PREDICTING THREATENED ORCHID (*ISOTRIA MEDEOLOIDES* [PURSH] RAF.) HABITAT IN THE SOUTHERN APPALACHIAN REGION USING MAXENT MODEL

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By

Ashley Dallas Montgomery

Director: Dr. Laura E.
DeWald Professor of
Biology
Biology Department

Committee Members: Dr. Beverly Collins,
Biology Dr. Thomas Martin, Biology
Dr. Duke Rankin, USDA Forest Service

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

PREFACE

This thesis is organized in manuscript format. Chapter 1 is an introduction focused on the project's relevance in the Southern Appalachian Mountain region. Chapter 2 is a review of scientific literature related to the topics in Chapter 3. Chapter 3 is a manuscript to be submitted to the journal *Biological Conservation*. Literature Cited lists references from all chapters.

ABSTRACT

PREDICTING THREATENED ORCHID (*ISOTRIA MEDEOLOIDES* [PURSH] RAF.) HABITAT IN THE SOUTHERN APPALACHIAN REGION USING MAXENT MODEL

Ashley Dallas Montgomery, M.S.

Western Carolina University (October 2014)

Director: Dr. Laura E. DeWald

Isotria medeoloides (Pursh) Raf. or small whorled pogonia is one of the rarest orchids in the eastern U.S. and is currently threatened by habitat loss in the southern Appalachians. The purpose of this study was to improve our understanding of where *I. medeoloides* is found and to develop a habitat suitability model so that monitoring and conservation efforts can be prioritized in the southern Appalachian region of the orchid's range. Habitat characteristics, including topographic, soil, and vegetation variables were measured at 15 extant locations during the initial habitat characterization. The results of the habitat characterization were used to guide development of the habitat suitability model. The maximum entropy modeling method (Maxent) was used to predict potential suitable habitat for *Isotria medeoloides* in the southern Appalachian region of NC, SC, TN, GA, and AL.

In general, *Isotria medeoloides* occurred in mid-successional, mixed acidic cove forests with a sparse herbaceous layer, high canopy cover (81-98%), moderately-sloped terrain (5-40%), low soil pH (4.0-4.9), and soils with a hardpan layer. Additionally, one-way ANOVA tests between small and large *Isotria medeoloides* populations revealed that overstory snag density was significantly higher (0.029 #/m^2) at large *Isotria medeoloides* populations. These habitat characteristics were used to determine the type of digital environmental data to include in the development of the habitat suitability model.

The Maxent jackknife test determined that annual average precipitation was the most

important environmental predictor of suitable habitat for *Isotria medeoloides*, suggesting that moisture may be one of the most critical factors controlling survival of the orchid species. The Maxent model had high predictive performance with a statistically significant Area Under the Curve (AUC) score of 0.954. It predicted that 0.74% of the total study area was suitable habitat for *Isotria medeoloides*. Field validation to evaluate the predictive performance of the model revealed differences in habitat characteristics at predicted high suitability sites compared to predicted low suitability sites, and these differences were similar to habitat characteristics that differed between large and small extant *Isotria medeoloides* sites. Additionally, a new population of 19 plants was found in an area predicted as highly suitable by the model indicating that the Maximum Entropy modeling method is a valuable tool for predicting suitable habitat for *Isotria medeoloides* in its southern Appalachian range thus contributing to conservation of the species.

CHAPTER 1: INTRODUCTION

Species are becoming extinct at what may be the highest rate in the past 65 million years (Wilson 1988). The majority of species losses are due to loss of habitat associated with increasing urban development (Gilliam 2014). There is a need to conserve threatened and endangered species as well as their habitats to alleviate continued species loss and protect biodiversity and functioning of our ecosystems. In the southern Appalachian region, one such species threatened primarily by habitat loss is *Isotria medeoloides* (Pursh) Raf., known as the small whorled pogonia. It was discovered by Frederick Pursh in 1814 and is considered the rarest orchid east of the Mississippi River, excluding Florida (Sperduto & Congalton 1996). *Isotria medeoloides* is widely distributed throughout the eastern forests of North America. However, there are three main population centers: New England; Virginia; and the southern Appalachian mountain region. Habitat loss from residential development and habitat changes from forest management have been the primary threats to this species (USFWS 1992). As a result of these threats, *Isotria medeoloides* was listed as endangered at the federal level in 1982 (USFWS 1982).

Since its listing in 1982, the habitat requirements of *Isotria medeoloides* have been more extensively studied to improve our understanding of its distribution and to locate additional populations. In 1994, *Isotria medeoloides* was reclassified from endangered to threatened after additional populations were discovered (USFWS 2008). Delisting of this species will only be considered when at least 61 sites that are uniformly distributed throughout the species range are protected (USFWS 1992). As of 1996, there were 13 protected sites across North Carolina, South Carolina, and Georgia (USFWS 1996). Trends in population sizes at these 13 sites are unknown; however, populations throughout the southeastern region have been observed as steadily decreasing (Kauffman pers. comm.; Schwartzman pers. comm.). Furthermore, most of the *Isotria medeoloides* populations in the Southeast contain fewer than 25 plants and could be more sensitive to local extinction than more common herbaceous species with larger population sizes in the region (USFWS 1996).

Species habitat models are widely used by land managers in species conservation projects as they have numerous benefits. Maps of potential suitable habitat contribute to the management of threatened and endangered species and can be used to identify potential sites for restoration, protection, or reintroduction of a species; target optimal monitoring areas; significantly decrease search time; and increase the likelihood of locating new species populations (Cleve et al. 2011; Razgour et al. 2011; Hernandez et al. 2006).

To better understand habitat requirements of *Isotria medeoloides* and facilitate location of additional populations in the southern Appalachian region, this study used a species habitat modeling program called Maximum Entropy (Maxent) to create a spatial habitat model for the orchid. This type of habitat model combines locations of known species occurrence with spatially continuous environmental layers to predict the potential geographic distribution of a species (Hernandez et al. 2006). Maximum entropy has become one of the most commonly used habitat suitability modeling methods because it has demonstrated robust predictive performance and high success in identifying locations of previously undiscovered species populations (Fuller et al. 2012; Cleve et al. 2011).

The purpose of this study was to improve our understanding of where *I. medeoloides* is found and to develop a habitat suitability model so that monitoring and conservation can be prioritized in the southern Appalachian region of the orchid’s range. The specific objectives were to: (1) characterize the habitat where *Isotria medeoloides* is found, and (2) evaluate the Maxent modeling approach for predicting suitable habitat of *Isotria medeoloides* in the southern Appalachian region

CHAPTER 2: LITERATURE REVIEW

Species Loss and Conservation

Every biome on Earth is experiencing species loss at an unprecedented rate (Pimm et al. 1995). As a result, studies have sought to understand the role of species diversity in maintaining ecosystems (e.g., Chapin et al. 2000; Symstad 2000). According to Ehrlich & Mooney (1983), all species should be assumed to contribute significantly to their ecosystems. Unfortunately, most studies have not addressed less common or rare species in ecosystem function and maintenance, and thus their role is poorly understood. Nevertheless, a few studies have shown that rare species can have large impacts on ecosystem processes (Lyons & Schwartz 2001; Marsh et al. 2000; Walker & Langridge 1999; Menges & Kimmich 1996; Theodose et al. 1996). For example, Marsh et al. (2000) determined that a rare Alaskan horsetail (*Equisetum spp*. L.) acts as a keystone species in soil resource dynamics by functioning as a nutrient pump moving limiting nutrients throughout soil horizons. In another study, the removal of less common species from sites successfully reduced diversity and thus increased the invasion of an exotic grass, *Lolium multiflorum* Lam. (Lyons & Schwartz 2001). These types of results highlight the importance of studying all types of species to understand their role and the role of biodiversity on ecosystem functioning.

Understory forest herbaceous species account for the majority of plant species richness in eastern deciduous forests of North America (Gilliam 2007; McCarthy & Bailey 1996; Huebner et al. 1995). This is significant because plant diversity creates habitat diversity to support a variety of other organisms (Hunter 1990). In addition, although the understory herbaceous layer accounts for only a small amount of biomass and annual energy fixation in a forest, nutrient cycling by herbaceous species contributes significantly to total ecosystem nutrient flux (Muller 2003; Lapointe 2001; Peterson & Rolfe 1982). For example, nutrients absorbed by herbaceous, spring ephemerals before canopy leaf-out can be released back into the soil through decomposition subsequently supporting early tree growth (Muller 1978; Muller & Bormann 1976). Additionally, the herbaceous layer can account for as much as 25% of total annual foliar litter that readily decomposes to release nutrients to available nutrient pools (Muller 2003).

Species loss continues to increase worldwide as the intensity and magnitude of threats to plant diversity increase (Gilliam 2014; Havens et al. 2014; Falk 1990). These threats include fragmentation, overexploitation, alien species, global climate change, and human use of land (Gilliam 2014; Falk 1990). Rare species that have been designated as threatened or endangered are of particular concern as they may be more sensitive to these threats than more common species due to their smaller population sizes, specific habitat requirements, or narrow geographic ranges (Whigham 2004; Jolls & Whigham 2003).

The continued loss of multiple species has initiated broad, multi-scale conservation strategies and the creation of several organizations such as the International Union for Conservation of Nature and Natural Resources (IUCN), the Nature Conservancy, Conservation International, World Wildlife Fund, and the Center for Plant Conservation. These organizations emphasize an integrated conservation approach to slowing species loss based on (i) determining the biological entity of concern; (ii) identifying specific threats; and (iii) coordinating conservation resources to address the threats (Falk 1990; Conservation International 1988; IUCN 1980). A few organizations such as NatureServe and IUCN have created ranking systems based on rarity of a particular plant or animal species, but the ranking does not mandate legal protection (NatureServe 2014; IUCN 2012). However, the Endangered Species Act (ESA) does legally mandate the protection of species and their habitats that have been designated as threatened or endangered until they are considered successfully recovered and no longer need protection (Havens et al. 2014;

Schemske et al. 1994; USFWS 1988). Other kinds of conservation strategies include protection and management of plant species of concern on federal or state lands, additional acquisition of land through research or private stakeholders to facilitate geographic range expansion (Kelly & Goulden 2008; Fiedler et al. 2007), species re-introductions (Havens et al. 2014; Schemske et al. 1994), and seed banking (Guerrant et al. 2014; Maunder et al. 2004). To keep pace with the escalating losses of species and biodiversity, a multidisciplinary approach to conservation involving scientists, government officials, land managers, agencies/private organizations, and the general public is recommended (Havens et al. 2014; Schemske et al. 1994; Falk 1990).

Factors Affecting Growth and Distribution of Forest Understory Herbaceous Species

The herbaceous layer in southern Appalachian forests is commonly defined as the forest stratum composed of all vascular plant species ≤ 1 m in height (Gilliam 2014). The growth and distribution of the herbaceous layer are affected by biotic and abiotic factors (Jackson et al. 2012; Elliot & Knoepp 2005; Searcy et al. 2003; Roberts & Gilliam 2003), natural and anthropogenic disturbance (Jackson et al. 2013; Carlson & Groot 1997; Huston 1994), and rarity attributes of herbaceous species (Gilliam 2014).

Biotic and Abiotic Factors

The biodiversity of the herbaceous layer can be affected by abiotic factors (e.g., water, temperature, nutrients, light), forest canopy composition, reproductive strategies, and seed dispersal mechanisms (Gilliam 2014; McEwan & Muller 2011; Whigham 2004). These factors can increase species diversity when they provide adequate conditions for growth, or they can result in a decrease in species diversity when they are not providing sufficient conditions for long-term survival of the species. In eastern deciduous forests these factors often result in a patchy or clumped distribution in the herbaceous layer where the distance between neighboring species is minimized (Rogers 1983; Bratton 1976) because the plants tend to “clump” together around patchy, crucial resources or exhibit clonal reproduction (Muller 2003).

Light is the primary limiting resource for the understory layer of the forest. Both the quantity (photon flux density [PFD]) and quality (spectral distribution) of light vary daily with changes in the sun’s position, seasonally by canopy foliage area, aspect, and geographic location (Warren 2008; Neufeld & Young 2003; Pitelka et al. 1985). Understory herbaceous species have evolved six distinct phenological forms due to the combination of both light and temperature stress. These are: 1) spring ephemerals; 2) summer-greens; 3) winter-greens; 4) heteroptics (i.e., summer-green and overwintering leaves); 5) evergreen (leaves last one year); and 6) evergreen (leaves last > two years (Tessier 2008; Uemura 1994; Chabot & Hicks 1982). Uemura (1994) determined the majority of understory plants in Japanese temperate forests were summer-greens (69%), followed by evergreens (19%), spring ephemerals (6%), winter-greens (3%), and heteroptic plants (1%). This is also most likely the case for all temperate deciduous forests (Gilliam 2014). Plant heights also differ between species of differing phenology. Data from flora of the Carolinas (Radford et al. 1968) showed mean maximum height of summer-green species was greater than spring ephemerals and evergreens. Growth rates of spring ephemerals and evergreens tended to be higher when light levels were greatest during the year, and growth of summer-greens was higher when competition with woody species increased.

Spring ephemerals leaf-out before the canopy develops when light levels are at maximum (Neufeld & Young 2003) and usually become dormant before mid-summer (Tessier 2008). Summer-greens are generally shade tolerant and thus flush during or after canopy closure because they are able to continue assimilating carbon under low-light conditions (Neufeld & Young 2003). Uemura (1994) defined winter-green plants as forming leaves in late-summer and senescence occurs early the following summer. This form differs from heteroptics, which have some kind of foliage on the plant at all times through the year and they live longer than winter-greens (Neufeld & Young 2003). True evergreen plants retain their leaves for more than one year (McCarron 1995; Koizumi & Oshima 1985) and use both autumn and spring canopy leafless periods to acquire most of their carbon (Rothstein & Zak 2001; McCarron 1995; Graves 1990). Chabot & Hicks (1982) proposed evergreen leaves evolved as an adaptation to drought, herbivory, or where soil nutrient levels are low.

Sunflecks are transitory increases in photon flux density that last only seconds to minutes throughout the day and contribute a significant proportion of the light received by forest understory herbaceous species (Vierling & Wessman 2000; Tang et al. 1999; Horton & Neufeld 1998; Koizumi & Oshima 1993; Chazdon & Pearcy 1991). For example, Horton & Neufeld (1998) determined nearly 80 percent of total PFD was supplied by sunflecks in the Great Smoky Mountains National Park. However, the growth response of herbaceous species to sunflecks varies. Koizumi & Oshima (1993) studied sunfleck dynamics of a warm temperate forest in Japan and determined that sunflecks contributed 7-10 percent of the carbon gained from May-July for *Syneilesis palmata* Maxim. but only 2-3 percent for *Pyrola japonica* Maxim. Sunfleck duration, frequency, and intensity (including background levels of diffuse PFD) likely determine different plant responses (Pearcy & Way 2012; Porcar-Castell & Palmroth 2012; Neufeld & Young 2003).

Forest canopy structure, which refers to the physical arrangement of tree species by canopy level, also impacts growth and distribution of understory forest herbaceous species through its affect on light quantity and quality, nutrients, and moisture (Roberts & Gilliam 2003; Hill & Silander 2001; McGee 2001; Rogers 1981; Tamm 1956). Up until the thinning phase in stand development, the size of tree crowns is largely determined by competition for light while the herbaceous layer is primarily responding to moisture and nutrient availability (Morris et al. 1993; Wilson & Shure 1993; Gilliam & Turrill 1993). Hill & Silander (2001) found the distribution of ferns in mixed hardwood-hemlock stands varied with proportion of different tree canopy species due to variation in light levels among contrasting stand structures. As forest stands move toward later successional stages, the canopy becomes dominated by shade tolerant species with greater leaf areas which generally decreases light available to the herbaceous strata (Brown et al. 1994; Huston 1994). In addition, suppression of wildfire in eastern deciduous forests has allowed a dense midstory of shade tolerant tree species to develop, further inhibiting light penetration to the understory (Abrams 1998; Cowell 1995; Jones 1974). These midstory canopies create a very different light microclimate than from the overstory (Smith et al. 1989) because the mean duration of sunflecks decreases with canopy height (Pearcy et al. 1990; Pearcy 1988). Spatial heterogeneity of the forest understory created by pit and mound microtopography can cause variation in light levels, soil moisture, nutrients, and temperature that affect herbaceous species distributions (Clinton & Baker 2000; Peterson & Campbell 1993). For example, McGee (2001) found that decaying logs provided important habitat for the establishment of several herbaceous species.

In addition to light, nutrient deficiencies can limit herbaceous species growth and distribution, and thus many species have developed mechanisms to overcome this limitation. For example, nutrients allocated early in the growing season to aboveground biomass (leaves and reproductive structures) can be moved to belowground structures thus allowing plants to survive through the colder months (Whigham 2004; Nault & Gagnon 1988). Other understory herbaceous species in resource poor environments have a greater uptake of nutrients when flushing (Muller 2003), then immobilize these nutrients in their biomass before the canopy closes (Muller 2003; Zak et al. 1990). Additionally, many understory herbaceous species develop mycorrhizal associations to facilitate nutrient absorption

(Lapointe & Molard 1997; Whitbread et al. 1996). Levels of mycorrhizal fungi activity in the forest herbaceous layer differ across nutrient, moisture, and forest successional stage and may influence herbaceous species growth and distribution (Whigham 2004; Boerner et al. 1996; DeMars & Boerner 1995).

In addition to limiting plant growth, pollinator activity can be indirectly affected by light levels in the understory. When light availability is high, herbaceous plants generally grow large and produce larger floral structures which have been shown to attract pollinators and increase the number of floral visits (Kilkenny & Galloway 2008; Grindeland et al. 2005; Galloway et al. 2002). Variable pollination success can limit herbaceous species population growth and distribution. Therefore, vegetative reproduction is very common among species in the forest understory (Vallejo-Marin et al. 2010; Honnay et al. 2005; Whigham 2004) and herbaceous species populations are known to persist for several years relying only on vegetative modes of reproduction (Whigham 2004). When seed production does occur, seeds are usually dispersed a distance less than the height of the plant (Bierzychudek 1981; Barkham 1980; Ernst 1979). Many understory herbaceous species also have low fecundity and large seeds, which limits species distributions (Verheyen & Hermy 2001; Ehrlén & Eriksson 2000) and forces them to quickly adapt to changing conditions (Hermy & Verheyen 2007; Hermy et al. 1999; Matlack 1994). However, many invertebrate species, especially ants in the genera *Aphaenogaster* Mayr*., Camponotus* Mayr*., Myrmica* Latreille*., Formica* L.*, and Lasius* Fabricius (Kalisz et al. 1999; Handel et al. 1981; Beattie et al. 1979), and vertebrate species such as white-tailed deer (*Odocoileus virginianus* Zimmermann.) and moose (*Alces alces* L.) can be important dispersal vectors (Pakeman 2001).

Natural and Anthropogenic Disturbance Factors

Disturbances affecting the herbaceous layer range from microspatial such as frost and trampling (McCarthy & Facelli 1990) to macrospatial including herbivory (Thiemann et al. 2009; Tremblay et al. 2007; Whigham 2004; Campbell et al. 2004), canopy mortality (Whigham 2004; Roberts & Gilliam 2003; Moore & Vankat 1986), fire (De Grandpre & Bergeron 1997), wind damage (Roberts & Gilliam 2003; Peterson 2000), and agricultural (Jackson et al. 2013; Whigham 2004) or forestry practices (Jackson et al. 2013; Wyatt & Silman 2010; Whigham 2004; Gilliam et al. 1995). The herbaceous layer is sensitive to these disturbances due to the changes in resource availability, although the response varies among sites and disturbance type (Singleton et al. 2001; Meier et al. 1995). Compared to abundance, plant performance may be more informative for assessing species responses since plant distributions can take longer to respond to changes in conditions (Jackson et al. 2013; Farrington et al. 2009; Valverde & Silvertown 1998).

A macrospatial disturbance to forest herbaceous species that occurred throughout the 1900s was overgrazing from the large population increase in white-tailed deer (*Odocoileus virginianus* Zimmermann) which threatened many native plant communities in eastern deciduous forests (Thiemann et al. 2009; Whigham 2004). Most forest herbaceous species do not produce new tissues in response to herbivory and thus complete or partial removal can result in decreased flowering or death of the plant (Whigham 2004; Primack et al. 1994; Syrjänen & Lehtilä 1993; Whigham 1990). However, partial removal has also been shown to have no effect on growth and reproduction (Agren & Willson 1992).

Canopy gaps can be created by wind disturbance and subsequent canopy mortality can increase light levels to the forest floor (Whigham 2004; Roberts & Gilliam 2003). Most forest herbaceous species respond to these gaps by increasing growth and reproduction (Whigham 2004; Neufeld & Young 2003; Collins & Pickett 1988). Additionally, mounds and logs from canopy mortality can be important colonization sites for species such as the orchid *Tipularia discolor* (Pursh) Nutt. (Rasmussen & Whigham 1998). The average return rate of canopy gaps due to wind damage in eastern deciduous forests is generally once every 100 years (Runkle 1982), which can explain some of the long-term variation in population sizes of forest herbaceous species (Gilliam 2014).

Most eastern deciduous forests today are relatively young, second-growth stands that are the result of historic clearing for agriculture and timber harvesting (Whitney 1996). Despite higher light levels in these forests compared to older-growth stands, the understory herbaceous layer has been reduced in richness, cover, and abundance due to these past disturbances (Wyatt & Silman 2010; Vellend 2005; Bellemare et al., 2002; Singleton et al. 2001). A reduction in the abundance of herbaceous species from previous agriculture can be more severe than logging because of the complete removal of the herbaceous vegetation and seed banks (Fraterrigo et al. 2006; Vellend 2004). Timber harvesting is still a dominant form of land use in the forests of the Southern Appalachians (Jackson et al. 2013) and several studies have determined effects from logging on the understory herbaceous layer can persist for decades to centuries (Wyatt & Silman 2010; Elliott et al. 1997; Meier et al. 1995). However, responses of the herbaceous layer vary with disturbance intensity (Moola & Vasseur 2008; Roberts & Gilliam 1995). Roberts & Gilliam (1995) found that the response of the herbaceous layer to harvesting disturbance was site-specific in mesic and dry mesic aspen (*Populus* L.) stands while Fredericksen et al. (1999) reported only the most intense forest harvesting in northern hardwood and oak-hickory forests in Pennsylvania had an effect on herbaceous species richness, diversity, and cover.

Rarity Attributes

Attributes associated with herbaceous species rarity can also affect growth and distribution of species in the understory herbaceous layer. Species are generally defined as rare if they have specific habitat requirements, restricted geographic distributions, or small population sizes (Miller-Struttmann 2013; Maliakal-Witt et al. 2005; Gaston 1994; Rabinowitz et al. 1986). A rare species can be broadly distributed, but not locally abundant; narrowly distributed, but locally abundant; or, narrowly distributed, and not locally abundant (Rabinowitz et al. 1986). Rarity can be caused by a species' poor colonization ability, limited adaptability to environmental changes, and specific requirements for germination and growth (Swarts et al. 2010). For example, Swarts et al. (2010) reported the rare orchid, *Caladenia huegelii* Rchb.f., formed a symbiotic relationship with a specific mycorrhizae fungus required for its growth while the more common *Caladenia* R.Br. species formed relationships with a variety of mycorrhizal fungi species. Rarity attributes of herbaceous species are highly species-specific and rare plant species can be found across various habitat types and geographic locations (Crain et al. 2014; Essl et al. 2009; Lavergne et al. 2004; Murray et al. 2002; Kessler 2000; Bevill & Louda 1999; Gaston 1994; Fiedler 1987). However, there are some trends associated with plant species rarity. For example, habitat types consistently associated with rare plant species generally have an open forest understory with low herbaceous cover (Gabrielová et al. 2013; Lavergne et al. 2004) and are recently disturbed or in early ecological successional stages (Essl et al. 2009; Griggs 1940). Rare, herbaceous species often have less genetic variability (Cole 2003; Gitzendanner & Soltis 2000; Soltis & Soltis 1991) with higher degrees of self-incompatibility (Gabrielová et al. 2013; Lavergne et al. 2004; Kunin & Gaston 1993; Harper 1979), greater pollen failure (Ashman et al. 2004; Wilcock & Neiland 2002; Larson & Barrett 2000) and generally produce significantly fewer seeds (Miller-Struttmann 2013; Lavergne et al. 2004; Murray et al. 2002) than their more common congeners. In addition, seed predation is often higher in rare plant species (Münzbergová 2005) and they have low competitive ability in more productive habitats but can tolerate stressful conditions (Lavergne et al. 2003; Walck et al. 1999; Médail & Verlaque 1997; Drury 1974; Griggs 1940).

Isotria medeoloides

Isotria medeoloides (Pursh) Raf. is a rare and federally threatened orchid limited to small, isolated populations in eastern forests from Maine to Georgia with three main population concentrations: New England, Virginia, and southern Blue Ridge (Figure 1) (NatureServe 2014; USFWS 2008; Vitt 1991; Mehrhoff 1989b). The total population of this species is estimated to be less than 3000 plants (NatureServe 2014) and it is thus considered one of the rarest orchids endemic to eastern North America (Sperduto & Congalton 1996; Vitt 1991; Mehrhoff 1989b). This species is primarily threatened by habitat loss due to factors such as urban development (USFWS 2008; Sperduto & Congalton 1996; USFWS 1992). Like other understory forest herbaceous species, the survival and distribution of *I. medeoloides* depends on its life history, population dynamics, and habitat requirements.

Life History and Population Dynamics

Isotria is a genus with only two species: *Isotria medeoloides* (small whorled pogonia) and *Isotria verticillata* (large whorled pogonia). Similar to most orchids, *I. medeoloides* is a long-lived, perennial species (Mehrhoff 1989b; Tamm 1972). It is characterized by a whorl of five or six leaves at the apex of a glabrous, pale-green, hollow stem that is 6-35 cm tall. It produces a single, yellowish-green flower that is self-pollinated and lacks fragrance; pollination guides; and nectar. The leaves are pale green, average 8 cm long and 4 cm wide, and droop during flowering (Sperduto & Congalton 1996; Patrick et al. 1995). The species has three, green, linear sepals that are 20-30 mm long, 3-5 mm wide, and outwardly

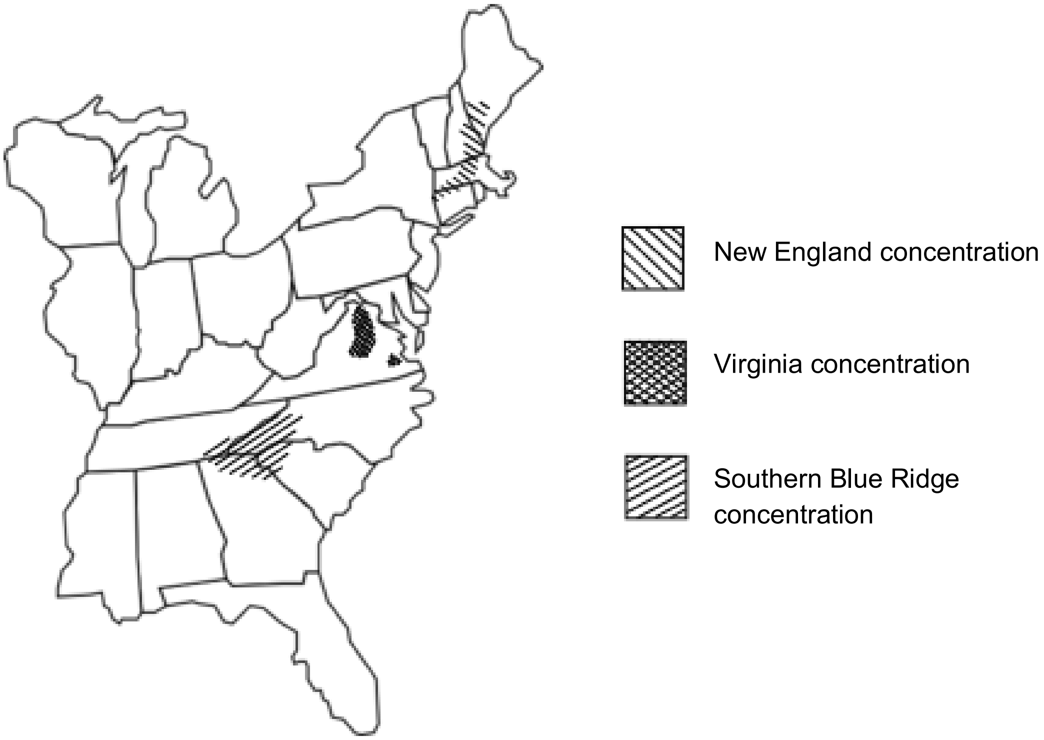


Figure 1. Three main population concentrations of *Isotria medeoloides* in North America.

spreading. The sepals are roughly equal in length, which is the attribute for the genus name (isos, equal; treis, three). Its two petals are pale yellowish-green, approximately as long as the sepals, and bend forward over the third petal. The third petal, known as the lip, is greenish-white, 3-lobed, and abraded on the top surface with small yellow projections. The petals and sepals of *Isotria medeoloides* are narrower than the typical orchid.

Individual *I. medeoloides* plants exist in four types of states: vegetative, flowering, with an abortive flower bud, or dormant (Mehrhoff 1989a, 1989b). *I. medeoloides* produces visible overwintering vegetative buds for the following growing season on the rootstock at ground level in August or September (USFWS 1992; Ware 1991; Mehrhoff 1983). The species usually emerges from winter dormancy in April and flowers through May with blossoms lasting 4-14 days (Chafin 2007; Ware 1991; Mehrhoff 1983). Flowering frequency and population sizes vary from year to year (McCormick et al. 2012; USFWS 1992; Ware 1991; Mehrhoff 1989a) but larger plants seem to flower more frequently than smaller plants (Chafin 2007).

Population monitoring is difficult due to dormancy in *I. medeoloides* (USFWS 2008), which varies by site and year (Vitt 1991) and can extend from less than three (Mehrhoff 1989b) or four years (USFWS 2008; Brumback & Fyler 1988) to 10-20 years (USFWS 1985). Most populations contain fewer than 20 individuals (USFWS 2008; USFWS 1992) that are widely scattered (Vitt 1991; Mehrhoff 1983), with about one-third of those individuals flowering per season (Mehrhoff 1983). Flowering frequency may be associated with resource accumulation in the previous season (Mehrhoff 1989a; Mehrhoff 1989b) or plant size (USFWS 2008; Chafin 2007; Mehrhoff 1989a). The flower exhibits high rates of self-fertilization and negligible gene flow is believed to occur between populations (Stone et al. 2012; Vitt & Campbell 1997; Mehrhoff 1983). The northern populations have the most genetic diversity, while southern populations seem to be genetically depauperate (Stone et al. 2012). Vitt & Campbell (1997) concluded that reproduction may be limited by resources as opposed to pollen availability. About 80% of flowers initiate fruit production (Vitt & Campbell 1997; Mehrhoff 1983) and the majority of those develop into mature capsules (Mehrhoff 1983). The fruit is an erect capsule about 2.5 cm long and 1 cm wide on a stalk nearly as long as the capsule that does not fully ripen and dehisce until autumn (Patrick et al. 1995; USFWS 1992). The seeds are dust-like and primarily dispersed by wind but generally land near the parent plant. Seed production is considered moderate as compared to other orchids (McCormick et al. 2012; USFWS 1992; Mehrhoff 1989a) and vegetative reproduction is also infrequent (Mehrhoff 1989a; Brumback & Fyler 1983; Mehrhoff 1983).

Mehrhoff (1983) concluded that no aspect of flowering, pollination, or seed dispersal should be limiting growth or distribution of *I. medeoloides* due to their high pollination rate and number of seeds produced. In contrast, temperate terrestrial orchids typically require specific conditions to germinate and establish. Similar to other orchids, *I. medeoloides* seed coats contain germination inhibitors (McCormick et al. 2012) and seeds contain only a small amount of food reserves or none at all, thus seeds depend on appropriate seedbed conditions and compatible mycorrhizal fungi to provide essential nutrients for germination and growth during the early stages of development (Swarts et al. 2010; Swarts & Dixon, 2009; Chafin, 2007; Rasmussen & Whigham 2002; Jackson & Mason 1984). Specific mycorrhizal fungi associated with *Isotria medeoloides* have not yet been identified (McCormick et al. 2012). However, ectomycorrhizae on oak (*Quercus* L.), hickory (*Carya* Nutt.), and beech (*Fagus* L.) roots have also been observed on *I. medeoloides* roots (McCormick et al. 2012; Ames 1922) with the majority belonging to the *Russula* Pers. genus in the Russulaceae family (McCormick et al. 2012). McCormick et al. (2012) reported *I. medeoloides'* seeds planted in the field or laboratory have never successfully germinated, but seed packets that have been deployed at several study sites are currently being monitored. Further studies are needed to understand seed germination and seedling establishment requirements (Mehrhoff 1989a; Gaddy 1985; Mehrhoff 1983) that may be affecting the survival and distribution of the species.

Habitat Requirements

Several studies have described habitat requirements of *I. medeoloides* based on where populations have been discovered (Sperduto & Congalton 1996; Mehrhoff 1989a; Rawinski 1986; Gaddy 1985). The soils at these sites are typically acidic, sandy loams (Sperduto & Congalton 1996; Vitt 1991; Ware 1991; Mehrhoff 1989a) with pH ranging 3.6 – 4.1 (Sperduto 1993; Mehrhoff 1989a; Brumback & Fyler 1983; Stuckey 1967) or up to 5.5 (Ware 1991). These pH ranges are similar for other orchid species (Sheviak 1983; Stuckey 1967). The low soil pH and subsequent low soil nitrogen, phosphorous, and potassium availability results in relatively infertile sites where *I. medeoloides* and associated species are found in New Hampshire and Maine (Sperduto 1993; Mehrhoff 1989a; Ware 1987; Brumback & Fyler 1983; Stuckey 1967). However, other associated species across the geographic range of *I. medeoloides* are indicative of more nutrient-rich situations (Sperduto 1993; USFWS 1992; Brownwell 1981). *I. medeoloides* is often observed growing near decaying organic matter such as stumps or logs (Whigham pers. comm.; Ware 1991; Brumback & Fyler 1983; Grimes 1921) and the organic accumulation generally present at *I. medeoloides* sites (Mehrhoff 1989a; Rawinski 1986; Homoya 1977) might provide nutrients not readily available from soils.

Moisture may be one of the most important factors controlling the establishment and survival of orchid species due to their specialized seed coats, large leaf areas, and few roots (Rasmussen 1995; Arditti 1967; Stuckey 1967; Correll 1978). The majority of *I. medeoloides* populations generally occur at the base of slopes or mid-slope positions with inclination ranging from 0 to 30 percent (Sperduto 1993; Ware 1991; Mehrhoff 1989a; Rawinski 1986). A common characteristic of soils at *I. medeoloides* sites is the presence of an impervious layer (Sperduto 1993; USFWS 1992; Rawinski 1986; Grimes 1921), with a mean depth of 58 cm (Sperduto 1993). This pan layer might slow downward percolation of water thus increasing lateral flow of water significantly. The relatively flat slopes combined with the impervious soil layer, could create lateral water flow conditions needed for the orchid's survival (Sperduto 1993; USFWS 1992; Rawinski 1986). *I. medeoloides* has been repeatedly observed growing in mesic to dry-mesic habitats (Sperduto & Congalton 1996; Mehrhoff 1989a; Ware 1989; Rawinski 1986; Homoya 1977) and near vernal streams throughout its range (Sperduto 1993; Mehrhoff 1989a; Rawinski 1986). Additionally, soil moisture measurements in Virginia have revealed that where *I. medeoloides* is found, soil moisture levels are consistently higher than the surrounding areas, even during drought conditions (Ware 1989). However, sustained drought will cause wilting of the whorl and drying out of the capsules (Ware 1989; Homoya 1977).

In addition to low percent slope, aspect tends to be east-facing (Ware 1991; Rawinski 1986) but varies (Sperduto 1993; Mehrhoff 1989a; Rawinski 1986) and Rawinski (1986) suggested aspect may only be critical at the extremes of the orchid's range. The apparent relatively high moisture requirement of *I. medeoloides* is likely why mycorrhizae fungi are important for successful germination and growth (Swarts & Dixon 2009; Rasmussen & Whigham 2002; Sperduto 1993). Because the seeds float, the consistent proximity of *I. medeoloides* populations with intermittent streams may also contribute to seed dispersal (Rasmussen 1995; Stuckey 1967).

I. medeoloides generally grows in mixed deciduous or mixed deciduous/coniferous forests (Sperduto & Congalton 1996; Ware 1991; Mehrhoff 1989a; Rawinski 1986; Brackley 1985). There does not seem to be specific indicator tree species associated with the species (Mehrhoff 1989a) but *Pinus strobus* L., *Tsuga Canadensis* (L.) Carrière (Sperduto 1993; Rawinski 1986; Gaddy 1985), *Acer rubrum* L., and *Quercus rubra* L. (Sperduto 1993; Mehrhoff 1989a; Rawinski 1986) are found throughout the range of *I. medeoloides*. Other orchids, including *Goodyera pubescens* (Willd.) R. Br. and *Cypripedium acaule* Aiton are also frequently present (Sperduto 1993; USFWS 1992; Rawinski 1986). There are several herbaceous species that commonly occur with the orchid in both its northern and southern range including *Medeola virginiana* L., *Thelypteris noveboracensis* (L.) Nieuwl., *Mitchella repens* L., and *Vaccinium angustifolium* Aiton (USFWS 1992; Mehrhoff 1989a; Rawinski 1986; Gaddy 1985). These relatively common herbaceous species may serve as reliable indicators of *I. medeoloides* habitat when found together in abundance (Rawinski 1986).

Most *I. medeoloides* populations occur in second or third-growth forests with a history of agricultural use (Sperduto 1993; Vitt 1991; Ware 1991; Mehrhoff 1989a). The ages of the trees in these forests have been estimated between 45 and 80 years at sites in Virginia (Ware 1987) and New Hampshire (Brumback & Fyler 1983) and as young as 30 years at sites in South Carolina (Gaddy 1985). Variation in the basal area and tree densities found where *I. medeoloides* is growing could relate to light required by the orchid because too much shading and low light levels could be a limiting factor. Total basal area per hectare consistently ranges between 12-44 m^2/ha (Sperduto 1993; Mehrhoff 1989a) although tree densities are more variable and generally range from 759-2925 stems/ha (Mehrhoff 1989a) but can be as high as 3237-6039 stems/ha (Brumback & Fyler 1983).

Flowering individuals of *I. medeoloides* have been reported to require more light (Mehrhoff 1989a; Stuckey 1967), although Brumback & Fyler (1983) did not find a significant correlation between herbaceous cover and reproductive class (vegetative, flowering, with an abortive flower bud, or dormant). Many populations have been found near semi-permanent canopy gaps caused by old logging roads, blowdowns, or streams (Brackley-Tolman 1991; Mehrhoff 1989a; Gaddy 1985). Studies have postulated that increased light levels may be one explanation for why terrestrial orchids seem to colonize and grow well in habitats with moderate disturbance frequency (Calder et al. 1989). Generally, understory and herbaceous cover is low where *I. medeoloides* is found (Sperduto 1993; Ware 1991;

Mehrhoff 1989a; Grimes 1921). However, a few studies have reported *I. medeoloides* growing in areas with high fern cover (Brackley-Tolman 1991; Rawinski 1986). Overall, the common habitat characteristics associated with *I. medeoloides* throughout its range include low herbaceous cover (except when among ferns), a relatively open understory canopy, and proximity to streams, logging roads, or other features creating semi-permanent breaks in the forest canopy (Sperduto & Congalton 1996; Patrick et al. 1995; USFWS 1992; Ware 1991; Mehrhoff 1989a; Rawinski 1986; Gaddy 1985).

Species Habitat Models

Species habitat models are important tools for research in spatial ecology; biogeography; and climate change, conservation planning, and land management (Elith et al. 2006; Guisan & Thuiller 2005; Rushton et al. 2004; Raxworthy et al. 2003). These models use associations between known species occurrence locations and environmental variables to identify habitat that is suitable for a particular species (Pearson 2009; Elith et al. 2006; Hernandez et al. 2006; Guisan & Zimmermann 2000). Species habitat models are labeled as ecological niche models (ENMs), species distribution models (SDMs), resource selection functions (RSFs), correlative models, and climate envelopes (Kearney & Porter 2009; Pearson 2009; Elith & Leathwick 2009). Regardless of the label, the basic modeling process is the same whereby the spatial distribution of suitable habitat is being modeled rather than the actual distribution of the species (Pearson 2009; Hijmans & Graham 2006; Elith et al. 2006; Guisan & Zimmermann 2000). These habitat models have successfully guided field surveys to identify undiscovered populations (Bourg et al. 2005; Graham et al. 2004; Raxworthy et al. 2003), determined potential impacts of climate change on population distributions (Thuiller et al. 2005a; Iverson & Prasad 1998), predicted species invasions (Thuiller et al. 2005b), and they have increased detection rates for rare species (Buechling &

Tobalske 2011; Guisan et al. 2006; Edwards et al. 2005; Sperduto & Congalton 1996).

Data Types and Sources Used in Species Habitat Models

Species habitat models use species occurrence data (known locations of the target species) and environmental data (habitat where the target species is found). Species occurrence data can be based on presence-only (i.e., locations where the species has been observed) or on presence/absence (i.e., locations where the species has been observed as present or absent) (Pearson 2009; Graham et al. 2004). Species occurrence data can be obtained from field surveys (Williams et al. 2009; Graham et al. 2004; Fleishman et al. 2001), herbaria collections (Pearson et al. 2007; Graham et al. 2004; Raxworthy et al. 2003), and online databases (Buechling & Tobalske 2011; Pearson 2009; Hernandez et al. 2006). Different modeling algorithms have been developed for both types of species occurrence data. In some cases, models that have included absence data have performed better than models that have not (Engler et al. 2004; Brotons et al. 2004; Stockwell & Peterson 2002). However, absence records are often not available or are unreliable (Razgour et al. 2011; Kumar & Stohlgren 2009; Pearson 2009) when absences were recorded when the species was not found even if the habitat was suitable.

Digital environmental data used in habitat suitability models are selected to optimally reflect three main types of influence on the target species: (i) limiting factors which are eco-physiological variables such as temperature, precipitation, and soil features, (ii) disturbances defined as natural or anthropogenic affecting ecosystem processes, and (iii) resources defined as compounds that can be assimilated by organisms (e.g., energy and water) (Guisan & Thuiller 2005; Guisan & Zimmermann 2000). The appropriate suite of environmental variables used and subsequent model performance is significantly improved when only predictors known to be ecologically relevant to the target species are used (Elith

& Leathwick 2009; Austin 2002). The most common type of environmental data used are variables that describe the abiotic environment related to climate, topography (e.g., elevation, aspect, slope), soil type, and land cover type (Yang et al. 2013; Pearson 2009; Kumar & Stohlgren 2009; Williams et al. 2009; Guisan & Zimmermann 2000; Sperduto & Congalton 1996). This digital data is obtained from a variety of sources depending on the data type and scale required. Climate data, including worldwide temperature and precipitation data are commonly obtained from WorldClim (http://www.worldclim.org; Yang et al. 2013; Razgour et al. 2011; Rebelo & Jones 2010; Pearson et al. 2007; Hijmans et al. 2005) or the National Oceanic and Atmospheric Administration (NOAA) (http://www.noaa.gov; Pearson 2009; Pearson et al. 2007). Topographic data including elevation, slope, and aspect are generally accessed from the United States Geological Survey (USGS) National Elevation Dataset (http://ned.usgs.gov; Williams et al. 2009; Pearson et al. 2007; Hernandez et al. 2006; Sperduto & Congalton 1996). Remotely sensed data obtained from USGS Landsat (http://landsat.usgs.gov; Buechling & Tobalske 2011; Williams et al. 2009; Sperduto & Congalton 1996) can provide land cover data, digitized maps, and other satellite imagery information. Regardless of the source, the environmental data are primarily manipulated using a geographical information system (GIS) to prepare it for use in the modeling program (Pearson 2009; Hernandez et al. 2006; Guisan & Thuiller 2005; Guisan & Zimmermann 2000).

Modeling Algorithms

There are a number of different modeling algorithms that classify the probability of species presence (and absence) based on the set of environmental predictor variables. These algorithms vary in their modeling approach, selection of relevant predictors, how they define fitted functions, the way they determine predictor contributions, if they allow interactions, and in how they predict geographic presence patterns (Pearson 2009; Elith et al. 2006; Burgman et al. 2005; Guisan & Zimmermann 2000). The most common modeling algorithms can be categorized into three approaches: (i) presence-only, (ii) regression-based, and (iii) machine-learning. The first group relies only on presence records and includes BIOCLIM and DOMAIN. BIOCLIM is an envelope-style method that summarizes up to 35 climate parameters throughout a species' range and identifies climatic suitable habitat under current and future climate scenarios (Booth et al. 2014; Beaumont et al. 2005). DOMAIN uses a distance-based method such as the Gower metric to predict environmental similarity between locations of occurrence and unvisited sites (Elith & Leathwick 2009; Elith et al. 2006; Carpenter et al. 1993).

Regression-based modeling methods extend the envelope and similarity approaches by modeling variation in species abundance or occurrence throughout a study area. Additionally, these models identify environmental predictors based on relative contributions to the prediction and require both presence and absence data (Elith & Leathwick 2009; Pearson 2009; Guisan et al. 2002). These models include generalized linear models (GLMs), generalized additive models (GAMs), and multivariate adaptive regression splines (MARs). GLMs are commonly used to fit parametric data or some combination of linear, quadratic, and/or cubic responses. GAMs use non-parametric smoothing functions to describe non-linear responses (Elith & Leathwick 2009; Elith et al. 2006; Lehman et al. 2002). GAMs are more capable than GLMs of modeling complex ecological responses (Lehmann et al. 2002; Yee & Mitchell 1991). GLMs and GAMs have been extensively used in many species distribution modeling applications due to their ability to model ecological relationships and their strong statistical foundation (Elith et al. 2006; Austin 2002). MARs are similar to GAMs except they use multiple linear functions instead of smoothing functions to describe non-linear responses. Additionally, MARs have an option to analyze plant community data by relating occurrences of multiple species to environmental predictors in one analysis. They are also faster to implement than GAMs and simpler to use in GIS applications (Elith et al. 2006; Leathwick et al. 2005).

The third group of modeling methods is based on machine-learning or data mining. Examples include artificial neural networks (ANNs), regression trees, genetic algorithms, and maximum entropy models. ANNs are machine-learning methods that have the ability to model complex ecological relationships by constructing a predictive model based on *a priori* examples of data with known outputs (Olden et al. 2008; Lek & Guégan 1999). Regression trees graphically explain the variation of a response variable by splitting the data repeatedly into more homogeneous groups using different combinations of explanatory variables (Prasad et al. 2006; De'ath & Fabricius 2000). Genetic algorithms predict species' distributions by using rule sets that attempt to mimic the action of natural selection (Hamblin 2013; Stockwell 1999). Maximum entropy (Maxent) models estimate species' distributions by determining the distribution of maximum entropy (i.e., closest to uniform) subject to the constraints of the environmental variables (Elith et al. 2006; Phillips et al. 2006). Most of these machine-learning methods control predictor selection and have the capability to automatically detect and fit interactions between variables, which often gives them higher predictive power than more conventional methods such as distance or regression-based methods (Elith & Leathwick 2009; Elith et al. 2006). A few of the machine-learning based algorithms are termed 'black box' because the model uses inputs and produces an output, while the internal functioning is unclear (Pearson 2009; Elith & Leathwick 2009). For example, ANNs have demonstrated high predictive ability (Segurado & Araújo 2004; Thuiller 2003; Pearson et al. 2002), but determining the relative contributions of each predictor variable is difficult and complex (Pearson 2009).

Regression-based and machine-learning modeling algorithms rely on both presence and absence (or pseudo-absence) data (e.g., ANNs, GLMs, GAMs, MARs, regression trees), pseudo-absence data (e.g., genetic algorithm), or presence-only with background data (e.g., Maxent). Methods that use ‘background’ environmental data compare the environment where the species is present to the environment across the rest of the study area (‘background’). Methods that use ‘pseudo-absences’ compare the environment between presence locations and a set of localities chosen from the study area used to replace real absence data (Pearson 2009).

Maximum Entropy (Maxent)

Maximum entropy (Maxent) is one of the most commonly used habitat suitability modeling methods because it has been shown repeatedly to outperform other presence-only and presence/absence methods (Razgour et al. 2011; Gibson et al. 2007; Elith et al. 2006; Hernandez et al. 2006). The model uses the principle of maximum entropy with presence-only data that relate environmental variables and habitat suitability to approximate the species’ niche (Warren & Seifert 2011). This method can predict habitat suitability with few presence records and it does not require absence data, which is often not available or unreliable (Razgour et al. 2011; Kumar & Stohlgren 2009). Maxent uses background information but does not interpret pixels without species records as absences and, therefore, this method may not suffer from biases that result from including absence data that may be inaccurate (Gibson et al. 2007). Maxent also has the ability to model complex relationships and interactions among ecological variables (Wisz et al. 2008). However, very rare species with few occurrence records and geographic sampling bias (e.g., accessibility from roads) can result in Maxent predictions that are prone to overfitting (Hernandez et al. 2006). These problems can be addressed in Maxent by applying regularization techniques to reduce overfitting for a dataset with few localities and sampling bias (Anderson & Gonzalez 2011; Phillips et al. 2006). This technique relaxes the estimated distribution so that the mean for a given environmental variable is close to the empirical average rather than being exactly equal to it and it can be set by the user (Phillips et al. 2006; Hernandez et al. 2006). Hernandez et al. (2006) showed that Maxent is able to compensate for small species occurrences by using regularization techniques and still perform near maximal accuracy level.

Assessing Predictive Performance

Assessing the predictive accuracy of a species habitat model is important for determining the suitability of the modeling method for a specific application, or to compare different models (Pearson 2009; Pearce & Ferrier 2000). There are several different approaches for model validation based on strategies used for generating test data and the use of various test statistics. Test data used to evaluate the predictions of the model differ from training data used to build the model (Pearson 2009; Pearson et al. 2007). Test data are collected independently from the data used to train the model to avoid over-fitting errors (Pearson 2009; Kumar & Stohlgren 2009; Pearson et al. 2007; Araújo et al. 2005a). Independent species location data can be generated from different time periods (Araújo et al. 2005a; Fleishman et al. 2003), different regions (Peterson & Shaw 2003; Beerling et al. 1995), or different spatial resolution (Araújo et al. 2005b; Pearson et al. 2004). Unfortunately, independent location data is not available in many cases, and it is common to partition the available data into test and training sets using bootstrapping, randomization, or k-fold partitioning (Elith & Leathwick 2009; Pearson 2009; Araújo et al. 2005a; Fielding & Bell 1997) where multiple models are built using training sets and predictive performance is determined using the corresponding test data. It is common to partition the data into 70% for training and 30% for testing the model (Yang et al. 2013; Huberty 1994). This approach may not be suitable when location data is limited and, therefore, the relative proportions of data may depend on the number of location records available (Pearson 2009; Pearson et al.

2007).

Several different test statistics have been applied to assess the performance of species habitat models (Pearson et al. 2007; Pearce & Ferrier 2000; Guisan & Zimmermann 2000; Fielding & Bell 1997). Most test statistics are derived from a contingency table that describes frequencies with which observed presences and absences are correctly and incorrectly predicted. When only presence data are available, test statistics are limited to those that do not rely on absence data (Pearson 2009; Pearson et al. 2007). Common test statistics derived from contingency tables include measures of accuracy (Pearson 2009; Cohen 1968), Kappa scores (Pearson 2009; Fielding & Bell 1997; Monserud & Leemans 1992), sensitivity and specificity (Pearson 2009; Guisan & Zimmermann 2000), or the Area Under the Receiver Operating Characteristic Curve (Yang et al. 2013; Razgour et al. 2011; Rebelo & Jones 2010; Elith & Leathwick 2009; Pearson 2009). Measures of accuracy require presence and absence data, and describe the proportion of test localities correctly predicted by the model. Kappa scores are similar to measures of accuracy but the proportion of correct predictions expected by chance is accounted for (Pearson 2009; Monserud & Leemans 1992). A few studies have suggested Kappa scores >0.75 represent excellent model performance (Fielding & Bell 1997; Landis & Koch 1977). Sensitivity is used when only presence data is available and measures the proportion of observed occurrences correctly predicted. An inaccurate, high sensitivity score can occur if the model predicts high suitability in an excessively large proportion of the study area (e.g., sensitivity would equal one if all habitat was predicted as suitable). Therefore, it is recommended to use statistical significance tests to determine whether the model predictions are greater than what would be expected by chance (e.g., Chi-square test, one-tailed binomial test [Zar 1996]) (Pearson 2009; Anderson et al. 2002). The specificity test statistic is rarely used because it relies solely on absence data and describes the proportion of observed absences that are correctly predicted by the model. Nevertheless, it is an important measure used in Receiver Operator

Curve (ROC) analyses (Pearson 2009). The ROC describes the relationship between the proportion of observed presences predicted correctly (sensitivity) and the proportion of observed absences predicted incorrectly (1 – specificity). The area under the receiver operator curve (AUC) ranges from 0.5 for models that perform no better than random, to 1.0 for models with perfect predictive accuracy (Pearson 2009; Phillips et al. 2006). AUC scores > 0.75 represent reasonable to high predictive accuracy (Elith et al. 2006; Pearce & Ferrier 2000). Although AUC requires both presence and absence data, Phillips et al. (2006) demonstrated this test can be used with pseudo-absences, which are randomly selected background points from the study area. In this case, AUC is measuring whether the model is predicting presence more accurately than a random prediction as opposed to the ability to distinguish presence from absence (Pearson 2009; Phillips et al. 2006). Additionally, it is argued that observed absence data should be excluded from model validation if the model is being used to predict *potential* suitable habitat since species absence may be due to factors such as dispersal limitation, biotic interactions, or land-use history that were not included during model development (Pearson 2009; Pearson et al. 2007; Pearson & Dawson 2003).

Ultimately, selection of a test statistic depends on the application of the model. If the goal of the model is to predict the *actual* species distribution, then test statistics requiring both presence and absence records may be the most appropriate (e.g., accuracy or Kappa scores). On the other hand, if the objective is to predict the *potential* species distribution then validation assessments using sensitivity and statistical significance tests to assess the model's ability to predict a high number of test localities while not predicting an excessively large area of suitable habitat, may be preferable (Pearson 2009). Therefore, without knowing the species *actual* distribution, it may be more informative to determine the usefulness of the species habitat model, rather than its accuracy.

CHAPTER 3: MANUSCRIPT

PREDICTING THREATENED ORCHID (*ISOTRIA MEDEOLOIDES* [PURSH] RAF.) HABITAT IN THE SOUTHERN APPALACHIAN REGION USING MAXENT MODEL

Introduction

Isotria medeoloides (Pursh) Raf., known as the small whorled pogonia, is a member of the orchid family (Orchidaceae). It was first described by Frederick Pursh in 1814 and is considered the rarest orchid east of the Mississippi River, excluding Florida (Sperduto & Congalton 1996; USFWS 1992). *I. medeoloides* was listed as federally endangered in 1982 (USFWS 1982) but reclassified to threatened in 1994 when additional populations were discovered (USFWS 2008). *I. medeoloides* is a herbaceous, perennial species characterized by a whorl of five or six leaves at the apex of a pale-green, hollow stem. It produces a single, yellowish-green flower that is self-pollinated, lacks fragrance and nectar, and blooms from 4 – 14 days. *I. medeoloides* can reproduce vegetatively as indicated by the presence of two or more stems originating from a single rootstock (USFWS 1992). Seed production is considered to be low to moderate compared to other orchids. *I. medeoloides* seeds contain a small amount of food reserves and must develop a mycorrhizal relationship to successfully germinate and develop into a mature plant (Chafin 2007).

Isotria medeoloides occurs in partially shaded gaps of mixed-deciduous or mixed-deciduous/coniferous forests with an open understory and sparse herbaceous layer (Patrick et al. 1995). These forests are generally in second or third-growth and early successional stages from 30-80 years old (Sperduto & Congalton 1996; USFWS 1992; Mehrhoff 1989a). The majority of *I. medeoloides* sites share characteristics such as indication of past land disturbances; sparse to moderate ground cover (except when growing among ferns);

proximity to logging roads, stream beds, or other features that create persisting breaks in the forest canopy that increase light availability; acidic, sandy loam soils that contain an impervious layer; a leaf litter layer; and locations at the base of steep slopes or on benches at mid-slope positions (Sperduto & Congalton 1996; USFWS 1992; Mehrhoff 1989a). Beyond these common characteristics, *I. medeoloides* sites exhibit a wide range of forest environmental conditions and associated species (Sperduto & Congalton 1996; Patrick et al. 1995; USFWS 1992; Mehrhoff 1980).

Although *I. medeoloides* is widely distributed throughout the eastern forests of North America, there are only three main population centers: Appalachian Mountain foothills in New England and northern coastal Massachusetts, the Coastal Plain and Piedmont of eastern Virginia, with outliers in Delaware and New Jersey, and its southernmost concentration located in the Blue Ridge Mountains where South Carolina, North Carolina, Tennessee, and Georgia join. The New England population center currently has the majority of the individuals although trends in population sizes are unknown (USFWS 1992; Mehrhoff 1989a). In the southern population center, there were 13 protected sites designated in 1996 (USFWS 1996). Trends in population sizes at these sites are also unknown but overall, population sizes throughout the southeastern region are believed to be steadily decreasing (Kauffman pers. comm.; Schwartzman pers. comm.). Furthermore, most of the *I. medeoloides* populations in the southeast contain fewer than 25 plants (USFWS 1996).

Specific factors that could be affecting population declines of *I. medeoloides* are unknown. In some instances declines may be associated with a decrease in light availability (Brumback et al. 2011; Mehrhoff 1989a; Brackley 1985; Stuckey 1967). Mehrhoff (1989a) found that sites with dense vegetative cover had many extinct or non-reproductive individuals and sites with greater light levels had flowering individuals that lived longer. Stucky (1967) reported more robust plants grew in almost full sun and were four times larger than plants at other sites where light intensity was less. Brumback et al. (2011) observed an increase in stem emergence of *I. medeoloides* six years after forest vegetation was cleared from a site adjacent to the orchid population. Changes in population sizes could also be related to the distribution of mycorrhizal fungi (Sperduto 1993) which could be affected by land disturbance.

Although the specific factors causing the decline in *I. medeoloides* populations are unknown, species habitat models can help to locate new populations. In general, modeling suitable habitat of a rare species helps determine optimal areas for future monitoring and reintroduction of a species, as well as increases the likelihood of locating previously unknown populations (Cleve et al. 2011; Razgour et al. 2011; Hernandez et al. 2006). These models combine locations of known occurrence with spatially continuous environmental layers to infer ecological requirements of a species (Hernandez et al. 2006). Maximum entropy (Maxent) is a commonly used habitat suitability modelling method which uses the principle of maximum entropy with presence-only data that relate environmental variables and habitat suitability to approximate the species' niche and potential geographic distribution (Warren & Seifert 2011). Compared to other models, Maxent is not as sensitive to sample size, and generally outperforms other models at very small sample sizes, which is usually the case for rare or endangered species. Many studies have demonstrated the strong predictive performance of Maxent, and its success in identifying locations of previously undiscovered populations has made it a recommended tool (Yang et al. 2013; Fuller et al. 2012; Reiss et al. 2011; Cleve et al. 2011; Kumar & Stohlgren 2009; Wisz et al. 2008).

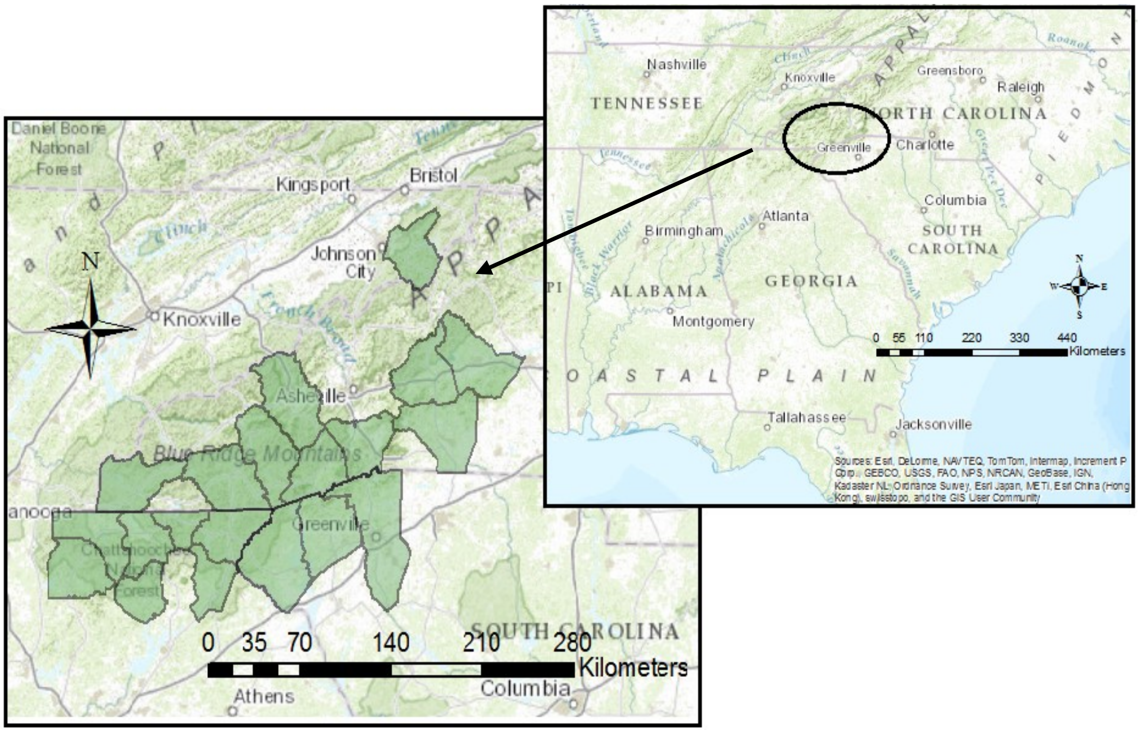
A habitat model using a geographic information system (GIS) for *I. medeoloides* was created for its New England population center and as a result, nine new populations of *I. medeoloides* were found (Sperduto & Congalton 1996). However, models currently do not exist for the southern Appalachian mountain region. The purpose of this study was to improve our understanding of where *I. medeoloides* is found in the southern Appalachian Mountains and to develop a habitat suitability model so that monitoring and conservation can be prioritized in the southern Appalachian region of the orchid's range. The specific objectives were to: (1) characterize the habitat where *Isotria medeoloides* is found, and (2) evaluate the Maxent modeling approach for predicting suitable habitat of *Isotria medeoloides* in the southern Appalachian region.

Methods

Study Area

There were two study areas chosen. The first study area included counties with extant locations of *I. medeoloides* in North Carolina, Tennessee, and Georgia which were visited during the habitat characterization. The second study area included the overall geographic distribution of the species in North Carolina, South Carolina, Tennessee, Georgia, and Alabama which was used to develop the habitat model (Figure 2). The climate in this region is described as modified continental, with warm summers and cool winters. Precipitation and temperature generally increase latitudinally from north to south, but precipitation is generally evenly distributed during the year. Much of the area consists of mountains separated by broad, somewhat hilly intermountain basins with elevations ranging from about 250 m in Guilford County, NC to 2038 m at Mt. Mitchell, in Yancey County, NC. Most soils are acidic (pH 4.5 to 5.5) and low in fertility except where the parent material consists of mafic or carbonate rock (Simon et al. 2005).

The southern Appalachian mountain region consists of about 2,250 vascular plant species. Approximately 31% of the region is mesic *Quercus*-*Carya* forest, 24% is acidic cove forest, 14% is xeric *Pinus*-*Quercus* heath, and 9% is rich cove forest (Simon et al. 2005). Natural forest disturbance that occurs is primarily from ice storms, drought, or



Sources: Esri, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community

Figure 2. Counties within North Carolina, South Carolina, Tennessee, and Georgia with historic and extant occurrences of *Isotria medeoloides* in the southern Appalachian Mountain region.

wind damage. Most of the forests were logged during the late 1800s and early 1900s with only small areas of old-growth forests remaining (Simon et al. 2005).

Initial Habitat Characterization and Analyses

To characterize the habitat where *I. medeoloides* is found, 75 geographic location records across the study area were obtained from the U.S. Forest Service and the Natural Heritage Programs in SC, NC, TN, and GA. Of these geographic locations, 15 that occurred on public land where permits were obtained (e.g., state parks and national forest land) were found using a GPS unit and sampled between August 6 and September 19, 2013. The habitat characteristics measured at each location included topographic, soil, and vegetation variables and were selected based on previous modeling studies in *I. medeoloides* more northerly population centers (Sperduto 1993; Mehrhoff 1989a). In addition, tree density by canopy layer was included as tree density can affect forest light levels and thus potentially affect *I. medeoloides* density. The vegetation sampling format was a modified version of the nearest neighbor approach (Clark & Evans 1954) and was selected to determine how the topographic, soil, and vegetation variables nearest to the *I. medeoloides* individuals were related to the orchid's density. One *I. medeoloides* individual at each location was chosen randomly for sampling. The nearest tree from the orchid was identified and the distance from the orchid to the nearest tree was recorded. That distance was multiplied by two and used as the dimensions for a macroplot, centered on the randomly chosen *I. medeoloides* individual. A microplot (2m x 2m) was centered on the same randomly chosen *I. medeoloides* individual to measure variables associated with the micro-habitat of the orchid. The number of *I. medeoloides* individuals in each macroplot was counted to determine how habitat variables were related to *I. medeoloides* density.

All trees were identified by species and canopy layer, and recorded as alive or dead to determine if openness of the tree canopy was associated with *I. medeoloides* density. To understand which phase of forest succession was associated with *I. medeoloides* habitat, the proportions of shade tolerant, intermediate, and shade intolerant (pioneer tree) species by canopy level were identified in each macroplot (Hall et al. 1995). A chi-square test of independence was used to test for association among shade tolerance and canopy level. Additionally, a Jaccard index of similarity was calculated to understand if tree species differed in each canopy level. The understory canopy level was defined as trees with canopy heights < 5 m, the midstory canopy level was defined as trees with canopy heights between 5 – 15 m, and the overstory canopy level was defined as trees with canopy heights > 15 m. For each tree, diameter at breast height (DBH) and level in the canopy (understory, midstory, and overstory) were determined in each macroplot to determine how tree basal area and level in the forest canopy which all affect the amount of light reaching the understory herbaceous layer, were related to *I. medeoloides* density. Percent canopy cover was estimated at the center of each macroplot by taking photos with a Canon (http://www.usa.canon.com) 15mm fish-eye lens. The camera was mounted on a tripod in the center of each macroplot at approximately one meter above ground-level. Canopy photos were analyzed using HemiView (http://www.delta-t.co.uk) 2.1 software to determine canopy cover values in each *I. medeoloides* plot.

Shrub (multi-stemmed, woody plant species < 6 m tall) and tree sapling (single stem < 10 cm DBH) species were identified and counted by number of clumps (shrubs) and number of stems (saplings) in each macroplot to calculate shrub and sapling density associated with *I. medeoloides* density. Shrub and sapling density were used to determine the openness of the forest understory where *I. medeoloides* occurs. Additionally, herbaceous plants were identified by species in each macroplot to determine frequency of herbaceous species commonly associated with *I. medeoloides* sites. Herbaceous plant cover was visually estimated as low (< 25%), medium (25-50%), or high (> 50%) in each microplot.

Habitat characteristics at large, extant *I. medeoloides* sites were assumed to be representative of optimal habitat as other studies have shown that high habitat quality may support larger species populations (Leimu 2010; Vergeer et al. 2003). Therefore, the 15 visited *I. medeoloides* locations were designated as large and small populations using the overall average number of individuals/location across all 15 locations as a separation threshold, which was 9. This threshold (9 plants/location) was compared to the average number of *I. medeoloides* individuals/location for all years of data recorded at each location to assign the population as historic large (> 9 plants/location) or historic small (< 9 plants/location).

Topographic features including percent slope, aspect (degrees), and elevation (meters) were measured in the center of each macroplot as these habitat variables relate to precipitation, temperature, and radiation levels that could affect the habitat quality at *I. medeoloides* sites. Percent slope was measured using a clinometer, aspect was measured in degrees using a compass, and elevation was measured in meters using a GPS unit.

Soil samples were collected from each microplot and pH determined in a laboratory using an ELE-511 bench top pH meter. Additionally, depth to a soil hardpan layer (centimeters) was determined using a soil penetrometer in each microplot. Soil samples were only collected at 14 plots because one plot was located on private property and permission was not obtained. Depth to the soil hardpan was only measured at nine sites because a hardpan was not detectable at the other six plots potentially due to the greater depths of the hardpan at those plots.

Information obtained from measurements of the above habitat variables in the 15 *I. medeoloides* plots was used to determine the habitat profile where *I. medeoloides* is found in the southern Appalachian region. The habitat information was also used to compare with habitat characteristics throughout the orchid's range and to analyze these variables for associations with *I. medeoloides* to determine the type of digital environmental data to include in the Maxent model. Finally, the habitat characteristics measured in the 15 plots were used to define the criteria for optimal *I. medeoloides* habitat in the southern Appalachian region to evaluate the accuracy of the Maxent model.

Pearson Product Moment correlations were calculated to determine associations between *I. medeoloides* density (#/m^2) and each habitat characteristic. All of the measured habitat characteristics were tested for normalcy with the Shapiro-Wilk test (Shapiro & Wilk 1965) and homogeneity of variance with Bartlett's test (Bartlett 1937) using R statistical software (R Development Core Team 2011). Spearman correlations were calculated if the habitat characteristics were non-normal. A one-way ANOVA was used to determine if there were differences between habitat characteristics by *I. medeoloides* population size. Pearson correlations and the one-way ANOVA were calculated twice for tree basal area to include dead hemlock in the analysis and then again without it to determine if the hemlock being alive or dead affected *I. medeoloides* density or population size through subsequent increases or decreases in light levels.

Maxent Model Development

Maxent is a machine learning program based on maximum entropy that estimates potential suitable habitat for a species based on environmental constraints (Phillips et al. 2006). It is a frequently used habitat suitability modelling method because it has repeatedly outperformed other methods (Razgour et al. 2011; Gibson et al. 2007; Elith et al. 2006; Hernandez et al. 2006). In addition, Maxent only requires presence data and environmental variables that are continuous or categorical as input layers for the study area. Maxent generates a logistic output of probability of presence that varies from 0 to 1, where 0 is the lowest and 1 is the highest probability. This logistic output can also be re-interpreted to represent habitat suitability, where 0 is low habitat suitability and 1 is high habitat suitability.

After the habitat variables at the *I. medeoloides* plots were analyzed, characteristics associated with *I. medeoloides* density and population size were used to guide selection of the type of digital environmental data most appropriate for the development of the Maxent model. The measured habitat characteristics from the 15 extant plots were not directly used to develop the Maxent model because they were collected at the micro-habitat level (finer spatial resolution) whereas the Maxent model was being developed for use at a regional scale (course spatial resolution) and thus required regional digital environmental data. Seven digital environmental data types available at a regional scale were used to develop the Maxent habitat model: annual average maximum temperature (˚C), annual average minimum temperature (˚C), annual average precipitation (cm), aspect (degrees), land cover, slope (%), and tree cover (%). Annual average maximum and minimum temperature, annual average precipitation, aspect, and land cover were chosen because these types of environmental predictor variables have been used in similar Maxent models and found to be important contributers to the predictive performance of the habitat model (e.g., Yang et al. 2013; Kumar & Stohlgren 2009). Slope and tree cover were chosen because they were related to the habitat variables (i.e., slope and overstory snag density) determined to be associated with large extant *I. medeoloides* populations visited during the habitat characterization, which may indicate optimal habitat for the species and thus provide important information to the habitat model.

Digital slope and aspect values were generated in ArcGIS 10.1 from elevation data downloaded from the United States Geological Survey (USGS) National Elevation Dataset (http://ned.usgs.gov) at a 30 meter resolution. Land cover data which relates to the forest types associated with the occurrence of *I. medeoloides* determined from the habitat characterization, and percent tree cover were downloaded from the National Land Cover Database 2011 (http://www.mrlc.gov/nlcd2011) at a 30 meter resolution. Annual average maximum and minimum temperatures, and annual average precipitation data between 1981 and 2010 were downloaded from United States Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS) Geospatial Data Gateway (http://datagateway.nrcs.usda.gov) at a 1km resolution. Digital soil data was not used in the Maxent model due to coarse resolution relative to the other variables. All the digital environmental variables were tested for multicollinearity in ArcGIS 10.1 using Pearson's correlation test because variables that are highly correlated can decrease the predictive accuracy of the model. Elevation data were subsequently not used to develop the model because it was highly correlated ($r > 0.90$) with slope data.

All seven digital environmental data layers (aspect, slope, percent tree cover, land cover, annual average maximum temperature, annual average minimum temperature, and annual average precipitation) were resampled using the nearest neighbor method in ArcGIS 10.1 to match the spatial resolution of the coarsest data layer (1 km). ArcGIS 10.1 was used to convert the digital environmental data layers into ASCII format, which was required for use in Maxent.

Of the original 75 location records, 80% (60) were used to build (train) the model and 20% (15) were used to test the model. The Maxent software program, version 3.1 (http://www.cs.princeton.edu/~schapire/maxent/) was used. All settings were kept as default other than the 60 location records and seven environmental predictors. The cross validation (k-fold) resampling method was used in Maxent to test the model (Pearson 2009). In this case, K was set to five thus creating five subsets of the total number of location records used in the model development (75) and ran five times, withholding the fifth subset in each run to use for testing the model.

The average Area under the Receiving Operating Characteristic Curve (AUC) over the five replicated runs was reported and used to evaluate the predictive performance of the Maxent model. The Receiver Operating Characteristic (ROC) Curve describes the relationship between the proportion of observed presences predicted correctly (sensitivity) and the proportion of observed absences predicted incorrectly (1 – specificity). The AUC ranges from 0.5 for models that perform no better than random, to 1.0 for models with perfect predictive accuracy (Pearson 2009). Although AUC requires both presence and absence data, Phillips et al. (2006) demonstrated this test can be used with pseudo-absences, which are randomly selected background points from the study area. In this case, AUC is measuring whether the model is predicting presence more accurately than a random prediction as opposed to the ability to distinguish presence from absence (Phillips et al. 2006).

Lastly, Maxent's internal jackknife procedure was generated to assess the importance and contribution of each digital environmental predictor variable to the model's training gain. The Maxent model training gain is a measure of how well the environmental predictor variables predict *I. medeoloides* presence or habitat suitability. During the Maxent jackknife test, several models are created for three different scenarios which include building a model by excluding each predictor variable and using the remaining variables, using each predictor variable in isolation, and using all variables to develop the model (Pearson 2009). The results of the Maxent jackknife test allowed comparisons to be made between the digital environmental predictor variables used to develop the model and the measured habitat characteristics of *I. medeoloides* plots.

The final Maxent model output of potential suitable habitat for *I. medeoloides* had a range of values from 0 to 1, which were regrouped into two suitability classes: low habitat suitability (0-0.5) and high habitat suitability (0.5-0.97). Environmental predictor response curves were generated in Maxent to show how each environmental predictor variable affected the prediction of the model, while holding all other environmental variables at their average digital value.

To validate the accuracy of the Maxent model, 20 habitat locations predicted by the model as high suitability (15 sites) and low suitability (5 sites) for *I. medeoloides* across the overall study area (NC, SC, TN, GA, and AL) were located using a GPS unit in June to mid-July 2014. Locations were selected by randomly choosing geographic coordinates on public land close to hiking trails (to allow for access to geographic coordinates) from the Maxent output map using ArcGIS 10.1. Habitat variables measured at these field validation sites included *I. medeoloides* density (#/m^2), tree canopy cover (%), aspect (degrees), slope (%), elevation (meters), soil pH, overstory snag density (#/m^2), overstory and midstory tree density (#/m^2), and herbaceous cover (low, medium, high). These particular habitat characteristics were chosen to validate the habitat model because they were either found to be associated with large, extant *I. medeoloides* populations in the initial habitat characterization and thus assumed to represent optimal habitat, or were measured in other habitat studies and thus could be used to compare environmental variables throughout the orchid's range. Habitat characteristics were measured in macroplots (30m x 30m) and microplots (2m x 2m). The macroplot dimension was chosen to equal the spatial resolution of the Maxent habitat model (1km) and the microplot dimension was chosen to maintain consistency with the microplot dimension used during the initial habitat characterization. All habitat variables were measured at the macroplot level except soil pH and herbaceous cover which were measured in the microplot. All habitat characteristics were measured using the same methods used during the initial habitat characterization.

Due to the broad range of suitable habitat predicted for *I. medeoloides* and the coarse resolution of the environmental data used to create the Maxent model there was potential for the model to over-estimate the amount of suitable habitat; thus, it was necessary to analyze differences between predicted high vs. low suitability sites as well as extant *I. medeoloides* sites vs. sites predicted by the model to evaluate the accuracy of the habitat model. All habitat characteristics measured in the field validation plots were tested for normalcy using the Shapiro-Wilk test (Shapiro & Wilk 1965) and homogeneity of variance with Bartlett's test (Bartlett 1937) using R statistical software (R Development Core Team 2011). A one-way ANOVA was used to determine if there were differences in habitat between predicted high vs. predicted low suitability *I. medeoloides* sites, extant *I. medeoloides* sites vs. predicted high suitability sites, and extant *I. medeoloides* sites vs. predicted low suitability sites to help validate the Maxent model.

Results

Initial Habitat Characterization

A summary of the characteristics measured in the 15 plots is in Table 1. Habitat characteristics in bold in Table 1 were also measured at sites visited during field validation. The mean density of *I. medeoloides* (#/m^2) was 0.09 across all extant plots. Tree canopy cover was greater than 90%, percent slope was variable but generally less than 40%, and soil pH was generally less than 5. A soil hardpan layer was present in 60% of all plots and the remaining 40% of plots lacked an impervious layer. Midstory snag and overstory density were high in all plots compared to the densities of the other forest canopy strata except for sapling and shrub density. Although not included in Table 1, herbaceous cover was low in all extant plots but one.

Approximately 50% of the total mean basal area was *Acer rubrum* L. and *Pinus strobus* L. with *Liriodendron tulipifera* L. and *Tsuga canadensis* L. contributing an additional 17% (Table 2). These latter three species were present in over 50% of the plots but *Acer rubrum* was present in only one-third of the plots. Although *Oxydendrum arboreum* L. DC. contributed < 2% of the total mean basal area, it was present 60% of the time (Table 2). *Acer rubrum* and *Liriodendron tulipifera* had the greatest stem diameters (34 ± 10.9 and 33 ± 4.1 cm) compared to the other tree species signifying they were the biggest trees but *Tsuga canadensis* had the largest percentage of total mean density indicating that *T. canadensis*

Table 1. Summary of habitat characteristics in 15 extant *Isotria medeoloides* (Pursh) Raf. plots in NC, TN, and GA. Habitat characteristics in bold were also measured during field validation of the habitat suitability model. (n=15 for all characteristics except soil pH [n=14] and soil hardpan depth [n=9])

Habitat Characteristic		Mean ± SE		Minimum	Maximum
I. medeoloides (#/m^2)		0.09	± 0.02	0.01	0.22
Canopy Cover (%)		90.30	± 1.08	81.90	97.90
Aspect (degrees)		108.69	± 31.10	18.00	325.00
Slope (%)		18.95	± 3.00	5.00	40.00
Elevation (m)		782.69	± 24.60	630.02	944.88
Soil pH		4.61	± 0.06	4.05	4.88
Soil hardpan depth (cm)		50.52	± 2.37	40.64	60.96
Basal area (m^2/ha) (trees > 10 cm DBH)		180.47	± 76.6	30.09	1208.72
Tree and shrub density (#/m^2)					
	Understory	0.04	± 0.01	0.00	0.14
	Midstory	0.04	± 0.007	0.01	0.13
	Overstory	0.06	± 0.01	0.02	0.16
	Sapling	1.48	± 0.17	0.35	2.93
	Shrub	0.59	± 0.23	0.06	3.40
Tree snag density (#/m^2)					
	Understory	0.00	± 0.002	0.00	0.03
	Midstory	0.06	± 0.03	0.00	0.32
	Overstory	0.01	± 0.01	0.00	0.17

Table 2. Forest composition of trees (> 10 cm DBH) and saplings in 15 extant *I. medeoloides* plots in NC, TN, and GA. DBH and Basal Area % of total mean data only includes trees > 10 cm DBH.

Species	Frequency (% of plots)		BA % of total mean	Stems % of total mean	
	Tree	Sapling		Tree	Sapling
Tsuga canadensis L.	73.3	80.0	4.9	16.9	5.1
Liriodendron tulipifera L.	66.6	40.0	12.2	7.7	1.5
Oxydendrum arboretum L. DC.	60.0	73.3	1.4	4.8	1.3
Pinus strobus L.	53.3	60.0	14.2	13.7	4.9
Betula lenta L.	46.6	46.7	3.1	5.1	2.3
Acer rubrum L.	33.3	86.7	35.2	7.8	23.8
Halesia carolina L.	26.6	46.7	1.1	6.5	2.9
Acer saccharum Marshall	20.0	20.0	3.7	9.3	2.3
Pinus virginiana Mill.	13.3	0	5.4	4.5	0
Carya spp. Nutt.	13.3	66.7	1.3	3.7	2.5
Quercus rubra L.	13.3	86.7	2.5	4.5	3.9
Cornus florida L.	6.6	13.3	0.1	0.5	0.6
Pinus pungens Lamb.	6.6	0	3.4	5.1	0
Sassafras albidum (Nutt.) Nees	6.6	13.3	2.5	4.0	2.4
Quercus velutina Lam.	73.3	0	n/a	1.3	3.2
Ilex opaca Aiton	66.7	0	n/a	2.0	0
Carpinus caroliniana Walter	0	26.7	n/a	0	2.3
Castanea spp. Mill.	0	20.0	n/a	0	2.6
Magnolia fraseri Walter	0	20.0	n/a	0	0.7
Quercus falcata Michx.	0	20.0	n/a	0	2.6
Betula alleghaniensis Britton	0	20.0	n/a	0	2.1
Cornus alternifolia L. f.	0	6.7	n/a	0	2.2
Symplocos tinctoria (L.) L'Hér.	0	6.7	n/a	0	20.1
Nyssa sylvatica Marshall	0	6.7	n/a	0	0.4
Asimina triloba (L.) Dunal	0	6.7	n/a	0	1.4
Prunus spp. L.	0	6.7	n/a	0	0.3
Robinia pseudoacacia L.	0	26.7	n/a	0	1.2

contributed the greatest number of smaller stems across all plots (Table 2). *T. canadensis* contributed to over 78% of the total snags followed by *Pinus strobus* (13%), *Oxydendrum arboreum* (6%), and *Liriodendron tulipifera* (3%). There were a total of 24 different sapling species. *Acer rubrum* saplings contributed the highest percentage (24%) of the total mean stem density across all plots compared to the other sapling species, although *Acer rubrum* occurred just as frequently as *Quercus rubra* L., which contributed a much lower percentage (4%) to the total mean stem density. Additionally, *Tsuga canadensis* and *Oxydendrum arboreum* saplings were present in over 70% of plots (Table 2).

Of the shrubs, *Gaylussacia ursina* (M.A. Curtis) Torr. & A. Gray ex A. Gray had the highest mean density and was present in over 60% of plots. *Rhododendron maximum* L. was also present in 60% of extant plots followed by *Euonymous americanus* L. (40%) however, *R. maximum* had fewer clumps/ha (1863.90 ± 1239) than *E. americanus* (3455.40 ± 2014) (Table 3).

The successional stage of the forests across the extant *I. medeoloides* plots is illustrated in Figure 3 as the proportion of shade tolerant trees by canopy level. The forest overstory was dominated by shade intolerant trees but the forest midstory and understory was dominated by shade tolerant tree species. The Chi-square test of independence revealed that there were no significant associations ($p < 0.001$) among all shade tolerance types (i.e., shade tolerant, intermediate, and shade intolerant) for tree species between the overstory vs. the midstory and understory canopy levels but there was an association among all shade tolerance types for tree species between the midstory vs. understory canopy levels (Figure 3). Additionally, Jaccard's index of similarity was 56% between the overstory and midstory tree species, 73% between midstory and understory trees, and 42% between overstory and understory trees. This indicated that similar tree species were present in both the midstory and understory, which differed from tree species present in the overstory.

The common herbaceous species that were present across the 15 extant

Table 3. Composition of shrubs in 15 extant *I. medeoloides* plots in NC, TN, and GA.

Species	Frequency (% of plots)	Density Mean ± SE (clumps/ha)		Clumps % of total mean
Gaylussacia ursina (M.A. Curtis) Torr. & A. Gray ex A. Gray	66.67	6340.70	± 3839	58.31
Rhododendron maximum L.	60.00	1863.90	± 1239	14.28
Euonymous americanus L.	40.00	3455.40	± 2014	15.89
Hamamelis virginiana L.	26.67	814.90	± 281	3.75
Kalmia latifolia L.	26.67	1089.50	± 801	3.34
Calycanthus floridus L.	20.00	492.20	± 233	1.13
Leucothoe fontanesiana (Steud.) Sleumer	13.33	1266.20	± 169	1.94
Vaccinium spp. L.	13.33	244.40	± 4.9	0.37
Clethra acuminata Michx.	6.67	478.50	± 478	0.36
Lindera benzoin (L.) Blume	6.67	638.00	± 638	0.49
Rubus spp. L.	6.67	159.50	± 159	0.12

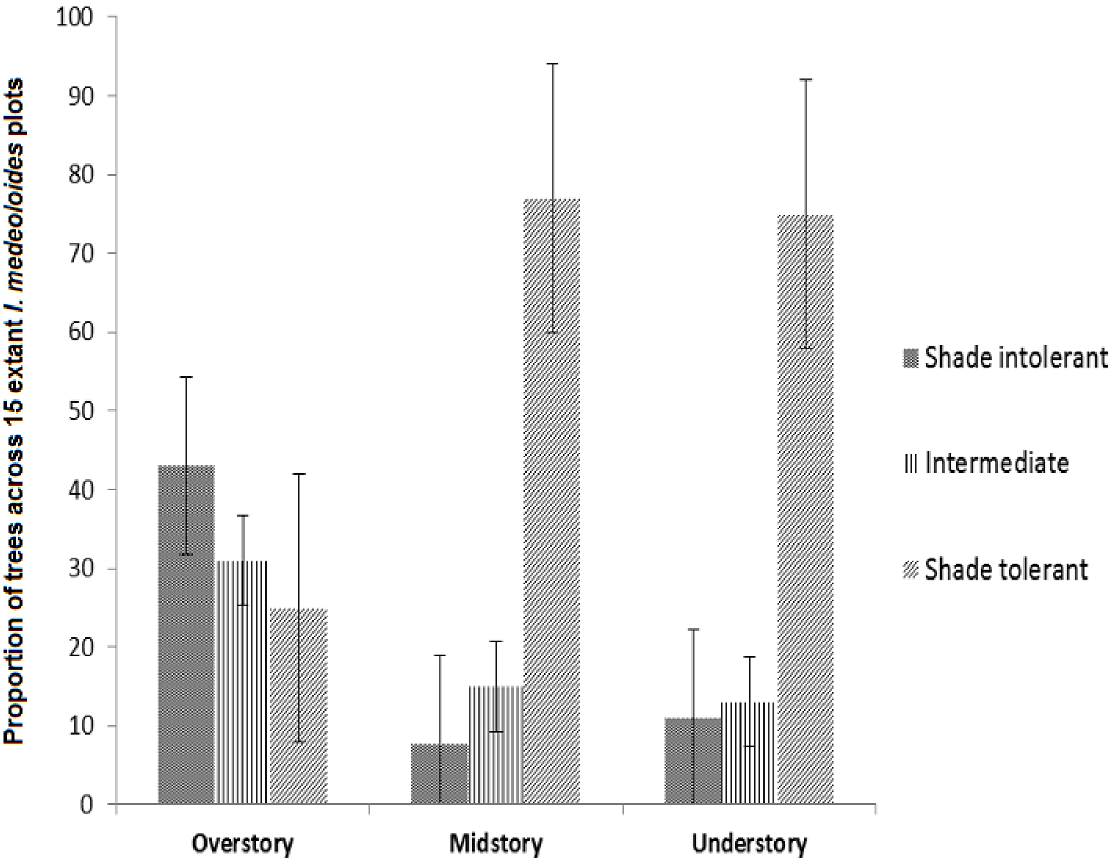


Figure 3. Proportion of trees in the overstory, midstory, and understory according to shade tolerance (Hall et al. 1995) across 15 *I. medeoloides* plots in the southern Appalachian region. Error bars are standard error for each shade tolerance type by canopy level.

I. medeoloides plots are listed in Table 4. *Polystichum acrostichoides* (Michx.) Schott and *Goodyera pubescens* (Willd.) R. Br. were present in greater than 85% of extant plots. Other common herbaceous species included *Viola spp.* L. (73%), *Chimaphila maculata (L.) Pursh (66%), Medeola virginiana L. (60%), and Thelypteris noveboracensis* (L.) Nieuwl. (60%).

The Pearson/Spearman correlations between *I. medeoloides* density and the measured habitat characteristics across 15 extant plots are listed in Table 5. Midstory snag density was the only measured habitat variable significantly correlated (r^2= 0.63, p=0.03) with *I. medeoloides* density. However, there were weaker correlations found for canopy cover (r^2= -0.45, p=0.09) and slope (r^2= 0.36, p=0.10).

Results of the one-way ANOVA among habitat variables between large (greater than 9 individuals/location) and small populations of *I. medeoloides* indicate that only overstory snag density differed (p=0.01) (Table 5) with greater number of snags occurring where *I. medeoloides* populations were larger (0.029 vs. 0 #/m^2 at large vs. small, respectively). Although not statistically significant, locations with larger populations of *I. medeoloides* tended to have lower midstory density (0.024 vs. 0.046 #/m^2, p=0.12), lower slopes (14.2 vs. 23.1%, p=0.16), and lower soil pH (4.54 vs. 4.79, p=0.16) (Table 5).

Environmental Predictors Used in Maxent Habitat Model

The results of Maxent's internal jackknife test of variable importance for the seven digital environmental variables evaluated are shown in Figure 4. Annual average precipitation contributed the most information for the Maxent habitat model compared to the other predictor variables. Compared to annual average precipitation, annual average maximum temperature contributed nearly half the amount and slope and tree cover contributed only one-quarter of the amount to the model. Land cover type, aspect, and annual average minimum temperature contributed small amounts to the model (Figure 4).

Table 4. Percent frequency of herbaceous species associated with *I. medeoloides* in 15 extant plots in NC, TN, and GA.

Herbaceous Species	Frequency (% of sites)
Smilax rotundifolia L.	93.33
Polystichum acrostichoides (Michx.) Schott	93.33
Goodyera pubescens (Willd.) R. Br.	86.67
Viola spp. L.	73.33
Chimaphila maculata (L.) Pursh	66.67
Medeola virginiana L.	60.00
Thelypteris noveboracensis (L.) Nieuwl.	60.00
Maianthemum racemosum (L.) Link	46.67
Fragaria virginiana Duchesne	46.67
Desmodium nudiflorum (L.) DC.	40.00
Mitchella repens L.	33.33
Trillium spp. L.	33.33
Toxicodendron radicans (L.) Kuntze	33.00
Galium spp. L.	26.67
Lycopodium digitatum Dill. ex A. Braun	26.67
Anemone quinquefolia L., *Clintonia umbellulata* (Michx.) Morong, *Arisaema triphyllum* (L.) Schott, *Cypripedium acaule* Aiton, *Tiarella cordifolia* L.	20.00*
Corallorhiza maculata (Raf.) Raf., *Dioscorea villosa* L., *Galearis spectabilis* (L.) Raf., *Huperzia spp.* Bernh., *Lobelia inflata* L., *Monarda spp.* L., *Uvularia perfoliata* L., *Lysimachia quadrifolia* L.	13.33*
Aster spp. L., *Actaea racemosa* L., *Celastrus orbiculates* Thunb., *Clintonia borealis* (Aiton) Raf., *Galax urceolata* (Poir.) Brummitt, *Geranium maculatum* L., *Geum spp.* L., *Houstonia purpurea* L., *Hypericum spp.* L., *Lonicera spp.* L., *Oxalis violacea* L., *Monotropa uniflora* L., *Shortia galacifolia* Torr. & A. Gray, *Tipularia discolor* (Pursh) Nutt., *Triphora trianthophora* (Sw.) Rydb., *Vitis rotundifolia* Michx., *Cypripedium parviflorum* Salisb., *Xanthorhiza simplicissima* Marshall	6.67*

* These percentages apply to each individual herbaceous species listed in a particular group.

Table 5. Pearson/Spearman correlations between *I. medeoloides* density (#/m^2) and measured habitat variables and ANOVA among habitat variables between large (> 9 individuals/location) and small populations of *I. medeoloides* across 15 extant plots in NC, TN, and GA. Habitat characteristics in bold were also measured during field validation of the habitat suitability model.

Habitat Characteristic	Pearson/Spearman with *I. medeoloides* density		ANOVA		
	r^2	*p*	# of large sites	# of small sites	*p*
I. medeoloides (#/m^2)	n/a	n/a	7	8	0.09
Canopy cover (%)	-0.45	0.09	7	8	0.46
Aspect (degrees)	-0.001	0.90	7	8	0.78
Slope (%)	0.36	0.10	7	8	0.16
Elevation (m)	0.27	0.30	7	8	0.30
Soil pH	-0.04	0.89	6	8	0.16
Soil hardpan depth (cm)	0.43	0.20	3	6	0.39
Basal area (m^2/ha) (trees > 10 cm DBH)**	0.25	0.40	7	8	0.56
Basal area (m^2/ha) (trees > 10 cm DBH)***	0.22	0.40	7	8	0.20
Tree and shrub density (#/m^2)					
Understory	-0.05	0.83	7	8	0.80
Midstory	-0.06	0.82	7	8	0.12
Overstory	0.40	0.20	7	8	0.24
Sapling	0.17	0.50	7	8	0.64
Shrub	0.33	0.20	7	8	0.24
Tree snag density (#/m^2)					
Understory	0.27	0.65	7	8	0.50
Midstory	0.63	0.03	7	8	0.30
Overstory	0.63	0.20	7	8	0.01

**with dead hemlock included in density
***without dead hemlock included in density

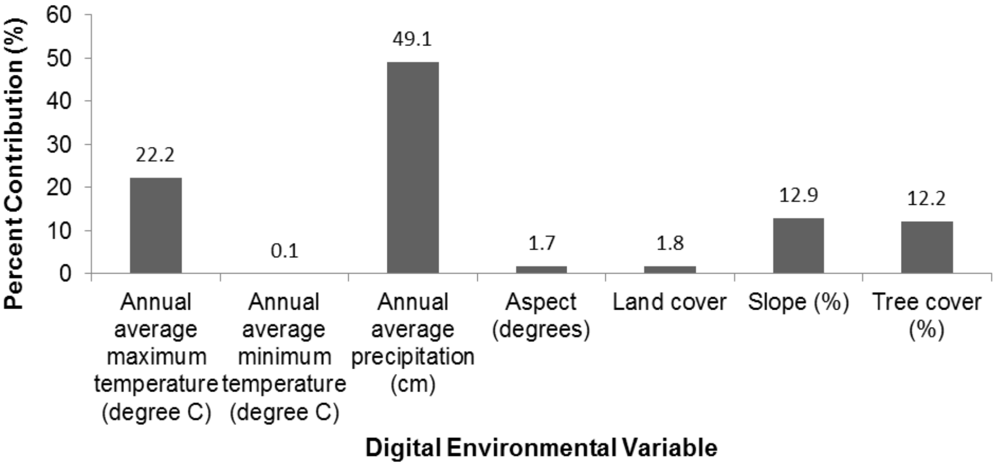


Figure 4. Results of jackknife test in Maxent showing percent contribution of environmental predictor variables for the *Isotria medeoloides* model.

The environmental predictor response curves generated in Maxent (Figures 5 and 6) show how each environmental predictor variable affects the prediction in Maxent when it is varied, while keeping all other environmental variables at their average sample value. Habitat suitability of *Isotria medeoloides* is highest (>0.6) when the annual average maximum temperature nears 20˚C (Figure 5a), when annual average minimum temperature is close to 7˚C (Figure 5b), when annual average precipitation nears 210 cm (Figure 5c), when aspect is close to 50 degrees (NE) (Figure 5d), when slopes range between 4-7% (Figure 5e), and when percent tree cover increases to 100% (Figure 5f). Figure 6 shows that habitat suitability of *Isotria medeoloides* is highest (0.60) in deciduous forest types, followed by evergreen forests (0.38).

Maxent Habitat Model Output and Predictive Performance

The Maxent model output for suitable habitat of *Isotria medeoloides* across the southern Appalachian region is shown in Figure 7. Using a commonly chosen suitability threshold of greater than 0.5 in which the species is more likely to be present, the Maxent prediction map was split into two suitability classes: low habitat suitability (0-0.5) and high habitat suitability (0.5-0.97). The most suitable habitat for *I. medeoloides* (>0.5) was predicted in the southern portion of the Appalachian mountains where North Carolina, South Carolina, Georgia, and Tennessee meet, as well as along sections of the Cumberland Plateau in Tennessee down into Georgia and Alabama. The high habitat suitability area was 5,254 km^2, of the total 708,163 km^2, making up approximately 0.74% of the overall study area (NC, SC, TN, GA, and AL).

The Maxent model had high predictive accuracy using independent test data (20% of original location data) with a mean area under the receiver operating characteristic (ROC) curve (AUC) value of 0.954 out of 1.0 and standard deviation of 0.025 (Figure 8).

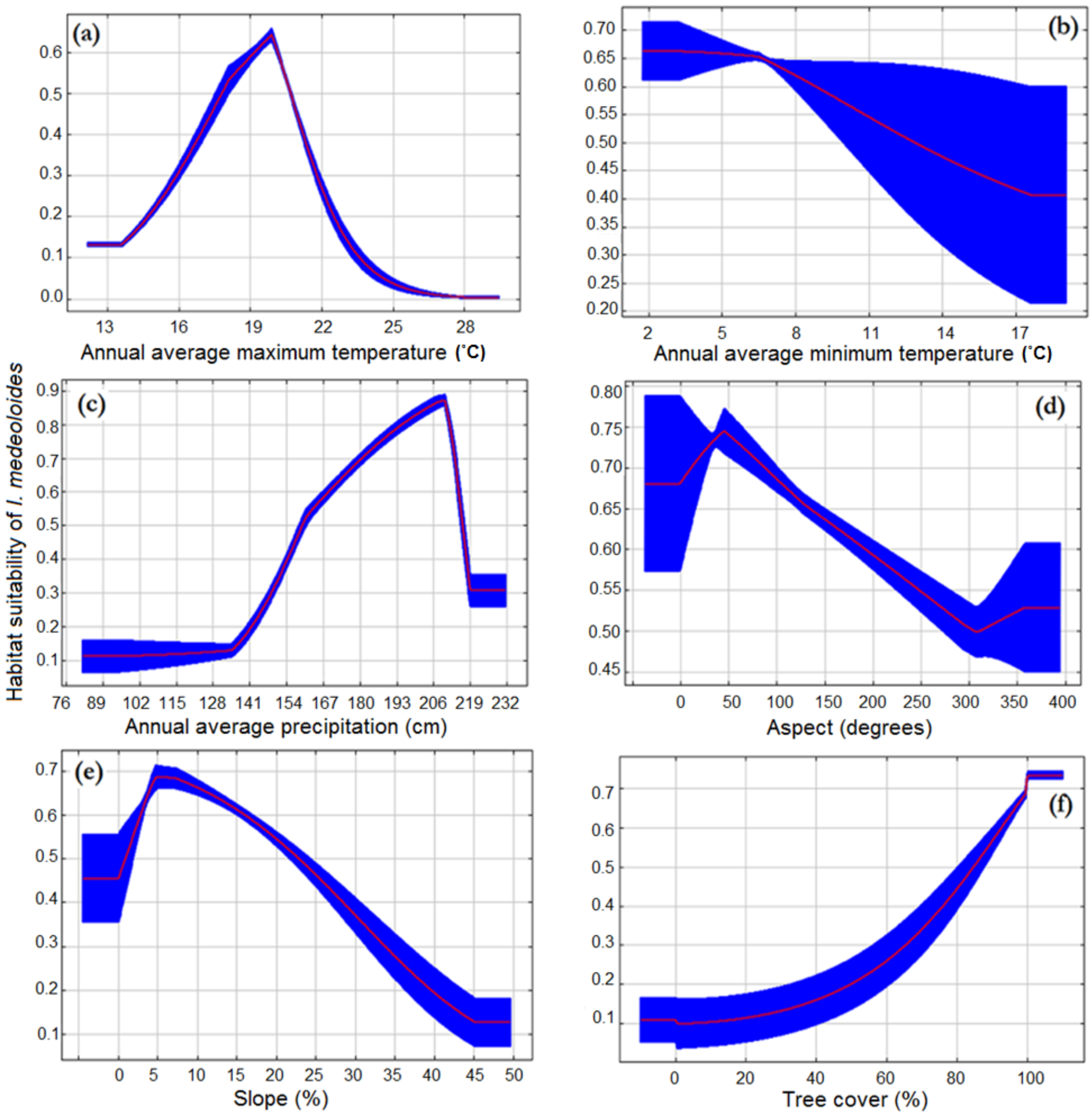


Figure 5. Environmental predictor response curves showing Maxent's output response (habitat suitability of *I. medeoloides*) as the digital environmental predictors vary. The mean response of the five replicated Maxent runs is shown by the white line and the mean +/- one standard deviation is shown by the dark curve area.

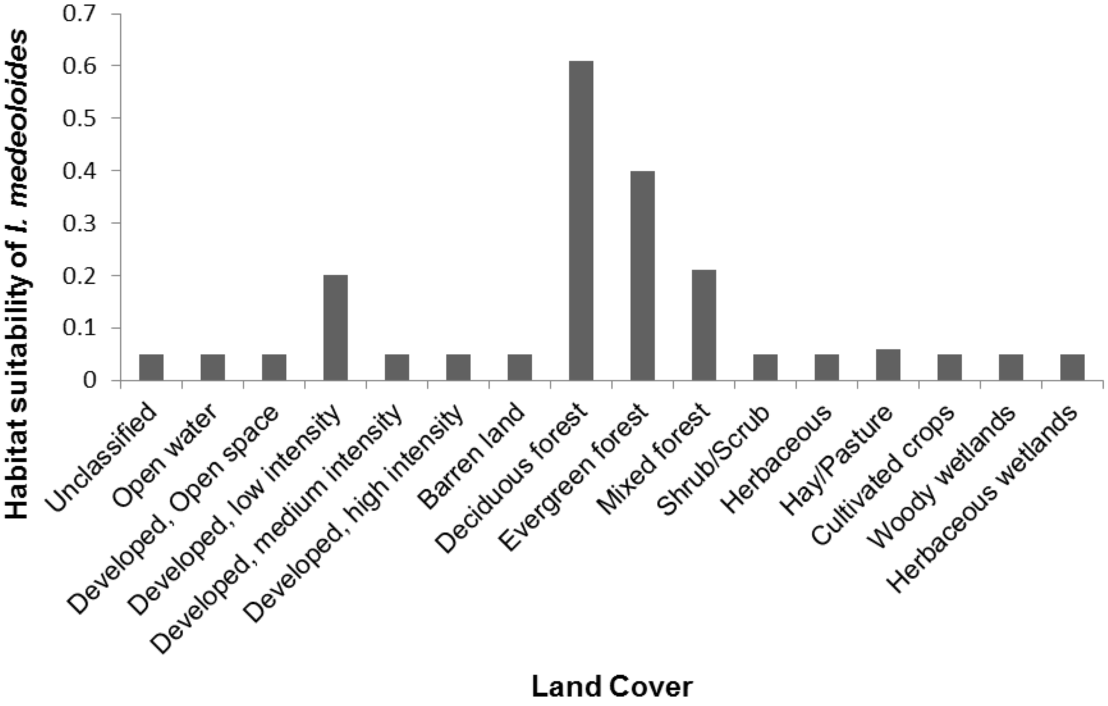


Figure 6. Environmental predictor responses for land cover showing Maxent's output response of habitat suitability for *I. medeoloides* for different land cover types.

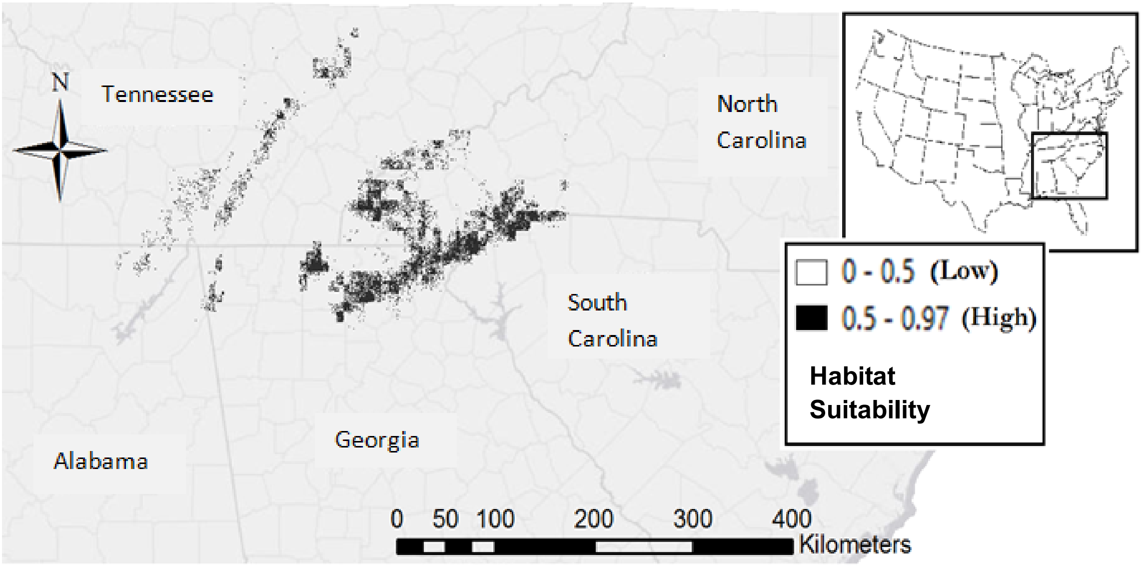


Figure 7. Predicted potential suitable habitat for *Isotria medeoloides* across the southern Appalachian mountain region in the southeastern United States. The logistic output representing habitat suitability for *I. medeoloides* is defined as low suitability (0-0.5) and high suitability (0.5-0.97) on the Maxent output map.

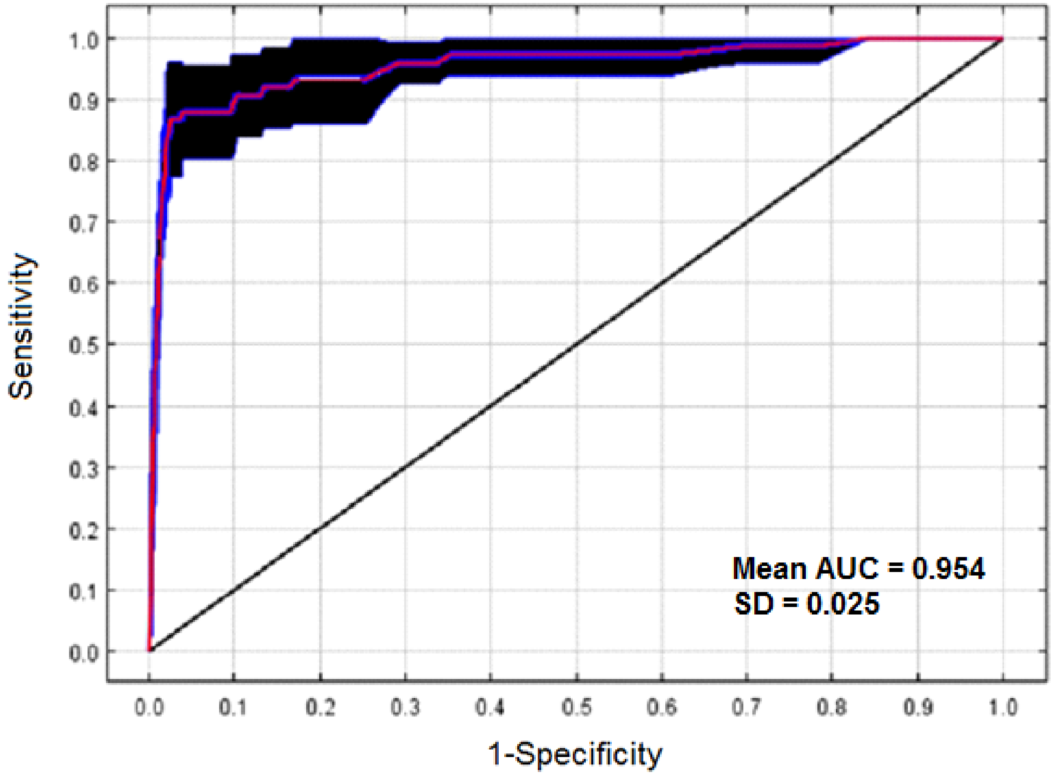


Figure 8. Area under the receiver operating characteristic (ROC) curve (Sensitivity vs. 1-Specificity) averaged over the five replicated Maxent runs describing the predictive accuracy of the model using independent test data. The mean AUC value is 0.954 and the AUC value of a random prediction (0.5) is shown by the linear line.

One new *I. medeoloides* population consisting of 19 individuals was found at a high suitability site out of 15 sites surveyed and none were found at predicted low suitability sites. A summary of the characteristics measured in the 15 predicted high suitability plots and 5 predicted low suitability plots during field validation of the Maxent model is presented in Table 6. Midstory tree density was significantly lower (0.01 vs. 0.02 #/m^2, p=0.02), overstory snag density was higher (0.01 vs. 0.004 #/m^2), and soil pH was lower (3.7 vs. 4.1) in predicted high suitability plots compared to the predicted low suitability plots. Additionally, herbaceous cover was low in all predicted high suitability plots but varied in the predicted low suitability plots. These results were similar to habitat characteristics that differed between large and small extant *I. medeoloides* populations visited during the initial habitat characterization, where midstory tree density was lower (0.024 vs. 0.046 #/m^2, p=0.12), overstory snag density was significantly higher (0.029 vs. 0 #/m^2, p=0.01), and soil pH was lower (4.54 vs. 4.79, p=0.16) in plots at larger populations (Table 5).

The one-way ANOVA among habitat characteristics between extant *I. medeoloides* plots from the initial habitat characterization and predicted high suitability and low suitability plots did not reveal any informative differences among habitat characteristics, with the exception of elevation (Table 7). Elevation was significantly (p=0.03) higher (1038 m) in predicted low suitability plots compared to extant *I. medeoloides* plots (782 m) and did not differ between predicted high suitability and extant *I. medeoloides* plots from the initial habitat characterization (Table 7). The lower elevation measured in the predicted high suitability plots is consistent with elevation ranges measured in extant *I. medeoloides* plots during the habitat characterization that were less than 944 m (Table 1).

Discussion

On average, *I. medeoloides* density in the southern Appalachian region was lower

Table 6. Summary of habitat characteristics in 15 predicted high suitability (> 0.5) *I. medeoloides* plots, predicted low suitability (< 0.5) *I. medeoloides* plots, and one-way ANOVA results of habitat characteristics between the 15 predicted high suitability (>0.5) and 5 predicted low suitability (<0.5) *I. medeoloides* plots.

Habitat Characteristic	Predicted high suitability (> 0.5) plots		Predicted low suitability (< 0.5) plots		*p*
	Mean ± SE		Mean ± SE		
I. medeoloides (#/m^2)	0.0003	± 0.0003	0	± 0	0.3
Canopy Cover (%)	99.9	± 0.01	99.9	± 0.03	0.7
Aspect (degrees)	246.5	± 63.7	187.1	± 83.6	0.8
Slope (%)	20.1	± 2.5	16.0	± 5.4	0.4
Elevation (m)	974.1	± 364	1038.7	± 638	0.8
Soil pH	3.7	± 0.1	4.1	± 0.3	0.2
Tree density (#/m^2)					
Midstory	0.01	± 0.001	0.02	± 0.005	0.02
Overstory	0.02	± 0.001	0.01	± 0.003	0.03
Tree snag density (#/m^2)					
Overstory	0.01	± 0.002	0.004	± 0.001	0.1

Table 7. One-way ANOVA results of habitat characteristics between 15 extant *I. medeoloides* plots in NC, TN, and GA vs. 15 predicted high suitability (>0.5) and 5 predicted low suitability (<0.5) *I. medeoloides* plots.

Habitat Characteristic	Extant sites	Predicted high suitability (> 0.5) plots		Predicted low suitability (< 0.5) plots	
	Mean ± SE	Mean ± SE	*p*	Mean ± SE	*p*
I. medeoloides (#/m^2)	0.09 ± 0.02	0.0003 ± 0.0003	<0.001	0 ± 0	<0.001
Canopy cover (%)	90 ± 1.08	99 ± 0.01	<0.001	99 ± 0.03	<0.001
Aspect (degrees)	108 ± 31.1	246 ± 63.7	0.48	187 ± 83.6	0.47
Slope (%)	18 ± 3.00	20 ± 2.5	0.76	16 ± 5.4	0.63
Elevation (m)	782 ± 24.6	974 ± 364	0.10	1038 ± 638	0.03
Soil pH	4.61 ± 0.06	3.7 ± 0.1	<0.001	4.1 ± 0.3	<0.001
Tree density (#/m^2)					
Midstory	0.04 ± 0.007	0.01 ± 0.001	0.004	0.02 ± 0.005	0.31
Overstory	0.06 ± 0.01	0.02 ± 0.001	0.001	0.01 ± 0.003	0.02
Tree snag density (#/m^2)					
Overstory	0.01 ± 0.01	0.01 ± 0.002	0.78	0.004 ± 0.001	0.62

than the density reported in its northern range (0.09 vs. 0.33 #/m^2) (Sperduto 1993). Soils were generally acidic which is similar to soil types in other portions of the orchid's range that have reported averages of 4.2 to 4.3 (Sperduto 1993; Mehrhoff 1989a). Additionally, the tree and shrub species that dominated extant sites in the southern Appalachian region (*Acer rubrum, Liriodendron tulipifera, Pinus strobus, Tsuga canadensis, Gaylussacia ursina,* and *Rhododendron maximum*) are typical of mixed, mesophytic, acidic cove forests across the southern Appalachian region (USDA 2011; Simon et al. 2005) which is also similar to forest types where *I. medeoloides* occurs in the northeastern portion of the species range (Sperduto 1993).

The dominance of forest overstories by shade intolerant/pioneer tree species, with shade tolerant tree species in the midstory and understory suggests that forests are in mid-succession moving toward late-succession (McEvoy 2000). This is supported by the Jaccard's index of similarity results. This mid-successional stage of the forests across the visited *I. medeoloides* sites is also typical in other portions of the species range (Sperduto 1993; Ware 1991; Mehrhoff 1989a).

Mean percent canopy cover at extant sites was greater than 90% and habitat suitability predicted by the model was highest at greater than 90% tree canopy cover which is similar to percentages (>85%) measured at sites in northern portions of the orchid's range (Sperduto 1993). Negative correlations between canopy cover and *I. medeoloides* density, lower midstory tree density at larger *I. medeoloides* populations, and positive correlations between overstory snag densities and *I. medeoloides* density could all indicate the species responds positively to gaps or openings in the forest canopy. This observation is consistent with other studies that have reported increases in *I. medeoloides* density with an increase in light levels following canopy thinning (Brumback et al. 2011). An increase in density is generally true for most herbaceous plants where light is the primary limiting resource in the forest understory (Warren 2008; Neufeld & Young 2003).

Consistent with the extant sites, *I. medeoloides* populations in northern portions of its range occur in habitats with relatively sparse herb layers (Sperduto 1993; Mehrhoff 1989a) and with several of the more common species such as *Polystichum acrostichoides, Goodyera pubescens, Viola spp., Chimaphila maculata, Medeola virginiana,* and *Thelypteris noveboracensis* (Sperduto 1993; Mehrhoff 1989a; Rawinski 1986; Gaddy 1985).

Suitable habitat predicted by the model was highest when aspect was close to 50 degrees (NE) and decreased as aspect neared 300 degrees (NW), which agreed with habitat measurements at extant *I. medeoloides* sites revealing that mean aspects were east-facing (108 degrees). Most report sites generally occur on eastern or southern exposures (Sperduto 1993; Ware 1991; Mehrhoff 1989a) and Rawinski (1986) suggested north facing slopes may be too cold to support *I. medeoloides* populations. Elevation varied at *I. medeoloides* sites but was generally higher (783 m) at extant sites in the southern Appalachian region compared to sites in the orchid's northern range (207 m) (Sperduto 1993). This is consistent with Hopkins' bioclimatic law that climatic conditions affecting vegetation zones at higher elevations typically correspond to similar climatic conditions at higher latitudes (Hopkins 1938).

Suitable habitat predicted by the model peaked when slopes were between four and seven percent, which agrees with the range of slopes measured at extant sites in the southern Appalachian region and at sites in New England that have reported average slopes of 4% (Sperduto 1993). Additionally, slopes were generally lower (14% vs. 23%) at larger vs. smaller extant *I. medeoloides* sites in the southern Appalachian region. It has been suggested that gently, sloped terrain may promote a hydrologic regime that favors *I. medeoloides* (Sperduto 1993). Specifically, lower percent slopes receive increased moisture and nutrients and a few of the most frequent herbaceous species (e.g., *Polystichum acrostichoides* and *Thelypteris noveboracensis*) occurring with *I. medeoloides* prefer the mesic conditions of these slopes (Whitney 1991). The hardpan soil layer found at many of the extant sites also occurs throughout the orchid's range (Sperduto 1993; Mehrhoff 1989a). The combination of sloped terrain and a hardpan layer may provide a suitable moisture regime for *I. medeoloides*. In this situation, water moves down the slopes above the pan layer causing these soils to be seasonally wet (Habecker et al. 1990; USDA 1977). Additionally, annual average precipitation was the most important digital environmental predictor in the habitat model and probability of suitable habitat peaked when average precipitation reached 210 cm, which corroborates other studies where populations were found in mesic habitat or near streams (Sperduto 1993; Mehrhoff 1989a; Rawinski 1986). This suggests that consistent moisture may be required for the establishment and survival of *I. medeoloides* due to their large leaf areas and few roots, which also occurs in other orchid species (Rasmussen 1995; Stuckey 1967). Finally, water can also successfully disperse the orchid seeds which float (Rasmussen 1995).

The Maxent habitat model had high predictive performance represented by a high AUC score of 0.954, which indicates the probability that the model will correctly discriminate between a presence record and a 'pseudo-absence' record is high (Pearson 2009). The model predicted the most suitable habitat in the southern portion of the Appalachian Mountains, in deciduous and evergreen forests with elevations less than 617 meters, percent slopes less than 15%, where tree cover exceeded 90%, and where temperatures were generally cooler (not exceeding 21˚C on average) which is consistent with *I. medeoloides* habitat throughout other portions of its range (Sperduto 1993; Mehrhoff 1989a; Ware 1989). Field validation, which is an especially critical step in assessing the predictive performance of rare species habitat models (Rebelo & Jones 2010; Jiménez-Valverde et al. 2008; Greaves et al. 2006), revealed similarities between habitat characteristics at predicted high suitability sites compared to large extant *I. medeoloides* populations visited during the initial habitat characterization. This indicates the Maxent model predicted suitable habitat for *I. medeoloides* accurately.

Overall, the Maxent model seemed to be useful in predicting suitable habitat for *Isotria medeoloides* based on similarities found among habitat characteristics between extant *I. medeoloides* sites and sites predicted by the model, and the high AUC score (0.954). Additionally, the new, relatively large population found in an area predicted as highly suitable by the Maxent model and none found in predicted unsuitable areas during the field validation of the habitat model supports the usefulness of the model. However, there were some limitations of the model. The resolution of the Maxent model was constrained by the available digital environmental predictor variables with the coarsest resolution (1 km) and it was not possible to use the habitat characteristics measured at the extant *Isotria medeoloides* sites directly to develop the model due to the requirements of the Maxent modeling program software. Therefore, the model could over-estimate the amount of suitable habitat because characteristics at the micro-habitat scale would not be included during model development. Other studies using Maxent to predict suitable habitat for a plant species have reported over-estimations of suitable compared to actual habitat of the species (Yang et al. 2013; Kumar & Stohlgren 2009). Despite these limitations, the Maximum Entropy modeling method provided a useful tool for predicting suitable habitat for *Isotria medeoloides* in its southern Appalachian range. Additionally, it has the potential to focus future monitoring efforts, and in combination with other threatened or endangered species data, identifying and conserving areas of significant biodiversity.

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