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ORCHID CONSERVATION IN THE AMERICAS—LESSONS LEARNED IN FLORIDA

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Introduction

Conservation can be a difficult concept to define, let alone apply. For some, plant conservation can simply mean the survey and cataloging of plant diversity within a defined conservation area. Others may define plant conservation as the collection and propagation of germplasm from one, or many, areas within a species' range. Still others may define plant conservation as the act of plant translocation for population reintroduction, establishment, or augmentation. Traditionally, many plant conservation researchers have focused efforts on only one or two of these actions and defined their efforts as effective species-level conservation. These researchers have treated species-level conservation as a set of individual steps that have little, or no, interconnection. Orchid species conservation has traditionally taken on this separated approach. Few of the many traditional orchid conservation efforts have taken into account one integrated picture of the orchid plants, their pollinators and reproduction, their mycobionts, their propagation, and their population genetic diversity.

Florida is home to approximately 120 orchid species and a number of other growth and color forms and varieties (Brown 2005). While nearly all of these species are considered endangered, threatened, or commercially-exploited, few have received any conservation attention and even less have received attention from those interested in integrated conservation. In 2002 the Plant Restoration, Conservation, and Propagation Biotechnology Program at the University of Florida (Gainesville, Florida) established a partnership with the Florida Panther National Wildlife Refuge (Naples, Florida) aimed at conserving the orchids of Florida through the implementation of integrated conservation efforts at the species level. To date, the partnership has initiated research examining various factors of the ecology, biology, and propagation of several native Florida orchids, including *Bletia purpurea* (Lamark) de Candolle, *Cyrtopodium punctatum* (Linnaeus) Lindley, *Dendrophylax lindenii* (Lindley) Bentham *ex* Rolfe, *Eulophia alta* (Linnaeus) Fawcett & Rendle, *Habenaria macroceratitis* Willdenow, *Prosthechea cochleata* (Linnaeus) W.E. Higgins var. *triandra* (Ames) W.E. Higgins, *Spiranthes floridana* (Wherry) Cory *emend*. P.M. Brown, and *S. odorata* (Nuttall) Lindley. Through the use of field observations of plants and pollinators, isolation and characterization of mycobionts, symbiotic and asymbiotic seed germination, and the study of population genetic diversity by amplified fragment length polymorphism (AFLP), the partnership has been able to demonstrate the applicability of integrated conservation methods for the conservation of orchid species in Florida.

The acceptance and application of these methods outside of Florida is now needed to further demonstrate their effectiveness on a global scale. The methods, successes, failures, and tribulations of identifying and addressing integrated orchid conservation needs in Florida will be discussed. Additionally, several case studies will be outlined will be used to elucidate these methods. One example case study on *H. macroceratitis* is presented here.

Case Study

Habenaria macroceratitis.—The following is a brief case study outlining the major areas of research concerning the integrated conservation of *H. macroceratitis* in Florida. At the time of writing, some data concerning the species' ecology, pollination biology, and population genetic diversity was not fully analyzed and, therefore, not presented in the current paper.

PLANT ECOLOGY. *Habenaria macroceratitis* is a rare sub-tropical terrestrial orchid native to central Florida, Mexico, the West Indies, and Central America (fig. 1). The species typically inhabits moist hardwood hammocks in Florida where the canopy is dominated by *Quercus virginiana* (live oak), *Magnolia grandiflora* (magnolia), *Liquidambar styraciflora* (sweet gum), *Pinus elliottii* (slash pine), *P. palustris* (long-leaf pine), and *Sabal palmetto* (cabbage palm) (S.L. Stewart, unpub. data 2003). The loss of hardwood hammock habitat throughout Florida has prompted interest by researchers and state agencies in the preservation and restoration of these critical habitats (Maehr & Cox 1995, Sprott & Mazzotti 2001). Many native Florida terrestrial orchids, including *H. macroceratitis*, inhabit this threatened habitat (S.L. Stewart, pers. observ. 2003) and unless effective species-level conservation methods are developed, these species are likely to face population decline or extinction. The integrated conservation of *Habenaria macroceratitis*, the long-horned rein orchis, was investigated through an examination of the species' ecology, mycology, propagation science, pollination biology, and population genetic diversity.

Four populations of *H. macroceratitis* were identified in west-central Florida representing various population sizes and conservation statuses. One of the populations was considered large (Hernando County #1, 270 plants) and existed on privately-owned land that was being adaptively managed for the orchid-appropriate habitat. The second population was considered of moderate size (Hernando County #2, 65 plants) and existed on privately-owned land with no management being applied. The final two populations were considered small (Marion County, 16 plants; Sumter County, 10 plants), with the Marion County population existing on managed state-owned land and the Sumter County population existing on privately-owned non-managed land. Data concerning flowering versus vegetative production, plant demographics, and habitat characterization were collected. At the time of writing, these data are currently being analyzed for associations among habitat/environmental factors and whole plant responses.

FIGURE 1. *Habenaria macroceratitis* inflorescence in habitat.

PLANT MYCOLOGY. — Fifteen mycobionts were isolated from the roots of *H. macroceratitis* collected at two of the previously mentioned populations (Hernando and Sumter Counties) following the methods outlined by Zettler (1997) and Stewart and Zettler (2002). Six of the mycobionts were assignable to the anamorphic fungal genus *Epulorhiza* Moore, while the remainder were assignable to the anamorphic genus *Ceratorhiza* Moore, based on cultural morphology, microscopic examination, and enzyme assays. These mycobionts were subsequently used in a number of studies investigating the *in vitro* symbiotic seed germination of *H. macroceratitis*. These data suggest that *H. macroceratitis* may demonstrate a degree of fungal specificity based upon geographic area in central Florida. Therefore, to insure the conservation of this species through symbiotic seed germination mycobionts from many populations throughout the species' Florida range should be isolated, characterized, and cataloged.

PLANT PROPAGATION. — Of particular interest was the development of a reliable symbiotic seed germination method that accounted for any potential mycobiont specificity based on seed collection and mycobiont collection sites. Current data suggest that symbiotic germination of this species is possible and that this species does demonstrate a degree of mycobiont specificity when seeds and mycobiont collected from the same sites versus geographically distinct sites were co-cultured (Stewart & Kane 2006b). These data suggest that the distribution of *H. macroceratitis* in west-central Florida may be closely tied with the distribution of specific mycobionts and not the species' typical hardwood hammock habitat. This finding has the potential to change state conservation policy concerning the management of hardwood hammock habitats throughout Florida to integrate management strategies for plants, animals, and soil microflora.

Concurrent with the symbiotic studies, the asymbiotic seed germination of *H. macroceratitis* was undertaken in an attempt to better understand the processes of seed germination and seedling development in the species. A simple asymbiotic media screen using six germination media was conducted to determine an optimal asymbiotic germination medium for further downstream studies. These media included Modified Lucke, Murashige & Skoog, Lindemann, Vacin & Went, Malmgren Modified Terrestrial Orchid Medium, and Knudson C. After 16 wks dark incubation, the Malmgren Modified medium supported the highest final percent germination and, therefore, was chosen to support further studies on seed germination and seedling development (Stewart & Kane 2006a). Subsequently, the effect of three photoperiod treatments (0/24, 16/8, 24/0 h L/D) on asymbiotic seed germination were evaluated. Seeds incubated in continual darkness (0/24 h L/D) exhibited the highest final percent germination (91.7%) and the most advanced protocorm developmental stage (Stage 4, fig. 2, Stewart & Kane 2006a). Although seed germination under both 16/8 and 24/0 h L/D treatments was stimulated, percent germination was statistically lower (fig. 2, Stewart & Kane 2006a). These data reinforce the notion that, in general, seeds of terrestrial orchid species are best germinated in continual darkness and that exposure to light may inhibit germination or protocorm development.

To further examine *in vitro* growth and development of *H. macroceratitis*, the effects of three photoperiod treatments (8/16, 12/12, 16/8 h L/D) on *in vitro* seedling development was evaluated. Asymbiotic seedlings grown under a 8/16 h L/D photoperiod produced the highest number of tubers (1.06), the tubers with the greatest fresh and dry mass (42.7 μg and 6.5μg, respectively), and the largest diameter tubers (3.1 mm) as compared to those seedlings grown under the other photoperiod conditions (Stewart & Kane 2006a). Interestingly, leaf number and size (length and width) significantly decreased as photoperiod increased from 8/16 to 16/8 h L/D (Stewart & Kane 2006a). These data suggest that the tuber formation response seen in *in situ* plants of *H. macroceratitis* in response to a decrease in photoperiod is maintained under *in vitro* conditions. This response could be harnessed to produce *in vitro* tubers of the species that may be better suited to translocation to restored habitats than leaved seedlings.

PLANT POLLINATION BIOLOGY. — The pollination biology of *H. macroceratitis* was investigated to determine what role an insect pollinator may play in the long-term conservation of the species. Following the methods outlined by Zettler *et al.* (1996) pollinator observations, nectar volume, and nectar sugar concentrations were conducted on 26-28 August 2004. Given that *H. macroceratitis* has white to cream colored flowers and produced a scent only at night, it was not surprising that a night-flying moth

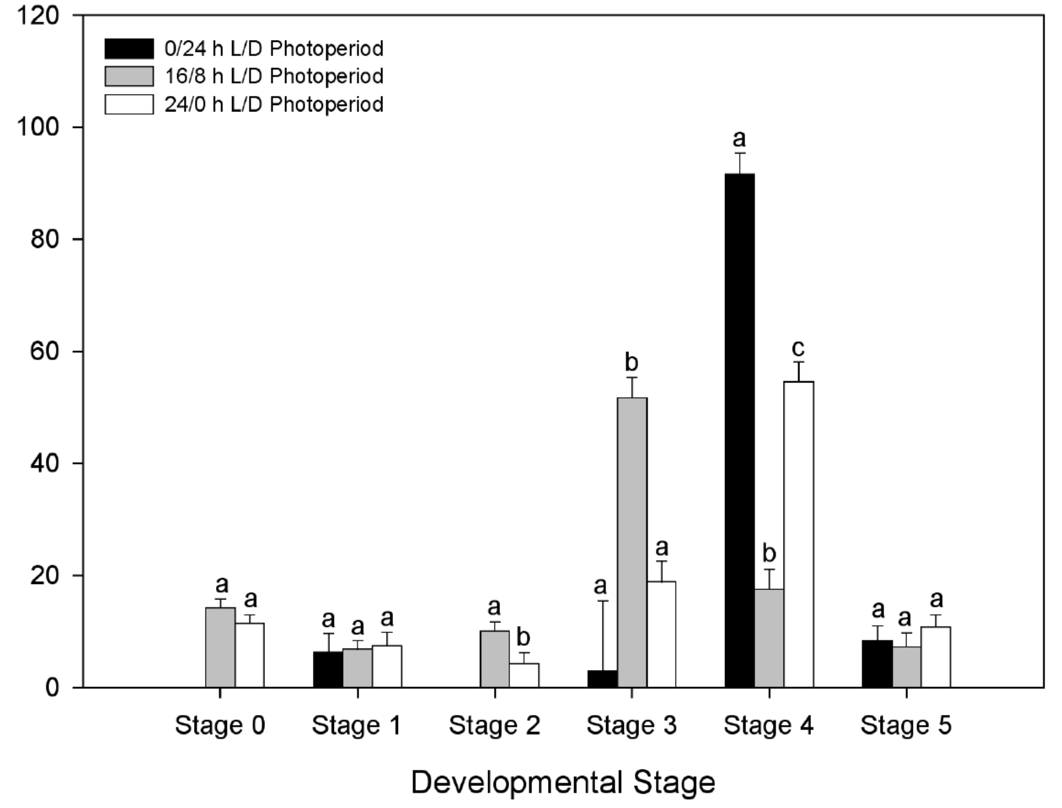

FIGURE 2. Effects of three photoperiod treatments (0/24, 16/8, 24/0 h L/D) on asymbiotic seed germination of *Habenaria macroceratitis* cultured on Malmgren Modified Terrestrial Orchid Medium after 14 weeks. Histobars with same letter are not significantly different within stage (? = 0.05).

(*Cocytius antaeus*, giant sphinx moth) was observed visiting and probing individual flowers. Furthermore, nectar volume (4.25 µl) and sugar concentration (max. 20%) were highest in the early evening, presumably in preparation for optimal attraction timing of *C. antaeus*.

Pollination mechanism experiments were designed to further investigate the breeding system of *H. macroceratitis*. Methods followed those outlined by Wong and Sun (1999), modified with the inclusion of seven experimental pollination mechanism conditions—open pollination (control), agamospermy, spontaneous autogamy, induced autogamy, artificial geitonogamy, artificial xenogamy, and induced xenogamy. Seed capsules were formed in four of the seven treatments—open pollination, induced autogamy, artificial geitonogamy, and artificial xenogamy. *Habenaria macroceratitis* does not appear to be self-fertile, agamospermic, or self-pollinating. Tetrazolium staining revealed high seed viability from three of the four successful pollination treatments, with open pollination being the most viable (91.0%) followed by artificial geitonogamy (86.3%) and induced autogamy (76.8%). The artificial xenogamy treatment resulted in 50.7% viable seed.

Examining the combined pollinator observation and pollination mechanism data, *H. macroceratitis* appears to rely on its night scent and nectar reward to attract nocturnal sphinx moths, such as *C. antaeus*. However, the data demonstrate that these nocturnal pollinators appear to transfer pollen within isolated populations of *H. macroceratitis*. This short distance pollen movement appears to result in highly viable seed within populations, but when pollen from a distant population is brought into an isolated population a decrease in seed viability results. These findings may indicate some level of historic isolation among *H. macroceratitis* populations in central Florida, and therefore result in a management plan that maintains this degree of population isolation.

PLANT POPULATION GENETIC DIVERSITY. — One of the final steps in the integrated conservation of *H. macroceratitis* is the examination of population genetic diversity within and among populations in central Florida. The AFLP technique (Vos *et al.* 1995), modified by Ranamukhaarachchi *et al.* (2000), will be used to assess the population genetic diversity of *H. macroceratitis*. Characterization of population genetic variability may help to better manage the conservation and restoration of *H. macroceratitis* habitat, as well as improve potential plant translocations of the species.

At the time of writing, a DNA library has been compiled representing 75 and 18 plants, respectively, from the Hernando County #1 and #2 populations, 14 plants from the Marion County population, and 7 plants from the Sumter County population. The AFLP technique is currently being applied to these samples. Genetic diversity in *H. macroceratitis* is expected to be representative of fit populations existing in isolated habitat pockets throughout central Florida. If this hypothesis is demonstrated as true, management for this species should focus on the maintenance of these isolated population and the conservation or restoration of the species' endangered habitat.

Conclusions

Integrated conservation represents a best management practice for the species-level conservation of orchid species. The effectiveness and applicability of this integrated approach has clearly been demonstrated in Florida through the research conducted on *H. macroceratitis* via the partnership between the Plant Restoration, Conservation, and Propagation Biotechnology Program at the University of Florida and the Florida Panther National Wildlife Refuge. In examining multiple levels of species plant conservation—from broad ecology to specific population genetic diversity—a holistic approach to the conservation of *H. macroceratitis* has been developed.

The outline of species-level integrated conservation, as presented here, represents a generalized scheme and should be interpreted as a set of guidelines only. Differences in species and habitats, as well as differences in conservation goals, political and social structure, and funding are all responsible for shaping the integrated approach and expected outcomes. Others interested in orchid conservation on either the species- or landscape-level are encouraged to design and implement this integrated approach.

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Michael Kane, Ph.D., Assistant Chair and Professor of Environmental Horticulture, conducts research on the application of plant *in vitro* culture techniques for production of native plants, including orchids, wetland and coastal plants for the purposes of habitat restoration and conservation. His research incorporates the use of genetic markers to characterize plants, *in vitro* culture procedures as well as greenhouse and field studies.