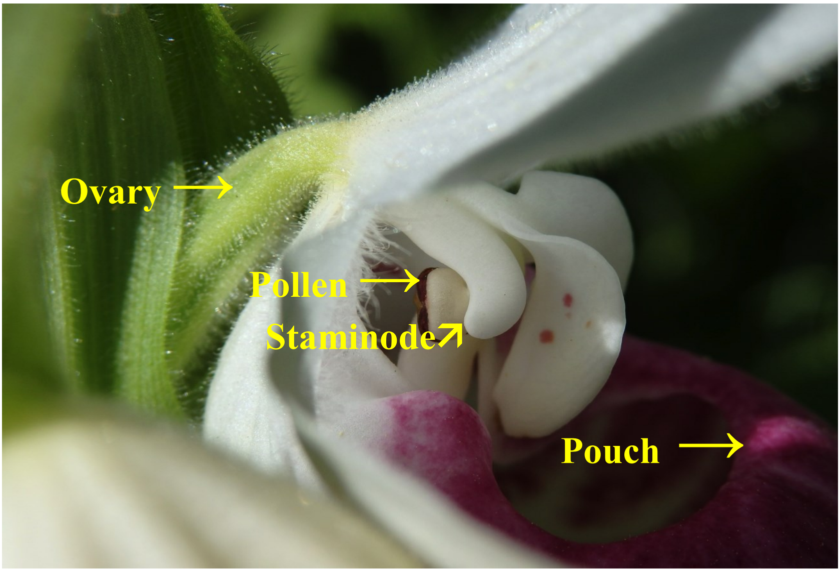

Fig. 3. The reproductive structures of the showy lady's slipper.

Experiments And Investigations

By understanding their life cycle, anatomy, histology, growth in laboratory conditions and the wild, and by raising public awareness, we hope to create a more complete model for rescuing plants from extinction. Since natural germination rates of showy lady's slippers are low, we are growing large numbers of plants using a technique called axenic seed culture. This procedure has the advantages of being fast and economical. Since axenic culture grows plants from seeds, it also ensures that the genetic variety needed to ensure a healthy population is maintained. We are also investigating the histological and developmental differences of the four species of lady's slippers in our region: *Cyp. reginae, Cyp. parviflorum, Cyp. acaule, and Cyp. arietinum.*

Fig. 4. Removal of seeds from a mature showy lady's slipper seed capsule.

Studies Of *Cypripdium reginae*

Seeds

In the wild, less than 20% of the flowers produce seed capsules. To ensure that we have a sufficient supply of seeds, we manually cross-pollinate flowers. In our experiments where we pollinate flowers artificially, we get between 25-30% of the pollinated flowers to produce seedpods. We collect mature, un-dehisced seedpods in mid-September. We allow the pods to dry for a week before removing seeds (Fig. 4).

In the wild, mycorrhizal fungi break down the seed coats and provide nutrients. In axenic seed culture, we obviate the need for fungi by pretreating seeds with bleach to weaken the integrity of the seed coats so that the nutrients in the medium can reach the embryo. The bleach also sterilizes the outer surfaces of the seeds.

After the seeds have been surface sterilized, we inoculate the seeds into nutrient agar. We monitor the germination and growth/development of the seeds every week. We use a staging system to track growth and development, which takes many hours since we have thousands of seedlings in hundreds of culture tubes.

Seedlings

Once a seed has germinated, it is considered a seedling. A seedling has a rhizome, which is an underground stem where the roots meet the shoots (Fig. 5). The rhizome stores starch to provide the energy to put up the next year's new shoots, which will grow the new leaves. With each successive year, the plant will produce more shoots from the rhizome. The seedling also has a coleoptile. Found in orchids and grasses, the coleoptile is a protective sheath that surrounds the developing shoot.

When the seedlings develop prominent roots and shoots, they can be vernalized. Lady's slippers must go through a cold dormancy (winter) in order to put up new growth the following spring (Faletra et. al., 1997). We vernalize seedlings by storing them in a refrigerator to mimic their natural cold dormancy.

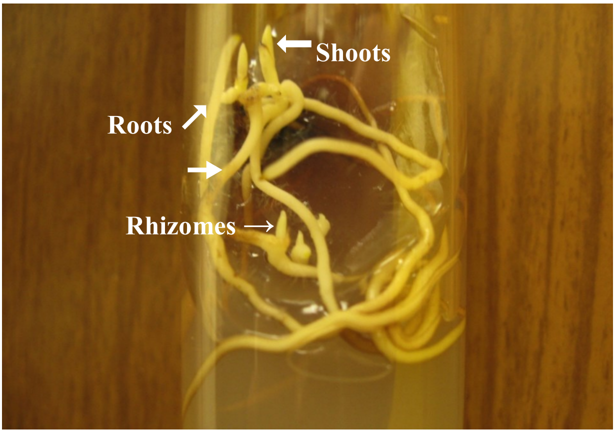

Fig. 5. Showy lady's slipper seedlings after about 6 months in sterile culture. The image shows five seedlings at various stages of development.

We have been studying methods that would reduce seedling death during vernalization. We tested two common methods of vernalization; one that stored bare-root seedlings in containers with small amounts of water, and the other that stored seedlings layered in a mixture of peat moss and compost. Less than 25% of the seedlings survived when layered in peat moss and compost. The bare-root, soilless method increased survival to about 50%. Recently, we have tested vernalizing seedings in their culture tubes. This is simple and efficient since we move the sterile cultures from their storage at room temperature to the 5° C refrigerator without transferring the seedlings to another medium. After two months in cold storage, almost 100% of the seedlings survived with this method. Seedlings that have been vernalized for at least two months will produce healthy shoots within a month after transferal to soil. In the past four years, we have grown over 8,000 seedlings. Most of the seedlings have been used for experimental purposes, such as the vernalization studies described above.

Planting In Natural And Artificial Fens

The showy lady's slipper's natural habitat is a fen. A fen has water moving through it and is typically alkaline (a pH of about 7 to 8). We have tried planting very small, 1 year-old seedlings into a natural fen, but none of the seedlings survived. This might be because the soil was acidic and the seedlings were not well developed. This was not surprising because all previous attempts to move very young showy lady's slipper seedlings to the wild have been unsuccessful.

To get a successful transfer to the wild, we believe that we need to allow the seedlings to become stronger and more accustomed to nature before we try to bring them out to the wild. We have designed a method of making a controlled environment for planting the seedlings. Once a suitable spot for the fen with about 6-8 hours of direct sunlight is located, we start by digging a hole about 8-10 inches deep. The dimensions will vary based on the number of seedlings to be planted. We try to mimic the moist conditions of a natural fen by lining the hole with plastic. We plant seedlings at least an inch apart. Before the first frost in late fall, we cover the fen with about 3-5 inches of straw to prevent frost heaving. Using this method, about 50% of the seedlings put up shoots the following spring. Through this entire lab procedure, we expect to shorten the showy lady's slipper's maturation from seed to flower from 8-10 years in the wild down to 4-5 years.

Histological Studies

Examining tissues of slipper orchids with light microscopy has revealed the structural details of stigma surfaces, pollinea, pollen, and ovules as well as locations of meristematic tissues. This has revealed substantial differences in the embryonic cells of the four species we are studying and suggested new approaches to culturing the ram's head lady's slipper. It has also revealed how many ovules are in lady's slippers of the various species. We have discovered substantial numerical differences in the intracellular organelles within the embryos of the various species.

Community Engagement

The showy slipper's habitat is rare. The few places where it thrives are being threatened by human development. One approach is establishing reserves in private and public lands for endangered slipper orchids. We call these reserves "sanctuaries".

We have built six sanctuaries on private properties where the owners have made a long-term commitment to be the stewards of our slippers. Every year we intend to visit the sanctuaries to track the growth and survival of the slippers. The New Hampshire Orchid Society has been a great help in this effort. In the event that we move any slippers to the wild, we will notify the New Hampshire Natural Heritage Bureau, which tracks endangered species in the wild.

Acknowledgements

This research was supported by grants from the Toyota Tapestry Foundation and The New Hampshire Orchid Society. It also received financial support from Crossroads Academy and Dr. Peter Faletra. A special thanks to Dr. Daniel Jones for his histological expertise in preparing specimens for light microscopy, and Dr. Markus Testorf and Elaine Faletra for their extensive help with digital photography. Credit and thanks is extended to Fiona Sweeney, Gabrielle Jarrett, Andy Kotz, Sam Seelig, Milo Wilcox, Claire Adner, Dan Jones, and Alexander Kish for their many months of work in helping to raise the thousands of lady's slippers used in this project. Lastly, we extend our thanks to Jean Stefanik for placing us in contact with the many orchid experts of North America.

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The Pollination Biology of *Cypripedium passerinum* Richardson (sparrow's egg lady's-slipper) [1]

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[1] Modified from *The Pollination Biology of North American Orchids*, vol. 1, Springer, New York (2012).

Distribution and habitat

Cypripedium passerinum is typically found in the moist acidic or neutral soils of coniferous forests or tundra, often along the shores of lakes and streams. It ranges from Alaska across Canada to Quebec and south into Montana with disjunct populations on the north shore of Lake Superior in Ontario and on the shore of Ille Nue in the Mingan Islands (Luer 1975, Catling and Catling 1991, Cribb 1997, Sheviak 2002). Some northern populations occur at higher and colder latitudes than any other North American lady's-slipper, with most populations occupying land that was for the most part covered by glaciers less than 10,000 years ago (Catling 1983).

Floral morphology

Cypripedium passerinum (Table 1) produces one or occasionally two small flowers on a leafy stem. Sepal color varies from green to white.

Table 1. Data on *Cypripedium passerinum* (Sheviak 2002).

Character	Dimensions
Plant height	12-38 (-50)
Number of flowers	1 (-2)
Dorsal sepal (mm)	11-20 × (7-) 9-15
Lateral sepal (mm)	6-12 × 6-15
Lateral petals (mm)	12[1]-20 × 3-6 [1]Misprinted as "2" instead of "12" in Argue (2012)
Lip length	11-20
Column length	6.8 8.0
Chromosomes (2n)	20

The dorsal sepal forms a hood over the lip (Fig. 1a), while the laterals, sometimes free almost to the base, can be connate to a bifid apex (Fig. 1b) (Luer 1975, Cribb 1997). The resulting synsepal is appressed to the bottom of the lip. The lateral petals, downward curved and spreading, are white, translucent, and flat (Fig. 1a, b). Also white, the lip is obovoid or subglobose with minute purple spotting at the orifice and on the inside of the pouch (Fig. 1a, b). The column is short with a relatively large stigma (Figure 1c, d). A longitudinally grooved staminode, about 6 mm long, has a white base and a yellow apex with purple to reddish-brown spots (Fig. 1b) (Luer 1975, Cribb 1997).

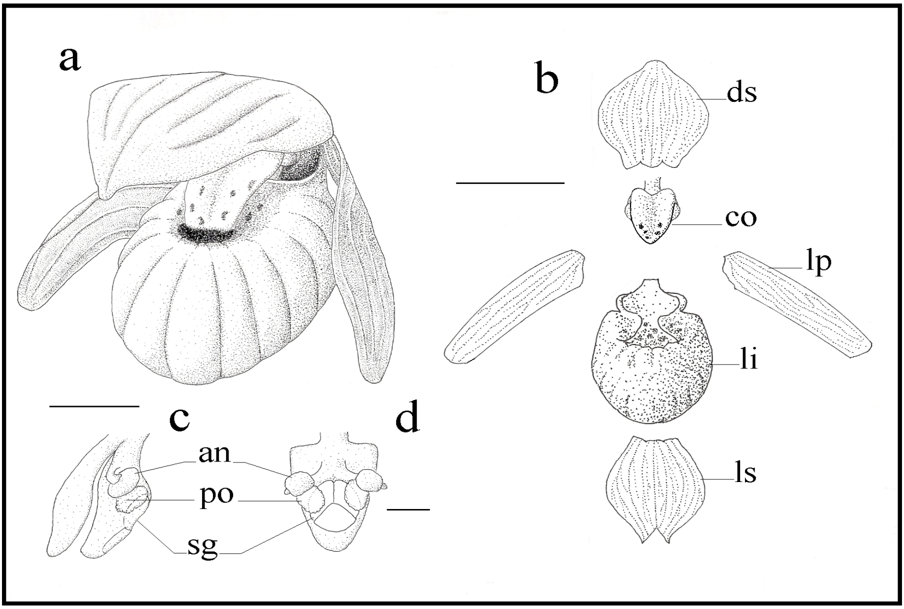

Fig. 1. *Cypripedium passerinum.* (a) Flower, slightly oblique view, scale bar = 5 mm; (b) Flower, exploded view, scale bar = 10 mm; (c) Column, side view; (d) Column. bottom view, scale bar (c,d) = 2 mm. *an* anther, *co* column, *ds* dorsal sepal, *li* lip, *lp* lateral petal, *ls* lateral sepal, *po* pollen mass, *sg* stigma.

Compatibility and breeding system

Cypripedium passerinum is self-compatible (Catling 1983, Keddy et al. 1983). Automatic self-pollination and subsequent fertilization (i.e., autogamy) occur over most of its range (Catling 1983).

Pollinators and pollinating mechanisms

Due to an alteration in the length and curvature of the stigmatic branch and a lateral convergence of the stamens, the anthers develop adjacent to the margins of the stigma (Fig. 1c, d) (Catling 1983). The pollen masses are consequently discharged directly onto the stigmatic surface, and no pollen vector is needed for pollination.

The presence of this orchid in areas that were occupied by the last continental glacier indicates a history of colonization. Autogamy is advantageous in a colonizing species because a single pioneering individual can set fertile seeds. Autogamy is also advantageous when pollinators are rare or inactive. The northerly distribution of this orchid suggests that bad weather might sometimes have adversely affected pollinator activity. Similarly, its establishment in small, isolated, pioneering populations might have affected pollinator attraction. Although the genetic similarity of seedlings to parents already adapted to extreme habitats should confer an average higher fitness on the products of autogamy as compared with the more variable progeny of outcrossing (Stebbins 1970), fertility assurance seems to provide the best explanation for the frequently observed high autogamy levels in plants such as *C. passerinum* found in colonial (unsaturated) or adverse pollinator environments (e.g. Hagerup 1951, 1952; Baker 1955; Arroyo 1973; Catling 1983; Hereford 2010). According to models proposed by Lloyd (1978, 1979a, b), autogamy can be selected in an unsaturated or colonial environment even when the average success of individual autogamous and cross-pollinated progeny is about equal, but less important than the number of seeds produced. It can also be selected if the agents of cross-pollination are unreliable even when individual progeny resulting from cross-pollination have an advantage in fitness. Given a combination of colonizing conditions and unreliable cross-pollination, strong selection for autogamy might be expected (Catling, 1990).

The flowers of *C. passerinum* open about a week after budding and wilt two to eight (usually four or five) days later (Keddy et al. 1983). Self-pollination, including penetration of the stigma by pollen tubes, may occur before the flowers are fully open, thus preventing subsequent removal by pollinators (Catling and Bennett 2007). However, not all the grains contact the stigma or germinate, and although the size of the exit holes, 2.5-3.0 mm wide, would clearly restrict pollinator size, the presence of sticky, ungerminated pollen and fragrant, open flowers with purple-doted and yellow-tipped columns suggest the possibility of occasional cross-pollination. The noted advantages of autogamy (assured fertility and a potential for rapid colonization) might thus occasionally be combined with the advantages of genetic recombination. In this connection, Catling and Bennett (2007) recently discovered a possible relict outbreeding morphotype in the Beringian region of southwestern Yukon. They attributed its occurrence here to the persistent advantage of outbreeding in an area not glaciated over the past several hundred thousand years.

Despite occasional anecdotal accounts to the contrary, only one other *Cypripedium*, *C. dickinsonianum* Hagsater, has, to my knowledge, been demonstrated to be primarily autogamous (Hagsater 1984, Cribb 1997). This species is restricted to the high central massif in eastern Chiapas (Mexico) where it occurs in juniper forest at about 5000 feet elevation (Cribb, 1997). All cultivated plants of this species produced capsules that contained seed with well-developed embryos. As in *C. passerinum*, changes in the structure and positioning of the stigmatic branch and stamens cause the pollinia to contact the stigma (Hagsater 1984, Catling 1990). The similarity is a result of convergence, and the species are not closely related (Albert 1994, Cox et al. 1997).

Fruiting success and limiting factors

In *C. passerinum* nearly all ovaries develop seed. Although seed production is largely, if not exclusively, the result of selfing, self-fertilizing species can retain a high degree of heterozygosity (Allard et al. 1968) with no trace of inbreeding depression, and the wide distribution of *C. passerinum* implies no disadvantage based on its level of genetic variability (Catling 1990).

Keddy et al. (1983) studied populations of this orchid at a location on the north shore of Lake Superior near the mouth of the Pic River in Ontario, a site some 400 km (ca. 250 miles) south of its more or less continuous range in the Hudson Bay lowlands. The terrain here is made up of dune complexes stabilized by a variety of vegetation types in various stages of succession and includes shrub-land, herbaceous communities, and forests. Only a part of the dune complex at any one time provides suitable habitat for *C. passerinum*, and only a minor fraction of this is favorable for seedling establishment. Seedlings are restricted to relatively rare, early successional sites, and most local recruitment of *C. passerinum* is a result of vegetative reproduction. As succession proceeds habitat conditions often improve, up to a point, for mature plants at the same time that they deteriorate for the establishment and growth of seedlings.

Given the 15 years or more required for *C. passerinum* to reach reproductive maturity, temporal variation in rainfall, temperature, and soil characteristics at Pic River undoubtedly influence the distribution and population size of this orchid. However, successful sexual reproduction here may be most closely tied to the availability of sufficient space to accommodate the changing mosaic of successional stages needed for both the establishment of seedlings and their persistence to reproductive maturity. At the same time, since conditions at other sites occupied by *C. passerinum* differ from those described for Pic River, additional studies across the geographic range of this orchid are needed to obtain a wider and more thorough assessment of its reproductive ecology and management needs.

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