Population ecology of the floodplain herb Macbridea caroliniana (Lamiaceae) with investigations on the species' habitat, breeding system and genetic diversity

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POPLATION ECOLOGY OF THE FLOODPLAIN HERB MACBRIDEA CAROLINIANA (LAMIACEAE) WITH INVESTIGATIONS ON THE SPECIES’ HABITAT, BREEDING SYSTEM AND GENETIC DIVERSITY

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Biological Sciences

by
Katherine Farrah Weeks
August 2009

Accepted by:
Dr. Joan Walker, Committee Chair
Dr. Craig Allen, Committee Co-Chair
Dr. David Tonkyn
Dr. Albert Abbott
The perennial herbaceous mint *Macbridea caroliniana* is known from 36 locations in discrete watersheds of the Carolinas and Georgia. It is one of relatively few conspicuously flowering herbs that occupy bottomland hardwood forests. The general project goal was to gain knowledge that is applicable to the species’ conservation both at the Congaree National Park (CNP) where the largest known population of this species occurs and range-wide. Specific objectives were to (1) quantify the population size and describe the distribution of *M. caroliniana* within CNP, and determine the extent of co-occurrence with wild hogs and the non-native *Murdannia keisak*; (2) identify habitat characteristics associated with the presence of *M. caroliniana*; (3) describe the breeding system; and (4) describe the genetic diversity and structure of the species across the range and within the CNP. Data relevant to the first three objectives were collected at CNP from field plots and experiments conducted in two large areas of seepage forest herein referred to as ECC and EDB. For the fourth objective, leaves were collected across the species range and allozyme systems were characterized using starch gel electrophoresis.

Important findings included the following: CNP has the largest known population of *M. caroliniana* with the greatest concentration near the center of the ECC seepage forest; new CNP locations of *M. caroliniana* were found; hog rooting activity negatively affects *M. caroliniana* patches in the short-term, but the long-term threat is unknown; the invasive plant, *Murdannia keisak*, is a frequent co-occurring plant, but is not a clear threat to the study species; in a patch-scale habitat study, the best model tested to predict the presence of *M. caroliniana* included the variables (+) herb richness, and two soil
nutrients, (+) phosphorus and (-) potassium; in a forest-scale study, the variables above were not statistically different between the two forested areas, but they were in the direction predicted, that is, ECC with the larger _M. caroliniana_ population has greater herb richness, more phosphorus and less potassium and this may explain in part why there is less _M. caroliniana_ at EDB; _M. caroliniana_ is not autogamous, but it is self-compatible, dependant on pollinators to set fruit, and likely pollen-limited; floral visitation was infrequent, but the most common floral visitors were _Poanes zabulon_ and _Bombus impatiens_; in the species-wide genetic study, the genetic structure of the species is greatly influenced by river basin; the ECC and EDB populations ranked highest for conservation priority based on genetic measures, further emphasizing their importance to the species; at ECC, gap patches are more like each other genetically than are the patches from closed canopies and this suggests more gene flow between the gap patches likely from floral visitors. Conservation implications based on this research may apply to other perennial, herbaceous, insect-pollinated species that occupy naturally fragmented or disjunct wetland habitats. Additional information needed to improve conservation efforts include an understanding of the relationship of _M. caroliniana_ to canopy gap dynamics, of its ability to compete for scarce resources, and of demographic patterns and processes.
DEDICATION

This work is dedicated to the Congaree National Park and all the Park personnel, friends, and volunteers who contribute to this very special place on our planet.
ACKNOWLEDGMENTS

This project was made possible with the help of many people. First and foremost, I thank my major advisor and mentor, Dr. Joan Walker of the U.S. Forest Service, for her friendship, unwavering support, hard work, and sage advice over the years; I know how lucky I am to have her in my life. I also thank my major co-advisor, Dr. Craig Allen, who brought me onto this project, helped obtain most of the funding for this research, and for his continued support even after he moved to Nebraska. I thank my other committee members, Drs. David Tonkyn and Bert Abbott for all of their contributions, great suggestions, and advice. I also thank Dr. Tonkyn for being the first to spark my interest in the science of ecology. I thank Congaree National Park, the main funding agency, and Park personnel including Bill Hulslander for their ongoing support of this project. I am thankful to the USDA Forest Service, Clemson University, the department of Biological Sciences including Dr. Hap Wheeler, and the SC Cooperative Unit including Dr. Pat Jodice for all of their support. I thank many folks for their field assistance including: Brian Weeks, David White, Bryan Mudder, Renee Martin, Theresa Yednock, Marcas Houtchings, Brad Friebel, and more! I also thank Shawna Reid for providing her vast knowledge of ArcGIS, Drs. Jim Hamrick and Dorset Trapnell for their collaboration on the genetics portion of this project, and Drs. Jim Reick, and Pat Gerard for their statistical expertise. Last, but certainly not least, I thank my friends and family including Lisa Eggert and Carolyn Wakefield, Helen Farrah (mom), Terry Duval and Tina Bazarian (sisters), Charlie Farrah (brother), Judy and Ronnie McCaig (my in-laws), and my husband, Brian “Duck-foot” Weeks, for all of their love and support through the years.
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CHAPTER ONE
INTRODUCTION

The decline in forest herbs and of floodplain habitats makes conservation and restoration important, but basic knowledge of floodplain herbs is lacking (Bierzychudek 1982), hindering conservation. Generally speaking, the decline of forest herbs is caused by a combination of habitat loss, non-native species invasions, over-exploitation, and indirect effects from other species (Jolls 2003). Although woody species are also affected by these factors, herbaceous species have extinction rates that are more than two times greater than woody species (Levin and Levin 2001). This risk to herbs of floodplain systems is likely exacerbated by the remarkable amount of habitat loss in these systems. Since European settlement, there has been an estimated loss of 69% of bottomland hardwood wetlands in the lower 48 states (Gosselink and Lee 1989). The declining number and increased extinction risk of forest herbs both complicate our ability to understand these populations (Jolls 2003) and emphasizes the timeliness of population biology studies to gain information for protection and restoration efforts.

The herb layers of major alluvial floodplains forests that are associated with either blackwater or brownwater rivers are sparse, variable and not well documented (Kellison et al. 1998). Herbaceous species cover in these bottomland forests is variable and patchy due mostly to differences in hydrology; coverage is up to 25 percent in blackwater river forests and up to 35 percent in the drier, brownwater river forests where the remaining areas of substrate are either bare or covered by leaf litter (Flinchum 1977 - cited in Kellison et al. 1998). The herb layer composition of brownwater systems is not well
documented and this may be due to the emphasis of research on woody taxa, the ephemeral nature of herbs or difficulty in identification (Kellison et al. 1998). Wetlands textbooks often describe the vegetation of bottomlands by the tree composition alone (e.g., Mitsch and Gosselink 1990).

**Study species description**

*Macbridea caroliniana* (Walt.) Blake is a rare, wetland herb of bottomland forests in the coastal plain in the Carolinas and Georgia (see Figure 1.1). *M. caroliniana* (Carolina birds-in-a-nest; Carolina bogmint) is a perennial rhizomatous herbaceous species that is easily identified when flowering, which occurs from early July through August. The flowers have 2-4 cm long purplish corollas that often have white to pinkish stripes on the lower 3-lobed lip (Radford et al. 1968). Plants over-winter in rosette form but stems grow upright during the spring. Stems are usually simple, but sometimes branched; they typically reach 30 cm and are square and pubescent with swollen nodes that are lighter green than the internodes. The leaves are opposite, 6-13 cm long by 1.5-4 cm wide with margins that are shallowly toothed to entire (Radford et al. 1968). The inflorescence occurs terminally on the stem with congested whorls of floral bracts that enclose the calyx and form the “nest” of birds-in-a-nest. Inflorescences are indeterminate and average six flowers each, but some stems can have more than 20 because one or more series of bracts and budding flowers sometimes develop as the stem continues to grow. Usually, two flowers bloom simultaneously opposite each other at the top of each inflorescence. *M. caroliniana* is one of only two species of the genus *Macbridea*. The
other species is the federally listed *M. alba*, a narrow endemic of the Florida panhandle (Godt et. al 2002).

*M. caroliniana* is recognized as a ‘species of concern’ federally by the U.S. Fish and Wildlife Service and as threatened by the state of North Carolina (Franklin and Finnegan 2004). It is tracked as a rare species by The Georgia Natural Heritage Program and the South Carolina Heritage Trust (LeBlond and Sorrie 2002). Most of what is known about *M. caroliniana* and its populations can be found in a species-wide survey conducted by LeBlond and Sorrie (2002). Generally speaking, *M. caroliniana* usually occurs in swamp forests of mainly blackwater floodplains and this habitat is especially vulnerable to negative anthropogenic impacts such as nutrient input and damming of associated rivers. There are 36 known locations, and the largest population occurs at the Congaree National Park (CNP) near Columbia, South Carolina (LeBlond and Sorrie 2002). Currently, very little is known about the population at CNP and the biology of the plant in general. Research reported in this dissertation was conducted to fill this information gap.

**Main objectives of the project**

The goals of this project were to (1) estimate the population size and distribution of *M. caroliniana* at the Congaree National Park and determine the extent of co-occurrence with invasive species, (2) describe the habitat of *M. caroliniana* in the CNP, (3) characterize the breeding system of the species, and (4) describe the genetic structure of *M. caroliniana* within CNP and across the species range. Work related to each of these goals is presented in Chapters 2, 3, 4 and 5 respectively.
References


Figure 1.1. Range wide distribution of *M. caroliniana* in North Carolina, South Carolina and Georgia. Counties in dark gray have extant populations and those in lighter gray have historical populations that have not been observed in more than 20 years (modified from LeBlond and Sorrie 2002).
CHAPTER TWO

*M. CAROLINIANA* POPULATION SIZE AND DISTRIBUTION WITH THREATS FROM FERAL HOGS AND INVASIVE PLANT SPECIES IN CONGAREE NATIONAL PARK, SOUTH CAROLINA, USA

**Introduction**

Fifteen of the 36 known occurrences of *Macbridea caroliniana* are in South Carolina and two of these are at the Congaree National Park (CNP) near Columbia, South Carolina. One of the CNP populations is estimated to have the greatest number of plants of all known populations while the other has a relatively large number of individuals (Leblond and Sorrie 2002). Therefore, the populations in two seepage forest areas at the Park are likely to be very important to the long-term existence of the species (Figure 2.1). There is concern that these populations in particular may be harmed by feral hog activity and the invasive herb *Murdannia keisak* (LeBlond and Sorrie 2002). Prior to this study, very little was known about the populations at CNP, and the species in general.

At CNP, *M. caroliniana* occurs in forested areas known as “seepage forest” that are permanently saturated by groundwater seepage (Zengel 2008). The two large areas of seepage forest at CNP are about the same size, but the densities of plants differ. The forested area that is east of Cedar Creek (ECC) is about 80 hectares and it was estimated to have several thousands of stems (19,000+); the area east of Dry Branch (EDB) is about 67 hectares and was estimated to have fewer than 1,000 stems (LeBlond and Sorrie 2002). These 2001 population size estimates were made by counting stems in a few relatively small sections of both populations and then extrapolating the data to get stem
estimates for both seepage forest areas (personal communication with Bruce Sorrie of the North Carolina Natural Heritage Program).

To better understand the populations at the Park, monitoring is needed to document the size of the populations over time. Three types of rare plant monitoring of increasing effort and cost are recognized: inventory studies that simply count all plants in populations at intervals, surveys that use repeated sampling methods to estimate population sizes, and demographic studies that track the fates of individual plants in a sample (Palmer 1987). These different types of monitoring provide managers with increasing levels of information. Inventories provide a quick and inexpensive indication of the stability of small adult populations over time while survey studies require greater effort and are used for sub-sampling larger populations (Palmer 1987). Demographic studies are the most costly and time consuming of the three types, but provide information about life history traits (Hamann 2001; Garcia 2003).

I used a sampling approach to estimate the size of the two CNP populations because their large sizes are impractical for either an inventory or a demographic study. Different methods can be used for sampling to estimate population sizes of species, but for those that are spatially clustered, an adaptive cluster sampling method has proven to be an effective choice (McDonald 2004; Talvitie 2006). This method gives a relatively precise estimate of abundance for the sampling effort and is especially beneficial to use for species that are rare, clustered and hard to detect (Silletti and Walker 2003). Within the seepage forest areas of CNP, the plants are somewhat difficult to detect because they are low growing and usually occur clustered in small patches that range from one to
hundreds of stems (personal observation). Therefore, I chose to use the adaptive cluster sampling method to estimate the population sizes at CNP.

My goal for this study was to sample the two populations at the Park in three consecutive years to (1) obtain population size estimates, (2) estimate the frequency of *Sus scrofa* disturbance and the presence of *Murdannia keisak* (an invasive plant) occurring in close proximity to *M. caroliniana* patches; and (3) make recommendations for conservation strategies based on the results.

**Methods**

Within each seepage forest area, five parallel transects each approximately 500 meters long were established perpendicular to the main bluff line (where the adjacent upland forest drops off to the seepage forest). The distance between transects was determined systematically depending on the width of seepage forest area. ECC is approximately 1500 m wide, and transects were spaced 300 meters apart. EDB is about 1250 m wide and transects were spaced 250 m apart. The locations of start points for all transects were collected by a Garmin e-Trex unit and mapped based on the GPS data. New start points were established for each sample year (2003-2005). For each transect, the main sampling unit was 2 meters wide and approximately 500 m long with the center line being the transect itself. The main sampling unit was subdivided into 1 m long x 2 m wide plots. All of these plots were scanned for *M. caroliniana* stems while walking along each transect. When stems were observed, they were counted, and then the adjacent 1 x 2 m plots surrounding the initial plot were searched for stems (see Figure 2.2). When a secondary plot had stems, the stems were counted and all surrounding plots
to that one were searched and stems within them counted (Thompson and Seber 1996). This procedure was repeated until no more stems were observed in the adjacent plots.

A map of each cluster and its position in the main sample unit (distance in meters from the start point) were recorded in a gridded notebook. I also recorded the GPS locations of clusters greater than ten stems, occurrences of hog disturbance, and presence of *Murdannia keisak* along each transect during 2004 and 2005. In 2003, I recorded the start point for each transect using a GPS unit and used the locations (in meters) of stem clusters > 10 stems, occurrences of hog disturbance, and presence of *Murdannia keisak* on the azimuths of the transects to prepare a map. During 2007, I conducted meandering searches of other, much smaller areas of seepage forest within the Park. ArcGIS 9.2 (ESRI) was used to prepare maps. The Hansen-Hurwitz (Thompson and Seber 1996) estimator and McCarthy and Snowden’s bootstrap-with-replacement method (1985) were used to construct the percentile confidence intervals of population size for ECC and EDB. This method was recommended by Christman and Pontius (2000) for studies using adaptive cluster sampling with the Hansen-Hurwitz estimator. The sample size used for the bootstrap procedure was based on the formula \( m = \left[ \frac{(n-1)}{(1-n/N)} \right] \) where \( n=5 \) for both populations and \( N=750 \) for ECC and \( N=625 \) for EDB (\( n = \) number transect estimates; \( N = \) number of units wide). Because \( m \) was between 4 and 5 for both populations, I followed Shao and Tu (1995) to determine the bootstrapping sample size ratios (\( P \)) where the proportion (\( P \)) of \( m_1 = \left[ \frac{(1/m_2 - 1/m_1)}{(1/m_2 - 1/m_1)} \right] \) and in this case \( m_1 = 4 \) transect estimate samples and \( m_2 = 5 \) transect estimate samples. To test the effects of population on cluster size, I fit generalized linear mixed models assuming a Poisson distribution for
the response variable using PROC GLIMMIX in SAS 9.2 (SAS Institute). I tested the
effect of forested area on number of clusters per transect and number of stems per
transect using student’s t-tests.

**Results**

During the three year study, I found 6995 stems in 232 clusters that ranged in size
from one to 1116 stems. There were a total of 179 clusters found at the ECC site; I found
60 in 2003, 58 in 2004 and 61 in 2005. At the EDB site I found a total of 53 clusters, 18
in 2003, 25 in 2004 and ten in 2005. Although the ECC and EDB forest seepage areas
were sampled with an equal number of transects during the study, I found more clusters
with > 10 stems at ECC and the greatest concentration of clusters was near the center of
ECC (Figure 2.5). Most of the EDB clusters with > 10 stems were found during 2004
(Figures 2.6 - 2.8). Over all years, the ECC population had a greater average number of
clusters found per transect (p < 0.001) and greater variability than did the EDB
population (Table 2.1). The average cluster size (number of stems per cluster) over all
years was 36 stems at ECC (SD = 126.9) and 10 stems at EDB (SD = 19.6), but this was
not significantly different (p = 0.20). There was a greater total number of stems
pertransect in ECC (433 compared to 34; p = 0.016) (Figure 2.3).

The 90% confidence intervals for the ECC population were 25,406 to 86,500
stems in 2003, 34,396 to 183,951 stems in 2004, and 55,656 to 163,866 stems in 2005
and the intervals for the population at EDB were 2,500 to 13,985 stems in 2003, 11,250
to 41,250 stems in 2004 and 469 to 10,469 stems in 2005 (Figure 2.4). Despite the wide
intervals at ECC, they do not overlap the EDB intervals in 2003 and 2005. However, in
2004 the intervals from the two populations do overlap (5% of the ECC interval is overlapped by the EDB interval).

Hog rooting activity was evident at both populations although there was variability between years and transects (see Table 2.2). I recorded a total of 44 instances of hog disturbance not including two transects that had continuous coverage of upturned substrate due to hog activity. Nineteen *M. caroliniana* patches were within two meters of hog disturbance. There was no evidence of hog disturbance at ECC during 2003 (Figure 2.6), but during 2004 and 2005, I found hog disturbance at both seepage forest areas (Figure 2.7 and 2.8). The Asian invasive plant, *Murdannia keisak*, was not recorded at the EDB forest area (Figures 2.6 - 2.8). *M. keisak* was almost always found co-occurring with *M. caroliniana*; however, this relationship was not reciprocal as *M. caroliniana* was not always found with *M. keisak* (Table 2.3).

I located six patches with a total of 128 *M. caroliniana* stems in areas where previously the study species was not known to occur (Figure 2.9). The western-most patch was found in a Bald cypress-Water Tupelo/Water Ash Forest as opposed to the more typical Swamp Tupelo- Red Maple / American Holly / Coastal Doghobble / Howe Sedge Forest or Swamp Blackgum Floodplain Seepage Forest community.

**Discussion**

The results support previous estimations that the Congaree National Park population at ECC is the largest *M. caroliniana* population. LeBlond and Sorrie (2002) estimated the population in ECC to have a minimum of 19,000 stems and my results largely concur with that finding as the smallest lower limit for this population over all
years is 25,406 stems in 2003. In comparison, the next three largest *M. caroliniana* populations outside of CNP are estimated to have many fewer stems: Savannah River Site – Upper Three Runs in South Carolina has ~11,000 stems, Fort Gordon in Georgia has ~2,700 stems, and Aiken State Park in South Carolina has up to 1,500 stems (LeBlond and Sorrie 2002). Following ECC, EDB may be the next largest population but the broad estimates do not clarify this population’s size ranking amongst the others.

Although a small portion of the ECC and EDB confidence intervals overlapped in 2004, it is reasonable to conclude the population at ECC is larger than EDB. In 2003 and in 2005, the large ECC confidence intervals did not overlap with those of EDB. Also, I found there to be significantly more stems per transect and more clusters per transect at ECC. The wide confidence intervals at ECC indicate the uncertainty of the estimate that is a result of the patchiness of the species, variation among patches sampled, and variation among transects. However, increased sampling can not overcome the natural patchiness and large variation of patch size so the estimates would still not be very precise.

A number of factors could be responsible for the population size difference between the two CNP populations. The habitat may be more suitable at ECC, for example, if more sunlight reaches the forest floor in more areas. Increased light is thought to be positively correlated with larger patches and greater plant vigor (LeBlond and Sorrie 2001). In a companion study, less light was found to reach the forest floor at EDB where there is significantly greater canopy coverage compared to the ECC area (Chapter 3). Results presented in Chapter 3 also indicate that there was significantly
more shrub cover at EDB than ECC and this finding suggests more suitable habitat at ECC according to LeBlond and Sorrie (2001), who described *M. caroliniana* microhabitats as mostly shrub-free. Other habitat factors including disturbance history, soil nutrients and texture, and hydrological factors are likely to influence the number, size and spread of *M. caroliniana* patches (e.g., different flooding patterns could affect seed dispersal).

Feral hogs affect both CNP populations and although hogs do not appear to seek out *M. caroliniana*, their uprooting activity causes such substrate disruption that stems and basal rosettes of *M. caroliniana* get tilled under the soil. Stems were frequently found lying on top of or half-buried in upturned soil (personal observation). Where *M. caroliniana* occurs, there are other plants and fruits that are important in the feral hog diet. In coastal South Carolina, the major components of their mostly plant-based diet has been found to include the fruits of *Quercus*, *Vitis* and *Nyssa* species, and roots including those of *Saururus cernuus* (Wood and Roark 1980), all found in the seepage forest areas. In particular, *S. cernuus* is a frequent co-occurring species of *M. caroliniana* (Leblond and Sorrie 2002, see Chapter 3 also) and hogs may be seeking out *S. cernuus* roots. At the CNP, acorns are probably an especially important food source as hog habitat use has been positively correlated with *Quercus* species (Friebel 2008). Interestingly, it has been suggested that hog rooting may act as a periodic disturbance that contributes to species richness since seepage forest areas with recent rooting were found to have the most diverse understory (of the four types of forest studied) at the Park.
(Zengel 2008). However, the relationship could be a result of hogs simply selecting the most diverse habitats but not creating them (Zengel 2008).

Hogs appear to preferentially select areas of the seepage forest with many of the same characteristics as *M. caroliniana* habitat. Hogs root in areas of the seepage forest with greater herb richness, more moss and fewer woody shrubs (Zengel 2008) and these qualities are also preferred by *M. caroliniana* (see Chapter 3). Because hog activity is so disruptive to the wet depressions that are prime *M. caroliniana* habitat (Figure 2.10), I believe that hog control would benefit the species at the Park. Elsewhere, efforts have been successful in protecting a rare plant species (*Hellonias bullata*) by excluding hogs with fencing (pers. communication with Mary Bunch, Preserve Manager at South Carolina Department of Natural Resources). Fences consisted of a series of low (10 - 15 cm high) cables attached at the corners to rebar staked into firm substrate surrounding patches of *H. bullata*. Some level of hog disturbance is expected to continue because hunt clubs (e.g., Cedar Creek Hunt Club) with wild hogs are adjacent to the Park. The effects of hog control and/or exclusion on *M. caroliniana* can be tested by sampling multiple patches before and after applying control and/or exclusion.

Hog activity has a clear negative effect on *M. caroliniana* patches, but the effect of *Murdannia keisak* is not so apparent. Often the largest patches of *M. caroliniana* were found growing interspersed with *M. keisak* (Figure 2.11). Understanding this relationship would require weed removal experiments that include before and after sampling. Interestingly, *M. keisak* was not found at EDB, but it may occur there. ECC may have more suitable habitat for this invasive as it does for the study species.
In conclusion, the ECC population is the largest *M. caroliniana* population and is therefore very important for the long term existence of the species. Based on my estimates, it is possible that the population consists of 100,000 stems. Conservation management should consider hog control while weed control of *M. keisak* requires more investigation.

**References**


Figure 2.1. Congaree National Park with areas of seepage forest highlighted in light aqua blue. The study areas that were sampled for population size estimates over three seasons are labeled as A (East of Dry Branch) and B (East of Cedar Creek).
Figure 2.2. Demonstration of adaptive cluster analysis technique. Grid A shows an example of a primary sampling unit with O’s where no *M. caroliniana* is found and X’s where it is. The question marks indicate the next 2x1 m plots to be searched. Based on the presence of stems, Grids B and C show the sequence of where subsequent plots would be added in bold X’s and O’s and question marks indicate the next 2x1 m plots to be searched. For each plot added to the cluster that contains stems, surrounding plots are checked in all cardinal directions. This process continues until no more stems are found in adjacent plots with stems.
Table 2.1. Total number of *M. caroliniana* clusters found along each transect in both populations by year sampled (1 = 2003, 2 = 2004, 3 = 2005) and average number of clusters for both forested areas over the course of the study. ECC (East of Cedar Creek) had a greater average number of clusters found per transect (# clusters) compared to EDB (East of Dry Branch).

<table>
<thead>
<tr>
<th>Year</th>
<th>Transect 1</th>
<th>Transect 2</th>
<th>Transect 3</th>
<th>Transect 4</th>
<th>Transect 5</th>
<th># Clusters (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECC</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>16</td>
<td>15 (2.9)</td>
</tr>
<tr>
<td>EDB</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>5</td>
<td>3 (0.8)</td>
</tr>
</tbody>
</table>

Figure 2.3. Total number of *M. caroliniana* stems sampled per transect at ECC (East of Cedar Creek) and EDB (East of Dry Branch). There was a greater total number of stems per transect in ECC versus EDB.
Figure 2.4. Ninety percent confidence intervals of the population sizes using adaptive cluster sampling and bootstrapping for East of Cedar Creek (A) and East of Dry Branch (B) forested areas at Congaree National Park over three years. The 2004 confidence intervals overlap where 5% of the East of Cedar Creek interval is overlapped by the East of Dry Branch interval. Note the difference in scales.
Table 2.2. Number of hog disturbance observations in East of Cedar Creek (ECC) and East of Dry Branch (EDB) forested areas per transect in 2003 (year = 1), 2004 (year = 2), and 2005 (year = 3). Although evidence hog disturbance was not found at ECC during sampling in 2003, it was found while sampling 2004 and 2005. The number of observations within 2 meters of *M. caroliniana* stems is in parentheses. Note: transects were not sampled in exact same locations each year. Some transects had continuous disturbance (CD) along the entire length.

<table>
<thead>
<tr>
<th>Transect 1</th>
<th>Transect 2</th>
<th>Transect 3</th>
<th>Transect 4</th>
<th>Transect 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECC</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>EDB</td>
<td>1 CD (3)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>4</td>
</tr>
<tr>
<td>Total number of observations (not including transects with continuous disturbance)</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of observations within two meters of <em>M. caroliniana</em></td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.3. Number of *Murdannia keisak* observations within *Macbridea caroliniana* patches and the percentage of all *M. caroliniana* observations with *M. keisak* present (%) for each study season. All observations of *M. keisak* were in East of Cedar Creek; there were no *M. keisak* plants found at East of Dry Branch during the study.

<table>
<thead>
<tr>
<th>ECC</th>
<th>Transect 1</th>
<th>Transect 2</th>
<th>Transect 3</th>
<th>Transect 4</th>
<th>Transect 5</th>
<th>Total <em>M. keisak</em> occurrences (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>0</td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>1</td>
<td>25 (41)</td>
</tr>
<tr>
<td>2004</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>8 (14)</td>
</tr>
<tr>
<td>2005</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>13 (21)</td>
</tr>
</tbody>
</table>
Figure 2.5. Distribution of *M. caroliniana* at East of Cedar Creek (ECC) and East of Dry Branch (EDB) forest seepage areas of the Congaree National Park from three years of surveys. The azimuths show the approximate transect locations for each year’s study season. Note the difference in the number of *M. caroliniana* clusters indicated by the green circles between ECC and EDB.
Figure 2.6. Distribution of *M. caroliniana*, hog disturbance and *Murdania keisak* of East of Cedar Creek (ECC) and East of Dry Branch (EDB) forest seepage areas of Congaree National Park during 2003. There was only one patch with more than 10 stems at East of Dry Branch (EDB - seepage forest on the right) and evidence of hog disturbance was only found at EDB. *M. keisak* was not found at EDB during the course of the study.
Figure 2.7. Distribution of *M. caroliniana*, hog disturbance and *Murdania keisak* of East of Cedar Creek (ECC) and East of Dry Branch (EDB) forest seepage areas of Congaree National Park during 2004.
Figure 2.8. Distribution of *M. caroliniana*, hog disturbance and *Murdania keisak* of East of Cedar Creek (ECC) and East of Dry Branch (EDB) forest seepage areas during 2005. There were no patches with more than 10 stems at EDB (seepage forest on the right).
Figure 2.9. Locations of *M. caroliniana* stems found during 2007 survey at Congaree National Park where the study species was previously not known to occur.

Figure 2.10. An example of hog rooting disturbance. Note the wallow indicated by the arrow.
Figure 2.11. The invasive plant *Murdannia keisak* in a large patch of flowering *Macbridea caroliniana* at Congaree National Park. A stem of the more slender-leaved *M. keisak* is indicated by the arrow.
CHAPTER THREE

HABITAT FACTORS ASSOCIATED WITH M. CAROLINIANA

Introduction

Populations of Macbridea caroliniana occur in swamp forests of blackwater floodplains and less frequently in brownwater floodplains (Leblond and Sorrie 2002). Blackwater floodplains are associated rivers that are colored by decaying organic matter and originate in the Coastal Plain while brownwater rivers have their origin in the mountains or Upper Piedmont and get their color from sediments (Kellison et al. 1998). Within these floodplain habitat types, M. caroliniana occupies a very small fraction of what is considered suitable habitat and the reasons for this rarity are unknown. I found the study species to be patchily distributed across two seepage forest areas of CNP during population sampling conducted over three consecutive summers (Chapter 2). I also found a greater abundance of M. caroliniana in the seepage forest east of Cedar Creek (ECC) than in the area east of Dry Branch (EDB) (Chapter 2). Both areas have been classified as the same forest type (Swamp Tupelo- Red Maple / American Holly / Coastal Doghobble / Howe Sedge Forest or Swamp Blackgum Floodplain Seepage Forest community) (The Nature Conservancy 2001). Understanding the differences between two areas that are classified as the same forest type and why M. caroliniana occurs only patchily in these areas that are considered suitable habitat would provide valuable information on which to base the management decisions regarding this species.

When not in bloom, M. caroliniana is easily confused with other wetland species. If habitat features associated with M. caroliniana can be easily identified, then better
informed decisions could be made with respect to managing and protecting the suitable
habitat for the species. Hodgson (1986) states that species’ abundance can be determined
exclusively by how much suitable habitat there is; however defining “suitable” is the
challenge. A plant species distribution can be limited by abiotic factors and further
limited by biotic factors interacting in complex ways (Kruckeberg and Rabinowitz 1985).
For example, some rare plants are limited by their inability to disperse seeds to potential
habitat (Coates et al. 1999). Seed dispersal and other biotic factors such as vegetative
reproduction and competition may also influence distributions (Schwarz et. al 2003).

Characteristics of suitable M. caroliniana habitat have not been quantified;
however observations by trained botanists suggest some possibly important factors.
LeBlond and Sorrie (2002) speculated that habitat occupancy might be related to large
canopy gaps, low shrub cover and/or high moss cover. Most of these are consistent with
my observations (Chapter 2). Areas with low vegetative cover may be necessary for
populations to become established and maintained as was found in Aeschynomene
virginica (Griffith and Forseth 2003), another wetland herb. Stems frequently grow on
mossy hummocks (personal observation) so there may be a positive association with
moss cover.

Edaphic factors have been shown to be important in determining plant
distributions (Bowles et. al 2005). M. caroliniana was frequently observed in small
depressions of the seepage forest where minerals may be deposited during rain events
making conditions more favorable for herbaceous species (Sluis and Tandarich 2004). In
wetlands, nitrogen and phosphorus often are primary limiting nutrients (Aerts et al.
1992), but calcium and potassium may also limit plant productivity (Bridgham 1996).

Within the seepage forest of the Congaree National Park, Dorovan muck is the major soil type and in this environment and phosphorus is a likely limiting factor (Graeme Lackaby, Auburn University, personal communication).

To model species-habitat relationships, multivariate approaches such as multiple regression, principal components analysis (PCA), or logistic regression are commonly used depending on the research question and the number and type of dependent and independent variables (Morrison et al. 1992). Ordination techniques have been used to compare habitats of rare plant species with sites where the target species does not occur. Prober and Austin (1990) used detrended correspondence analysis (DCA) and global non-metric multidimensional scaling (NMDS) to compare sites where *Eucalyptus paliformis* occurred with similar sites where it does not. To compare the vegetation of sites with and without *Spiranthes romanzoffiana*, Henderson (2001) also used DCA in addition to two-way indicator species analysis (TWINSPAN; Hill 1979). The problem with these and other multivariate techniques is that they can be difficult to interpret in a biologically meaningful way because these techniques combine several environmental parameters into one collapsed mathematical function that is correlated with the species of interest (Morrison et al. 1992). Sometimes it is not clear how to interpret the collapsed functions or correlations especially when axes are rotated and data are transformed.

Information-theoretic approaches to multi-model selection using Akaike’s information criteria (AIC) have recently been used in ecological studies that seek to identify habitat associations. To quantify evidence for models and choose the best
predictive models for species occurrence, AIC is often used with logistic regression (Carrie et al. 2002; Connor and Godbois 2003; Welch and McMahon 2005). To choose the best predictive models for species abundance (or richness), AIC may be used with linear regression (LeDee et al. 2008).

Here, I measured habitat characteristics of paired sample plots, one with and one without *M. caroliniana*, and used regression techniques to model presence, abundance, density and total patch area. An information-theoretic approach was used to ask the following general research questions: Within the study area, which model of habitat characteristics is the best predictor of *M. caroliniana* presence? Are the factors in the best models for predicting *M. caroliniana* similar to those for patch abundance, density and total patch area?

Second, I sampled two areas of seepage forest in CNP which supported very different population sizes of *M. caroliniana* (Chapter 2). I asked the following question: Is ECC different than EDB with respect to the tree composition, canopy cover, shrub cover, herb composition and abundance, or soil nutrients? My final goal was to use both sets of analyses to explain, if possible, the difference in abundance in ECC and EDB.

**Methods**

**Patch-scale study sampling**

I randomly located a total of 72 sites with between one and 1012 *M. caroliniana* stems during the summer of 2005 (May 27 to August 18). Nineteen of these sites met my minimum criterion of stems (10) and I matched each of these with a site approximately 15 m due south with no *M. caroliniana* stems. All of the observations were from ECC
(East of Cedar Creek, see Figure 2.1 for location at Park), because none of the 11 observations at the East of Dry Branch (EDB) area contained the minimum number of stems. I measured seven biotic and 13 abiotic habitat variables (Table 3.1).

In each area of seepage forest, sample sites as described above were located along each of the five 500-m transects while surveying the population at the Park (described in Chapter 2). All patches that contained > 10 stems within a 2 x 2 m area were sampled along the transects. A 2 x 2 m plot was centered on the greatest concentration of stems (presumably indicating the best habitat) and this plot was subdivided into four 1 x 1 m sub-plots. In each sub-plot, the percent cover of shrubs, moss and sedge were recorded using a scale of cover classes modified from Braun-Blanquet (1932): 1=up to 1%, 2=1-5%, 3=6-25%, 4=26-50%, 5=51-75%, 6=76-100%. All herbaceous stems were identified and counted in each sub-plot and soil samples were taken to a depth of 20 cm using a 7 cm diameter mud/clay auger. The means of all sub-plot measurements were analyzed.

Canopy openness was measured using a Model-A densiometer positioned on a 1 m stake located near the highest density of *M. caroliniana*. To determine the percentage of canopy cover, four densiometer readings were taken (one per cardinal direction) at each location (Lemmon 1956). This procedure was repeated for matched plots without *M. caroliniana*. A total of 19 plot pairs were sampled. Patch area and number of stems were based on a concurrent study of population size (Chapter 2).

**Forest area-scale study sampling**

In each forested area, my goal was to establish 30 sample locations distributed evenly along six north-south transects (five samples per transect). These transects were
not the same as those used in the above patch-scale study. At ECC, I sampled 30 locations, and at EDB I sampled 34 locations. I mapped the locations during the study and realized that one of the locations at EDB was just outside the sampling area and that I missed sampling the center of this area. To correct this, I removed the one point that was outside and added a seventh transect (five new points) in the center of EDB, giving a total of 34 points sampled. At each point, I sampled the herb layer, canopy cover and soil as described above except that at each sampling point there was a single 1 x 1 m plot. Shrub cover (< 1.5 m tall) for each species was estimated (by cover class) in a 4 x 4 m plot. I used plot and plotless methods centered on the sample point to quantify the tree component. For the plotless method, I followed standard procedures for estimating basal area using a glass wedge prism with a basal area factor (BAF) of 10 (West 2004). From the same point, I also measured the diameter at breast height (dbh; approximately 1.4 m above ground) of all trees within a 10 m radius and identified stems by species. I used a rangefinder to verify if trees were within the 10 m radius plot.

Laboratory analysis

Soil samples were dried at room temperature for several days and delivered to the Agricultural Service Laboratory of Clemson University for analysis, where the samples were dried for 24 hours at 60º C, ground, and finally sieved through a 2mm screen. Samples were extracted with Mehlich 1, a weak double acid solution (0.05M HCl + 0.0125M H$_2$SO$_4$); the extract was analyzed by ICP (induction coupled plasma) and the pH was determined in a 1:1 soil to water mixture. The percentage of organic matter was determined using a dry combustion method. The samples were weighed and heated to
about 900°C in a stream of ultra-pure oxygen, samples were combusted (C oxidized to CO₂), and the CO₂ was measured (Soil Analysis Procedures). I determined the cation exchange capacity (CEC) by calculating the milliequivalents of H, K, Mg, Na and Ca per 100 g of soil and summing these values (Lippert). Nitrate was not tested because it is especially unstable under anaerobic conditions such as in wet muck soil and is quickly depleted (Vepraskas and Faulkner 2001).

Data analysis

For the patch-scale study, I grouped all the variables according to forest strata layers or as soil nutrients. Variables were placed into the following categories: canopy and subcanopy layer, shrub layer, herb/fern layer, litter layer, and soil chemistry (Table 3.1). I used an information theoretic approach to model selection (Burnham and Anderson 2002). I considered a total of 34 plausible models: 21 single variable models and 13 multiple variable models (Table 3.2). All models were based on a priori hypotheses of environmental variables that are thought to be associated with M. caroliniana habitat suitability. I used logistic regression (Allison 2000) to model M. caroliniana presence. I used linear regression in the habitat prediction models to analyze the following dependent variables: number of stems in patch (patch abundance), density of stems in plot and total patch area. Linear correlations of habitat factors with patch abundance, patch area and plot density were determined using Proc CORR (SAS 1999b) to avoid using highly correlated variables in the same models. I used Akaike’s Information Criterion corrected for small sample size (AICc), ΔAIC and Akaike weights (w) to identify the most parsimonious models (Burnham and Anderson 2002). The
Akaike weight ($w_i$) of a model estimates the relative likelihood that it is the best model out of the set of candidate models. Models with a $\Delta$AIC of less than two are also supported. I compared models using evidence ratios that indicate which fitted model is better in a Kullback-Leibler information sense which is a measure of the similarity between the statistical model and the “true” distribution (Burnham and Anderson 2002). For the forest area scale study, I used t-tests and when data could not be transformed to fit normality assumptions I used non-parametric one-way median tests to determine if there are differences in the characteristics sampled at ECC and EDB.

**Results**

**Patch-scale study**

The top three models with the lowest AIC values were all multiple variable models. The model with the lowest AIC contained the variables herbrich (number of herbaceous species), K (potassium ppm), and PHOS (phosphorus ppm) (Table 3.3A). This model explained 91% of the variation and had an evidence ratio of 27 ($w_1/w_2 = 0.9065/0.0335$) when compared with the next closest model. Sites with *Macbridea caroliniana* had a mean of 2.63 other herb species (SD=1.1), a mean concentration of 87.4 ppm potassium (SD = 23.3), and 6.5 ppm (SD = 3.4) of phosphorus. Sites without *M. caroliniana* had a mean of 1.2 herb species (SD = 0.70), 109.9 ppm potassium (SD = 19.7), and 5.5 ppm (SD = 1.1) of phosphorus.

I modeled several indicators of abundance: number of stems per patch, patch area, and stem density. My sample areas had between 12 and 1012 stems per patch ($\bar{X} = 138$, SD = 221). The best model to predict *M. caroliniana* abundance measured by total
number of stems per patch contained the variables Saur (*Saururus cernuus* abundance), Triad (*Triadenum walteri* abundance), Woodwardia (*Woodwardia areolata* abundance), moss (% *Sphagnum* spp. cover), PHOS and K and had an Akaike weight of 0.67 (Table 3.3B). The total patch area varied from 4 to 110 m² ($\bar{x} = 28$, SD = 24). The best model of patch area with a $w_1$ of 0.60 included the variables Saur, Triad, and Woodwardia (Table 3.4A). Plot stem density (in 4 m² plots) varied from 12 to 128 stems per plot ($\bar{x} = 44$, SD = 33). The best model was a single variable model with Triad ($w_1 = 0.33$) (Table 3.4B).

Forest area scale study

There were several significant differences in tree composition between forest areas ECC and EDB (Table 3.5A). The total basal area (all species combined) was greater at ECC than EDB. The basal area of three individual species, *Fraxinus caroliniana*, *Ilex opaca* and *Liquidambar styraciflua*, shared the same pattern (Figure 3.1). Two of these species (*Fraxinus caroliniana* and *Liquidambar styraciflua*) along with *Fraxinus pennsylvanica* were also found to have greater density at ECC (Figure 3.1). However, there was greater density of *Nyssa biflora* and *Persea borbonia* at EDB. Only *Acer rubrum* showed a significant difference in mean dbh, larger at ECC.

In addition to the differences in tree composition, there were other differences between forest areas (Table 3.6). Most notable were the differences in canopy and shrub cover, with EDB having greater cover for both of these components. The three variables comprising the best predictive model of *M. caroliniana* presence are also shown in this table, but none are significantly different between the populations. The general trends are
in agreement with the model, that is, ECC (with more *M. caroliniana*) has higher herb richness, less potassium and more phosphorus, at the forest level, a result that also is consistent with the patch level study.

I also compared herbs that were in the top models to predict *M. caroliniana* abundance, total patch area, and stem density (Table 3.5B). Although only significant at the 0.05 level for *Woodwardia areolata*, the trends, *Saururus cernuu* and moss coverage are in agreement with these local predictive models. There was more *S. cernuus*, greater moss coverage and less *W. areolata* at ECC. Due to a lack of data, I could not test if there were differences in the number of *M. caroliniana* nor *Triadenum walterii* stems between the populations. *M. caroliniana* was only found in two of the 30 randomly located sample sites at ECC and one of the sites at EDB (ECC = 5 and 10 stems; EDB = 6 stems) while *T. walterii* was only found in 2 of both areas’ sample sites (ECC= 4 and 1 stems; EDB = 1 and 1 stems).

**Discussion**

Other herb species including the variable for herb species richness dominate the strongest models for presence, abundance, patch area and density of *M. caroliniana*. It is likely that *M. caroliniana* responds to the same environmental characteristics as other seepage forest herbs including higher phosphorus and lower potassium availability in the soil. When growing conditions are good for *M. caroliniana*, they are good for many of the other herbs. Because *M. caroliniana* is difficult to notice when not in bloom, searching for the study species should include investigating any areas of the seepage forest with an abundance of herbaceous species.
Interestingly, the variables for canopy and shrub coverage were not in any of the top models for predicting *M. caroliniana*. Contrary to expectations, these two variables were not in the best predictive models of the patch-scale study; however, there were significant differences in the forest area-scale study (Table 3.8). I expected them to be negatively correlated with the presence of *M. caroliniana*, which is what was found: EDB overall had more shrub and canopy cover and less *M. caroliniana*. The difference in scale may account for the discrepancy and I did not sample EDB in the patch-scale study. In other words, the differences between shrub and canopy cover within a forest area may be smaller than between the overall cover in the ECC and EDB forest, and hence not appearing significant based on my samples.

Collectively, the results from the patch-scale and forest area studies show several trends that suggested overall better *M. caroliniana* habitat at ECC than EDB. Although I did not find significant differences in the forest-scale study for the variables that were in the strongest models for presence, abundance, density and patch area, the relationships were in the expected directions. There was greater herb richness, more phosphorus, less potassium, less *Woodwardia areolata*, and more *Saururus cernuus* at ECC.

Although ECC and EDB are classified as the same forest type, there are differences in the habitat that likely lead to differences in the tree composition and the abundance of the study species. The combination of higher levels of organic matter in the soil, lower pH, and greater density of *Nyssa biflora* and *Persea borbonia* indicate wetter conditions for *M. caroliniana* at EDB (Tables 3.5, 3.6). Although *M. caroliniana* is usually found in small wet depressions of the seepage forest, many areas of EDB may
be too wet for the study species to become established and survive. An alternative explanation is that there is more competition in those wet depressions and *M. caroliniana* can not compete under those conditions. Despite these unknowns, it is likely that there are fewer areas within EDB that are suitable for *M. caroliniana*.

**References**


Lippert, Bob. How are the cation exchange capacity (CEC) and percent base saturation calculated for the soil test report? Bob Lippert’s frequently asked questions regarding soil testing, plant analysis and fertilizers. (accessed June 6, 2006) http://hubcap.clemson.edu/~blpprt/bobweb/BOBWEB13.HTM.


Table 3.1. – Variable categories, name abbreviations and definitions measured at *M. caroliniana* and non- *M. caroliniana* sites at the Congaree National Park, 2005 for patch-scale habitat study.

<table>
<thead>
<tr>
<th>Category</th>
<th>Variable Names</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.) Canopy and Subcanopy Layers</td>
<td>canopy</td>
<td>Canopy cover (%)</td>
</tr>
<tr>
<td>2.) Shrub Layer</td>
<td>shrub</td>
<td>Woody vegetation cover (%)</td>
</tr>
<tr>
<td>3.) Herb/Fern Layer</td>
<td>sedge</td>
<td>All Carex spp. cover (%)</td>
</tr>
<tr>
<td></td>
<td>herbrich</td>
<td>Herb richness (number of species/m²)</td>
</tr>
<tr>
<td></td>
<td>Saur</td>
<td><em>Saururus cernuus</em> (number of stems/m²)</td>
</tr>
<tr>
<td></td>
<td>Triad</td>
<td><em>Triadenum walteri</em> (number of stems/m²)</td>
</tr>
<tr>
<td></td>
<td>Woodw</td>
<td><em>Woodwardia areolata</em> (number of stems/m²)</td>
</tr>
<tr>
<td>4.) Litter</td>
<td>moss</td>
<td>All Sphagnum spp. cover (%)</td>
</tr>
<tr>
<td>5.) Soil</td>
<td>PH</td>
<td>Active acidity</td>
</tr>
<tr>
<td></td>
<td>Buffer</td>
<td>Stored acidity</td>
</tr>
<tr>
<td></td>
<td>PHOS</td>
<td>Phosphorus (ppm)</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>Potassium (ppm)</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>Calcium (ppm)</td>
</tr>
<tr>
<td></td>
<td>ZN</td>
<td>Zinc (ppm)</td>
</tr>
<tr>
<td></td>
<td>MN</td>
<td>Manganese (ppm)</td>
</tr>
<tr>
<td></td>
<td>MG</td>
<td>Magnesium (ppm)</td>
</tr>
<tr>
<td></td>
<td>CU</td>
<td>Copper (ppm)</td>
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<tr>
<td></td>
<td>B</td>
<td>Boron (ppm)</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>Sodium (ppm)</td>
</tr>
<tr>
<td></td>
<td>CEC</td>
<td>Cation Exchange Capacity (meq/100g soil)</td>
</tr>
<tr>
<td></td>
<td>OM</td>
<td>Organic Matter (%)</td>
</tr>
</tbody>
</table>
Table 3.2. Hypotheses underlying the multi-variable models, the categories of forest strata layers and/or soil nutrients they contained and the variables that made up each model. All models were based on *a priori* hypotheses of environmental variables that are thought to be associated with *M. caroliniana* habitat suitability. These models plus single-variable models were used to find the best models to predict presence and abundance of *M. caroliniana* using logistic and linear regression.

<table>
<thead>
<tr>
<th>#</th>
<th>Hypotheses</th>
<th>Categories</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Is the structure above <em>M. caroliniana</em> best at predicting <em>M. caroliniana</em> presence?</td>
<td>Canopy, Understory and Shrub Layers</td>
<td>Canopy and shrub</td>
</tr>
<tr>
<td>2</td>
<td>Are forest floor species best at predicting presence of <em>M. caroliniana</em>?</td>
<td>Herb/Fern and Litter layers</td>
<td>Sedge, Saur, Triad, Woodw, and moss</td>
</tr>
<tr>
<td>3</td>
<td>Are seepage forest herbs best at predicting presence of <em>M. caroliniana</em>?</td>
<td>Herb/Fern Layer</td>
<td>Sedge, herbrich, Saur, Triad, and Woodw</td>
</tr>
<tr>
<td>4</td>
<td>Is the combination of all forest strata variables best at predicting presence of <em>M. caroliniana</em>?</td>
<td>Canopy, Understory, Shrub, Herb/Fern and Litter layers</td>
<td>Canopy, shrub, sedge, Saur, Triad, Woodw, and moss</td>
</tr>
<tr>
<td>5</td>
<td>Can the presence of <em>M. caroliniana</em> be predicted best by herbs thought to be good indicators?</td>
<td>Herb/Fern Layer (subset)</td>
<td>Saur, Triad, and Woodw</td>
</tr>
<tr>
<td>6</td>
<td>Can the presence of <em>M. caroliniana</em> be best predicted solely on soil nutrients?</td>
<td>Soil (subset)</td>
<td>PH, Buffer, PHOS, K, MN, CU, B, NA</td>
</tr>
<tr>
<td>7</td>
<td>Are the other soil factors best at predicting presence of <em>M. caroliniana</em>?</td>
<td>Soil (subset)</td>
<td>ZN, OM, CEC</td>
</tr>
<tr>
<td>8</td>
<td>Can the presence of <em>M. caroliniana</em> be best predicted solely on the soil nutrients thought to be most limiting in this environment?</td>
<td>Soil (subset)</td>
<td>PHOS and K</td>
</tr>
<tr>
<td>9</td>
<td>Can the presence of <em>M. caroliniana</em> be best predicted by forest floor species and the most limiting soil nutrients?</td>
<td>Herb/Fern (Subset), Litter layers and Soil Nutrients (subset)</td>
<td>sedge, Saur, Triad, Woodw, moss, PHOS, and K</td>
</tr>
<tr>
<td>10</td>
<td>Can indicator herbs, the litter layer and the most limiting soil nutrients best predict the presence of <em>M. caroliniana</em>?</td>
<td>Herb/Fern (subset) + Litter layer + Soil Nutrients (subset)</td>
<td>Saur, Triad, Woodw, moss, PHOS, and K</td>
</tr>
<tr>
<td>11</td>
<td>Can the presence be best predicted by the herb diversity and the most limiting soil nutrients?</td>
<td>Herb/Fern (subset) + Soil Nutrients (subset)</td>
<td>Herbrich, PHOS, and K</td>
</tr>
<tr>
<td>12</td>
<td>Can the presence of <em>M. caroliniana</em> be best predicted by one variable thought to be most important from each category?</td>
<td>Canopy, Understory, Shrub, Herb/Fern and Litter layers and Soil Nutrients (subset of all)</td>
<td>Canopy, shrub, Saur, moss, PHOS</td>
</tr>
<tr>
<td>13</td>
<td>Can the canopy opening and herb/fern layer best predict the presence of <em>M. caroliniana</em>?</td>
<td>Canopy, Understory and Herb/Fern layers</td>
<td>Canopy, sedge, herbrich, Woodw, Triad, and Saur</td>
</tr>
</tbody>
</table>
Table 3.3. 95% of the Akaike weight for A.) The best logistic regression models for *M. caroliniana* presence and B.) The best linear regression models for total number of stems. The first model for *M. caroliniana* presence has an estimated 90% probability of being the best model tested while the first model for total number of stems has a 67% probability of being the best model. With their ΔAIC values > two, the other two models are not supported. The minus signs indicate a negative relationship between the variable and the presence of *M. caroliniana*.

<table>
<thead>
<tr>
<th>A.) Models</th>
<th>$K^a$</th>
<th>$AIC^b_c$</th>
<th>$ΔAIC_i^c$</th>
<th>$w_i^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbrich PHOS (-K)</td>
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<td>27.51</td>
<td>0.00</td>
<td>0.9065</td>
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<tr>
<td>Saur Triad (-Woodw) moss PHOS (-K)</td>
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<td>34.10</td>
<td>6.59</td>
<td>0.0335</td>
</tr>
<tr>
<td>(-Canopy) (- sedge) herbrich (-Woodw) Triad Saur</td>
<td>8</td>
<td>35.22</td>
<td>7.71</td>
<td>0.0192</td>
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</table>

<table>
<thead>
<tr>
<th>B.) Models</th>
<th>$K^a$</th>
<th>$AIC^b_c$</th>
<th>$ΔAIC_i^c$</th>
<th>$w_i^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saur Triad (-Woodw) (-moss) PHOS –K</td>
<td>8</td>
<td>194.21</td>
<td>0.00</td>
<td>0.6719</td>
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<td>199.57</td>
<td>5.36</td>
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<tr>
<td>Triad</td>
<td>3</td>
<td>200.85</td>
<td>6.64</td>
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<tr>
<td>(-MN)</td>
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<td>201.13</td>
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</tr>
<tr>
<td>(-K)</td>
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<td>201.21</td>
<td>7.00</td>
<td>0.0203</td>
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<tr>
<td>(-MG)</td>
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<td>201.51</td>
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<tr>
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</tr>
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<td>OM</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Buffer</td>
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</tr>
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</tr>
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<td>(-CU)</td>
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<td>(-B)</td>
<td>3</td>
<td>201.83</td>
<td>7.62</td>
<td>0.0149</td>
</tr>
</tbody>
</table>

*a* Number of parameters in model  
*b* $AIC_c = AIC$ corrected for small sample sizes  
*c* $ΔAIC_i = AIC_i – minimum AIC_c$  
*d* The Akaike weight or estimated probability of being the best model tested
Table 3.4. Best linear regression models with 95% of the Akaike weight for (A) total patch area and (B) plot density. The first model for total patch area has an estimated 60% probability of being the best model tested while the first model for plot density has only a 33% probability of being the best model. With their $\Delta AIC$ values $> 2$, the other models are not supported. The minus signs indicate a negative relationship between the variable and the patch area in (A) or plot density in (B).

### A. Models

<table>
<thead>
<tr>
<th>Model</th>
<th>$K^a$</th>
<th>$AIC_c^b$</th>
<th>$\Delta AIC_i^c$</th>
<th>$w_i^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saur Triad (-Woodw)</td>
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<td>112.34</td>
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<td>(-sedge) Saur Triad (-Wood) (-moss)</td>
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<td>115.76</td>
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<tr>
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<td>116.59</td>
<td>4.25</td>
<td>0.0714</td>
</tr>
<tr>
<td>(-Canopy) (-shrub) Saur (-moss) PHOS</td>
<td>7</td>
<td>117.13</td>
<td>4.80</td>
<td>0.0544</td>
</tr>
<tr>
<td>(-sedge) herbrich Saur Triad (-Woodw)</td>
<td>7</td>
<td>117.64</td>
<td>5.31</td>
<td>0.0422</td>
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<tr>
<td>lizard</td>
<td>3</td>
<td>118.70</td>
<td>6.37</td>
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<td>(-MN)</td>
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<tr>
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<td>120.78</td>
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<td>3</td>
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<tr>
<td>(-K)</td>
<td>3</td>
<td>121.98</td>
<td>9.64</td>
<td>0.0048</td>
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</table>

### B. Models

<table>
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<th>Model</th>
<th>$K^a$</th>
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<th>$\Delta AIC_i^c$</th>
<th>$w_i^d$</th>
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<tbody>
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</tr>
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<td>herbrich</td>
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<td>130.11</td>
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</tr>
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<td>CA</td>
<td>3</td>
<td>130.29</td>
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<td>ZN</td>
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<td>NA</td>
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<tr>
<td>MG</td>
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</tr>
<tr>
<td>BUFFER</td>
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</tr>
<tr>
<td>K</td>
<td>3</td>
<td>131.24</td>
<td>4.38</td>
<td>0.0371</td>
</tr>
<tr>
<td>MN</td>
<td>3</td>
<td>131.47</td>
<td>4.61</td>
<td>0.0332</td>
</tr>
<tr>
<td>OM</td>
<td>3</td>
<td>131.48</td>
<td>4.62</td>
<td>0.0330</td>
</tr>
<tr>
<td>Saur Triad (-Woodw)</td>
<td>5</td>
<td>131.61</td>
<td>4.75</td>
<td>0.0308</td>
</tr>
<tr>
<td>(-CU)</td>
<td>3</td>
<td>132.08</td>
<td>5.22</td>
<td>0.0244</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>132.16</td>
<td>5.30</td>
<td>0.0234</td>
</tr>
<tr>
<td>(-PH)</td>
<td>3</td>
<td>132.17</td>
<td>5.31</td>
<td>0.0233</td>
</tr>
<tr>
<td>(-PHOS)</td>
<td>3</td>
<td>132.18</td>
<td>5.32</td>
<td>0.0232</td>
</tr>
</tbody>
</table>

---

*a Number of parameters in model  
*b $AIC_c = AIC$ corrected for small sample sizes  
*c $\Delta AIC_i = AIC_i – minimum AIC_c$  
*d The Akaike weight or estimated probability of being the best model tested
Figure 3.1. Mean basal area in m²/hectare (A.) and mean density (number of trees/hectare) (B.) by top nine species at ECC and EDB of Congaree National Park. Abbreviations are as follows: Nb=Nyssa biflora, Ar=Acer rubrum, Ls=Liquidambar styraciflua, Io=Ilex opaca, Ql=Quercus laurifolia, Fp=Fraxinus pennsylvanica, Fc=Fraxinus caroliniana, Cc=Carpinus caroliniana, and Pb=Persea borbonia. Stars indicate where significant differences were found between the tree species at ECC and EDB.
Table 3.5. Vegetation tables of tree and herb species of East of Cedar Creek (ECC) and East of Dry Branch (EDB) for forested-area scale study. A.) Mean density (trees/hectare) and basal area (BA; m$^2$/hectare) of tree species. B.) Average number of herb stems found per m$^2$ plot. The means shown in bold are statistically greater ($p$ or chi-square value < 0.05).

<table>
<thead>
<tr>
<th>A.) Tree Species</th>
<th>ECC Density (SE)</th>
<th>ECC BA (SE)</th>
<th>EDB Density (SE)</th>
<th>EDB BA (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nyssa biflora</td>
<td>198.41 (20.4)</td>
<td>22.8 (2.6)</td>
<td>261.2 (20.8)</td>
<td>26.06 (2.9)</td>
</tr>
<tr>
<td>Acer rubrum</td>
<td>67.91 (10.3)</td>
<td>4.29 (1.0)</td>
<td>75.83 (10.8)</td>
<td>4.32 (0.7)</td>
</tr>
<tr>
<td>Liquidambar styraciflua</td>
<td><strong>123.08</strong> (18.0)</td>
<td><strong>6.12</strong> (1.2)</td>
<td>58.98 (8.1)</td>
<td>1.89 (0.6)</td>
</tr>
<tr>
<td>Ilex opaca</td>
<td>141.12 (16.3)</td>
<td><strong>5.66</strong> (0.8)</td>
<td>172.26 (16.3)</td>
<td>2.3 (0.5)</td>
</tr>
<tr>
<td>Quercus laurifolia</td>
<td>42.44 (9.8)</td>
<td>1.07 (0.5)</td>
<td>34.64 (8.1)</td>
<td>0.81 (0.3)</td>
</tr>
<tr>
<td>Fraxinus pennsylvanica</td>
<td><strong>23.34</strong> (6.8)</td>
<td>0.61 (0.4)</td>
<td>2.81 (2.1)</td>
<td>0.27 (0.3)</td>
</tr>
<tr>
<td>Fraxinus caroliniana</td>
<td><strong>46.69</strong> (11.3)</td>
<td><strong>1.84</strong> (0.5)</td>
<td>1.87 (1.3)</td>
<td>0.27 (0.3)</td>
</tr>
<tr>
<td>Carpinus caroliniana</td>
<td>7.43 (3.6)</td>
<td>0.77 (0.5)</td>
<td>24.34 (7.0)</td>
<td>0.81 (0.3)</td>
</tr>
<tr>
<td>Persea borbonia</td>
<td>7.43 (2.5)</td>
<td>0.61 (0.4)</td>
<td><strong>67.41</strong> (12.0)</td>
<td>1.22 (0.4)</td>
</tr>
<tr>
<td>Quercus alba</td>
<td>5.31 (2.7)</td>
<td>0.15 (0.2)</td>
<td>11.23 (4.0)</td>
<td>0.27 (0.2)</td>
</tr>
<tr>
<td>Ulmus americana</td>
<td>4.24 (2.5)</td>
<td>0.15 (0.2)</td>
<td>0.94 (0.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Quercus michauxii</td>
<td>9.55 (4.9)</td>
<td>0.46 (0.3)</td>
<td>5.62 (2.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Magnolia virginiana</td>
<td>12.73 (4.5)</td>
<td>0.31 (0.3)</td>
<td>5.62 (2.5)</td>
<td>0.27 (0.3)</td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>0.00 (0.0)</td>
<td>0.15 (0.2)</td>
<td>0.94 (0.9)</td>
<td>0.27 (0.2)</td>
</tr>
<tr>
<td>Liriodendron tulipifera</td>
<td>1.06 (1.1)</td>
<td>0.00 (0.0)</td>
<td>3.74 (2.2)</td>
<td>0.00 (0.0)</td>
</tr>
<tr>
<td>Fagus grandifolia</td>
<td>0.00 (0.0)</td>
<td>0.00 (0.0)</td>
<td>0.94 (0.9)</td>
<td>0.00 (0.0)</td>
</tr>
<tr>
<td>Ulmus rubra</td>
<td>1.06 (1.1)</td>
<td>0.00 (0.0)</td>
<td>0.00 (0.0)</td>
<td>0.00 (0.0)</td>
</tr>
</tbody>
</table>

Total 691.79 (24.2) **45.69 (2.0)** 728.37 (29.1) 37.74 (1.8)
Table 3.5 (continued). Vegetation tables of tree and herb species of East of Cedar Creek (ECC) and East of Dry Branch (EDB) for forested-area scale study. A.) Mean density (trees/hectare) and basal area (BA; m$^2$/hectare) of tree species. B.) Average number of herb stems found per m$^2$ plot. The means shown in bold are statistically greater ($p$ or chi-square value < 0.05).

<table>
<thead>
<tr>
<th>B.) Herbaceous species</th>
<th>ECC</th>
<th>EDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woodwardia areolata</td>
<td>1.17 (0.3)</td>
<td>3.18 (0.7)</td>
</tr>
<tr>
<td>Saururus cernuus</td>
<td>0.33 (0.1)</td>
<td>0.29 (0.2)</td>
</tr>
<tr>
<td>Hydrocotyle verticillata</td>
<td>0.77 (0.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Boehmeria cylindrica</td>
<td>0.07 (0.1)</td>
<td>0.03 (0.0)</td>
</tr>
<tr>
<td>Mitchellia repens</td>
<td>0 (0.0)</td>
<td>0.37 (0.2)</td>
</tr>
<tr>
<td>Osmunda cinnamomea</td>
<td>0.1 (0.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Sagittaria latifolia</td>
<td>0.07 (0.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Viola villosa</td>
<td>0.32 (0.2)</td>
<td>0.06 (0.1)</td>
</tr>
<tr>
<td>Macbridea caroliniana</td>
<td>0.48 (0.4)</td>
<td>0.17 (0.2)</td>
</tr>
<tr>
<td>Persicaria punctata</td>
<td>0.03 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Triadenum walteri</td>
<td>0.16 (0.1)</td>
<td>0.06 (0.0)</td>
</tr>
<tr>
<td>Murdannia keisak</td>
<td>0.19 (0.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Asplenium platyneuron</td>
<td>0.03 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Mikania scandens</td>
<td>0.16 (0.1)</td>
<td>0.11 (0.1)</td>
</tr>
<tr>
<td>Botrychium biternatum</td>
<td>0 (0.0)</td>
<td>0.029 (0.0)</td>
</tr>
</tbody>
</table>
Table 3.6. Averages of descriptive habitat characteristics other than trees or individual herb species of East of Cedar Creek (ECC) and East of Dry Branch (EDB) at Congaree National Park for forested-area scale study. The means shown in bold are statistically greater ($p$ or chi-square value < 0.05).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ECC (SE)</th>
<th>EDB (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canopy (% cover)</td>
<td>87.0 (0.8)</td>
<td><strong>89.4</strong> (0.8)</td>
</tr>
<tr>
<td>Sedge (% cover)</td>
<td>12.58 (4.1)</td>
<td>5.34 (2.3)</td>
</tr>
<tr>
<td>Shrub (% cover)</td>
<td>17.82 (4.3)</td>
<td><strong>35.7</strong> (5.1)</td>
</tr>
<tr>
<td>Moss (% cover)</td>
<td>10.9 (2.8)</td>
<td>8.3 (2.8)</td>
</tr>
<tr>
<td>Herb richness (species/m²)</td>
<td>1.57 (0.2)</td>
<td>1.24 (0.1)</td>
</tr>
<tr>
<td><strong>Soil (ppm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td><strong>1121.40</strong> (80.9)</td>
<td>745.85 (85.8)</td>
</tr>
<tr>
<td>Copper</td>
<td>2.36 (0.2)</td>
<td>1.77 (0.1)</td>
</tr>
<tr>
<td>Magnesium</td>
<td><strong>290.53</strong> (14.1)</td>
<td>236.65 (20.6)</td>
</tr>
<tr>
<td>Manganese</td>
<td><strong>46.3</strong> (3.4)</td>
<td>25.5 (3.2)</td>
</tr>
<tr>
<td>Organic Matter</td>
<td>36.51 (2.2)</td>
<td><strong>44.36</strong> (3.6)</td>
</tr>
<tr>
<td>pH</td>
<td>4.27 (0.2)</td>
<td>4.24 (0.0)</td>
</tr>
<tr>
<td>Zinc</td>
<td><strong>10.73</strong> (0.7)</td>
<td>7.54 (0.5)</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>13.4 (0.7)</td>
<td>12.7 (0.8)</td>
</tr>
<tr>
<td>Potassium</td>
<td>177 (14.3)</td>
<td>182.5 (18.3)</td>
</tr>
<tr>
<td>Boron</td>
<td>0.177 (0.0)</td>
<td>0.162 (0.0)</td>
</tr>
<tr>
<td>Sodium</td>
<td>55.00 (4.5)</td>
<td>47.97 (3.8)</td>
</tr>
</tbody>
</table>
CHAPTER FOUR

BREEDING SYSTEM AND POLLINATION OF M. CAROLINIANA

Introduction

There have been no previous studies on the reproductive biology of M. caroliniana and information about the reproductive biology of rare species may be essential for their conservation. Although recruitment by seed is believed to be generally uncommon in shade-tolerant forest herbs (Abrahamson 1980), it has been shown to be vitally important to population persistence for some species (Beirzychudek 1982). Whenever possible, conservation biologists should determine the nature of a rare species’ breeding system (Karron 1991) because knowledge of a rare species’ breeding system may be a key to its recovery (Weekley and Race 2000).

Knowledge of breeding systems could be applied specifically to the conservation of genetic variability in rare species (Kearns and Inouye 1993). Additionally, this information may be used to develop management actions for species recovery. For example, if it is demonstrated that pollinator services are mandatory for a species to set seed, then management actions should include protecting nearby pollinator populations. Even if species are self-compatible and autogamous, they may benefit from increased fecundity as a result of insect-facilitated pollination (Evans et al. 2000). Managers may determine that supplemental hand-pollinations are necessary when pollinator populations are insufficient.

Inferences about the breeding system can be based on the study species’ floral morphology and associated pollination syndromes or on previous studies of related
species. I predict that the purplish-pink tubular flowers with nectar guides of *M. caroliniana* (Figure 4.1) are pollinated by bees and/or birds because pink or purple perianths are associated with bees as are those with nectar guides and with tubular perianths are associated with birds (Richards 1986). However, the perianths of flowers associated with birds are usually red or orange (Richards 1986). Members of the Lamiaceae have bilabiate (two-lipped) flowers with their reproductive surfaces on the ‘roof’ (i.e. pollen and stigma) and the ‘floor’ is at a fixed distance for legitimate visitors to contact the reproductive structures with their dorsal side; and this structure presumably evolved to protect pollen from pollen-collecting bees (Westerkamp and Claßen-Bockhoff 2007). However, members of the Lamiaceae are not restricted to bee-pollination as birds pollinate some *Salvia* species (Wester and Claßen-Bockhoff 2007). Predictions about the breeding system can also be based on what is known about *M. caroliniana*’s closest relative and only congener, *M. alba*, a federally listed narrow endemic of the Florida panhandle (Godt et al. 2004). This closest relative is self-compatible, but requires pollinator visitation to set fruit (Walker and Madsen 1997) and is visited by bumblebee species (Pitts-Singer et al. 2002). I could reasonably expect the same for *M. caroliniana*.

The goal for this study was to gain knowledge about the reproductive biology of *M. caroliniana* including (1) describing aspects of the breeding system, specifically whether or not the species is self-compatible, autogamous (spontaneously selfing), dependent on pollinator services, agamospermous (setting seeds without fertilization), and/or pollen-limited; and (2) identifying the potential pollinator species.
Methods

Study area

The study area is located within a floodplain seepage forested area of the Congaree River at the Congaree National Park in Hopkins, South Carolina, USA, approximately 20 km southeast of Columbia, SC. The Park protects the largest contiguous tract of old-growth hardwood bottomland forest in the United States (approximately 4,500 hectares) and has been designated as an International Biosphere Reserve and a Globally Important Bird Area. I studied two patches of blooming *M. caroliniana* within the “seepage forest” (*Nyssa biflora-Acer rubrum-Ilex opaca-Leucothoe axillaris-Carex atlantica ssp. capillacea* forest) (The Nature Conservancy 2001). The climate is characterized by hot and humid summers with average highs around 32º C and cool winters with average lows around 3º C; the average annual snow accumulation is less than 5 cm and freezing occurs only occasionally (Historical Climate Summaries for South Carolina). The forest surface is mostly level with little topographical relief although even minor variations in topography can have major effects on plant assemblages. Soils are usually permanently saturated by ground water with little or no fluvial flooding (LeBlond and Sorrie 2002). The soils are mostly the mucky histosol Dorovan series or the loamy inceptisol Johnston series (Ben Stuckey, USDA, Columbia, SC, personal communication).

Breeding system experiments

From mid-July to early September 2005, I conducted a breeding system experiment using naturally growing plants at the Congaree National Park (CNP) and
plants grown in pots from CNP field-collected seeds in a garden 16 km south of Clemson, South Carolina. In the first field study (Field 1 study) at CNP, I selected two large flowering patches and in the first patch, I selected 32 flowering stems and treated 114 flowers (described below); at the second patch, I selected 19 flowering stems and treated 50 flowers. Treatments included bagging inflorescences prior to anthesis using bridal veil circles (~ 25 cm diameter) that had their edges gathered with dental floss that was tied gently around the stem at the base of each inflorescence. The following treatments were applied to flowers in bagged inflorescences: A) No treatment applied, B) Excising anthers, C) Excising anthers and applying pollen from same flower, D) Excising anthers and applying pollen from another flower at the same patch, and E) Excising anthers and applying pollen from another flower from a different patch approximately 300 m away.

Before fruits were dispersed from the infructescences, I recorded the fruit set of treated flowers and the fruit set of randomly selected open-pollinated flowers (Field 1 in Table 4.1). In a separate experiment (Garden study), I treated 288 flowers in bagged inflorescences on 55 garden grown plants with treatments A-E above; fruit set was recorded from the treated flowers and from open pollinated flowers in open inflorescences on garden plants (Garden in Table 4.1). During July, 2006, I conducted a pollen-limitation study (Field 2 study) using pairs of flowers from 42 open-pollinated inflorescences selected from a patch of flowering stems at CNP. On each inflorescence, one flower received additional pollen by hand (from two flowers from two presumably different plants in the same patch), and another received no treatment (Field 2 in Table 4.1); fruit set was recorded at maturity.
Statistical significance between treatments C, D, E, and open-pollinated flowers was tested using Fisher’s protected least significant difference multiple comparison procedure. I used a one-tailed Wilcoxon signed-rank test to determine if open-pollinated flowers with additional pollen set more fruits than open-pollinated flowers. The data analysis was generated using SAS/STAT® software, Version 9.2 (SAS Institute Inc.). Statistical significance was determined at $\alpha = 0.05$ level.

Observations and identification of floral visitors

During July of 2006, I observed and identified floral visitors at three different sized patches of *M. caroliniana* in bloom at CNP. I made 71 15-minute observations of 20 flowering stems between the hours of 8:30 am and 12:30 pm when pollinators were most active (personal observation). For each timed observation, I recorded the type and number of floral visitors. I considered floral visitors to be any insect that could potentially make contact with the reproductive parts by going into the corolla of a flower; those that briefly landed on the outside of the flower were not counted or identified. Separate from the timed observations, I caught insect visitors using a butterfly net and delivered them to an entomologist for identification.

Results

None of the flowers that only had their anthers removed (treatment B) set fruit and just one of the 38 garden study flowers that were bagged only (treatment A) set fruit (Figures 4.2 and 4.3, Table 4.2). In the Field 1 study, the self-pollinated flowers set fewer fruits than the open-pollinated flowers and the within-patch pollinated flowers set fewer fruits than the open-pollinated flowers according to Fisher’s protected LSD
multiple comparison procedure (Figure 4.2). The trend was not the same in the garden as I found no differences between treatments C, D, E, and open-pollinated (Figure 4.3). In the Field 2 study, the open-pollinated flowers that had additional pollen set an average of 2.98 fruits (SE 0.20) and the open-pollinated flowers set 2.57 fruits on average (SE 0.25) ($p = 0.056$ in Figure 4.4).

During the timed observations, I observed 32 visitors including 12 bumblebees, nine skippers, eight sweatbees, two flies, and one hoverfly. I observed hummingbirds (*Archilochus colubris*) visiting flowers on three occasions, but not during the timed observations. I captured six insects that were identified by entomologist Ian Stocks (Clemson University). They were *Poanes zabulon* (skipper), *Vespula maculifrons* (yellow jacket), *Bombus impatiens* (bumble bee), *Copestylum barei* (fly), and a sweat bee of the Halictidae family, either *Halictus* or *Dialictus*.

**Discussion**

Plant families with complex animal pollinated flowers (including those of the Lamiaceae) are usually self-compatible, but incapable of automatic selfing (Richards 1986, however see Ruiz de Clavijo 1997) and the typical pattern holds true for both *Macbridea* species. Male and female functions are not separated in time (not dichogomous) as hand self-pollinations set fruits, but male and female parts are separated in space (herkogomous) as stigmas extend outward beyond the anthers (personal observation) in *M. caroliniana*. This separation of parts is likely the reason that automatic selfing does not occur and why pollinator activity is necessary for successful pollination. In addition, plants did not produce seeds without fertilization, but this is not
a surprise as almost all agamospermous species belong to the Asteraceae, Poaceae and Rosaceae families (Richards 1986).

Because fruits are produced as a result of selfing and outcrossing, *M. caroliniana* employs a mixed-mating strategy and this has genetic consequences. A mixed strategy balances selfing and outcrossing and the degree of outcrossing (or conversely selfing) is usually variable. There can be variability in the outcrossing rate between populations, days or seasons and this can be due to many factors such as weather, floral display and/or pollinator foraging activities (Richards 1986). The degree of outcrossing has implications for gene flow, levels of genetic diversity and population genetic structure. For example, levels of genetic diversity are significantly greater for predominantly outcrossed species than for species with a mixed mating system or those capable of selfing; the amount of total genetic diversity found amongst (rather than within) populations is greater for selfing species than for outcrossed wind-pollinated species (Hamrick and Godt 1989). These genetic differences can affect conservation management decisions. To conserve a species’ genetic diversity, more populations would need to be protected for a selfing rather than for an outcrossing species. Further analysis is required to determine how much selfing is occurring at these sites.

Interestingly, I found differences between the pollination treatments in the field (Field 1 study) but not between those of the garden study (Figures 4.2 and 4.3). The mean number of fruits produced by the open-pollinated flowers was greater than that of the self-pollinated and the within-patch crossed flowers in the field, but open-pollinated flowers in the garden did not set more fruits than any of the other pollination treatments.
The reason for the lack of differences in the garden may be because there were fewer floral visitors (although this was not measured), possibly because potential visitors had more foraging choices in the garden compared to the natural habitat. During July in the garden, there are other plants in bloom that pollinators visit. In contrast, there are probably fewer foraging choices in the seepage forest and although this is not ideal for the pollinators it probably is good for *M. caroliniana*’s reproductive success as a “harried, underfed, yet constant pollinator is ideal for the plant” (Crawley 1997). An alternative or possibly complimentary reason that there were no differences between pollination treatments in the garden is that the maternal plants were fertilized and were probably less stressed by limited nutrients than those in the field. Therefore, the garden plants set more fruits regardless of hand-pollinated treatment applied (Table 4.2).

Based on the floral traits of *M. caroliniana* and the species observed visiting flowers, the pollination syndrome matches expectations, that is, the species is most likely predominantly bee-pollinated. Bumblebees were the most frequent floral visitors and made up 38% of all observations, but lepidoperans made up 28% of observations. However, pollen was not collected and identified from floral visitors so visitors could not be distinguished from pollinators. Additionally, birds may play an important role as pollinators even though this was not evident during my observations. Hummingbirds were apparently more shy and reluctant to forage near us than the insects and they were only observed foraging from >10 m.

During the 71 timed observations, only 32 visitors were observed and this rarity of visitors can have ramifications for plants that are pollinator-limited. Although the
significance was marginal between the treatments of the Field 2 study (Figure 4.4), this experiment was carried out at one of the largest flowering patches (with more than 1000 flowering stems) at CNP where the grand display probably attracts more visitors than that of smaller flowering patches. With fewer visitors, the smaller flowering patches probably set even fewer open-pollinated fruits and are more pollinator-limited. Because this rare species is dependent on pollinators to set fruit and is pollinator-limited, conservation management will require additional information about effective pollinators, and may involve protecting or enhancing pollinator populations.

References


Figure 4.1. *Macbridea caroliniana* inflorescence with four flowers showing nectar guides on the lower flower lips that lead insect pollinators to nectar source.
Table 4.1. Experimental protocol and number of flowers in each treatment for breeding system study of *M. caroliniana*. Breeding system tests and their abbreviations are in the top row. Treatments included different combinations of bagging, emasculation, and hand-pollinations from flowers of different distances. Two field studies were conducted at the Congaree National Park and a garden study was conducted using plants grown from field-collected seeds in pots.

<table>
<thead>
<tr>
<th>Breeding system tests and their abbreviations</th>
<th>Autogamy (A)</th>
<th>Agamospermy (B)</th>
<th>Self-compatibility (C)</th>
<th>Xenogamy Within Patch (D)</th>
<th>Xenogamy Between Patch (E)</th>
<th>Control (O)</th>
<th>Pollen-Limitation (OPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagged</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Emasculated</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Hand-Pollinated</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Pollen source</td>
<td>Same flower</td>
<td>None</td>
<td>Same flower</td>
<td>Different flowers same population</td>
<td>Different flowers of different population</td>
<td>Open pollination</td>
<td>Different flowers same population</td>
</tr>
<tr>
<td>n Flowers-Field 1 Study</td>
<td>26</td>
<td>46</td>
<td>31</td>
<td>33</td>
<td>28</td>
<td>54</td>
<td>-</td>
</tr>
<tr>
<td>n Flowers-Garden Study</td>
<td>38</td>
<td>42</td>
<td>72</td>
<td>65</td>
<td>71</td>
<td>134</td>
<td>-</td>
</tr>
<tr>
<td>n Flowers-Field 2 Study</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>42</td>
<td>42</td>
</tr>
</tbody>
</table>
Table 4.2. Average number of fruits per flower for each treatment in the Field 1 and Garden breeding system experiments of *M. caroliniana*. Standard errors are shown in parentheses. The maximum number of fruits per flower is four.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field</td>
<td>Bagged</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.68 (0.24)</td>
<td>1.00 (0.23)</td>
<td>1.18 (0.27)</td>
<td>1.74 (0.22)</td>
</tr>
<tr>
<td>Garden</td>
<td>Anthers removed</td>
<td>0.03(0.03)</td>
<td>0 (0)</td>
<td>1.35 (0.19)</td>
<td>1.43 (0.19)</td>
<td>1.41 (0.18)</td>
<td>1.02 (0.13)</td>
</tr>
<tr>
<td></td>
<td>Selfed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crossed Within Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crossed Between Sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Open Pollinated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

Figure 4.2. Mean number of fruits produced per *M. caroliniana* flower and standard errors for treatments applied in the Field 1 experiment at Congaree National Park. Treatments include flowers that were bagged (A), bagged + emasculated (B), bagged + hand self-pollinated (C), bagged + hand pollinated using anthers from two flowers in the same patch (D), bagged + hand pollinated using anthers from two flowers in a different patch (E), and open pollinated flowers (O). Amongst treatments C-O, significant differences were found between C - O and D - O.
Figure 4.3. Mean number of fruits produced per *M. caroliniana* flower and standard errors for treatments applied in the Garden experiment. Treatments include flowers that were bagged (A), bagged + emasculated (B), bagged + hand self-pollinated (C), bagged + hand pollinated using anthers from two flowers in the same patch (D), bagged + hand pollinated using anthers from two flowers in a different patch (E), and open pollinated flowers (O). Amongst the pollinated treatments (C-O), there were no significant differences found between any treatment pairs.
Open pollinated treatments applied in Field 2 experiment

Figure 4.4. Mean number of fruits produced per _M. caroliniana_ flower and standard errors for treatments applied in the Field 2 experiment at Congaree National Park. OPA = open-pollinated flowers with pollen added from anthers of two different flower in the same patch, O = open-pollinated flowers. The difference in the mean number of fruits between treatments was marginally significant.
CHAPTER FIVE
GENETIC DIVERSITY AND STRUCTURE OF *M. CAROLINIANA*

**Introduction**

Knowledge of levels of genetic diversity found within species’ populations, and how that genetic variation is partitioned within and among populations, is critical for sound management of rare plant taxa (Lammi et al., 1999). Genetic diversity is considered essential to the long-term survival of species because populations and species are unable to adapt to changing environmental conditions in the absence of sufficient genetic variation (Frankel et al. 1995). Hence, population genetic analyses of rare plant species can provide important insights for species’ conservation. For example, populations with high genetic diversity or unique alleles can be identified and given priority for preservation. When populations of an endangered species exhibit high genetic differentiation, more populations will need to be preserved or sampled for *ex situ* propagation if genetic variation within the species is to be preserved (Schoen and Brown 1991).

Small populations often have low levels of genetic diversity (Hamrick and Godt 1989) and may experience negative effects of inbreeding (Frankham et al. 2002). Both factors can increase a species’ risk of extinction. Molecular methods such as protein electrophoresis can be used to determine if populations experience such negative effects. Management actions to offset these impacts may include artificial gene flow or the propagation of seeds collected from the site but germinated *ex situ* and grown until reintroduction.
On the other hand, higher levels of population genetic diversity have been linked to more flowering in canopy gaps (Kudoh et al. 1999). At the Congaree National Park there is an area (East of Cedar Creek) with several large flowering patches of *M. caroliniana* associated with large canopy gaps. If there is more sexual reproduction and seedling recruitment in these patches compared to those of closed canopy conditions, I would expect greater levels of genetic diversity in patches of canopy gaps.

Although a variety of molecular techniques are available to examine genetic variation within plant species, allozymes are often the method of choice because they are highly informative, efficient and cost-effective (Weir 1996). Sampling for allozyme analyses is largely non-destructive and usually requires only a small amount of vegetative tissue. Since the 1960’s, allozymes have been used to describe genetic diversity and structure within many rare plant species (e.g., Godt and Hamrick 1996; Allphin et al. 1998; Izawa et al. 2007). The availability of literature reviews of published allozyme studies permits comparisons of newly analyzed species with species having similar life history characteristics (Hamrick and Godt 1989; Godt and Hamrick 2001). Most importantly, the genetic diversity of *M. caroliniana*’s only congener, *M. alba*, has been studied using allozymes (Godt et al. 2004) allowing comparisons with this species’ closest relative.

This investigation was designed to (1) describe genetic diversity within and among populations of *M. caroliniana*; (2) compare *M. caroliniana*’s genetic diversity with taxonomically related species as well as those with similar life history traits; (3) compare genetic diversity levels of canopy gap and closed canopy patches *M. caroliniana*
and patch structure at ECC; and (4) propose recommendations for conservation strategies based on the genetic information.

**Methods**

**Sampling**

Eleven populations of *M. caroliniana* were sampled from across the species range during spring 2007 (Figure 5.1). Selected study populations occurred in representative habitat (Leblond and Sorrie 2002). Populations sampled were HOW (Howell Woods), BMS (Brown Marsh Swamp), and MMP (Meares Millpond) in eastern North Carolina, POS (Poston) in eastern South Carolina, ECC (east of Cedar Creek), EDB (east of Dry Branch) and ASP (Aiken State Park) in central South Carolina, SRS (Savannah River Site) and FGA (Fort Gordon) in eastern/central Georgia, JUN (Juniper Creek) in western Georgia and MOC (Mount Olive Church) in southern Georgia. To determine if populations occurring in the same river basin are more genetically similar than populations from different river basins, I selected two populations from each of three river basins (Fig. 4.1): Cape Fear river basin (MMP and BMS), Santee River basin (ECC and EDB) and Savannah River basin (SRS and FGA). Five to six leaves were collected from each of 24 - 48 plants randomly sampled from each population. The population at ECC was sampled by collecting leaves from 24 stems in each of four canopy gap patches and four patches under relatively closed canopy conditions (Figure 5.2). Samples were placed in sealed bags and kept chilled during transport to the University of Georgia.
Enzyme extraction and electrophoresis

Within 48 hours of collection, enzymes were extracted from the leaf samples. Two to three young leaves per stem were crushed in pre-cooled mortars with a pestle and a pinch of sea sand. Enzymes were extracted from the leaf tissue with a polyvinylpyrrolidone-phosphate extraction buffer (Wendel and Parks 1982) selected for its superior resolution during preliminary trials. Extracts were absorbed onto 4 x 6 mm wicks made from No. 3 Whatman chromatography paper. Wicks were stored in 96-well microtest plates at -70º C until they were used for electrophoresis.

Wicks were placed in horizontal starch gels (10%) for electrophoresis. Five gel-electrode buffer combinations and twelve enzyme stains were used to resolve 23 putative loci including the following: Buffer 4 (Soltis et al. 1983): aconitase (ACO1, ACO2), UTP-glucose-1-phosphate (UGPP); buffer 8- (a modification of system 8 of Soltis et al. 1983): diaphorase (DIA), fluorescent esterase (FE-1, FE-2, FE-3, FE-4), triose phosphate isomerase (TPI-1, TPI-2); buffer 11 (Soltis et al. 1983): 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucomutase (PGM-1, PGM-2); buffer 6 (Soltis et al. 1983): colorimetric esterase (CE), malic enzyme (ME), peroxidase (PER, CPER; EC); buffer morphiline citrate (Conkle et al. 1982): aspartate aminotransferase (AAT-1, AAT-2) and malate dehydrogenase (MDH-1, MDH-2, MDH-3, MDH-4). Stain formulations are from Soltis et al. (1983) except DIA (Cheliak and Pitel 1984) and UGPP (Manchenko 1994). Observed banding patterns were consistent with those expected for each enzyme system (Weeden and Wendell 1989).
Genetic analyses

Standard measures of genetic diversity, including the percentage of polymorphic loci ($P$), mean number of alleles per polymorphic locus ($AP$), total number of alleles ($Na$), effective number of alleles per locus ($Ae$), and observed ($Ho$) and expected heterozygosity ($He$) were calculated for the species as a whole and for each population using Lysprog, an unpublished statistical program written by M. D. Loveless and A. Schnabel (College of Wooster, Wooster, Ohio and University of Indiana, South Bend, Indiana, respectively). Lysprog was also used to estimate the distribution of genetic variation among populations using Nei’s (1973) genetic diversity statistics: total genetic diversity ($HT$), mean intrapopulation genetic diversity ($HS$), and the proportion of genetic variation distributed among populations and watersheds ($Gst$ and $Gsw$, respectively).

Genetic identity and distance (Nei 1972) between pairs of populations were calculated using Lysprog. $F_{IS}$ values ($1-Ho/He$) were used for Chi-square tests to determine whether genotype frequencies at each locus per population deviated from those expected under Hardy-Weinberg equilibrium using the program POPGENE (Yeh et al. 1997).

Pair-wise fixation indices ($FST$) were calculated using FSTAT (Goudet 1995). To test for isolation by distance, a matrix of converted pairwise fixation indices ($FST/(1-FST)$) was compared to a matrix of pair-wise geographical distances (transformed to ln distance in km) and subjected to a Mantel matrix randomization test (Mantel 1967) using the program TFPGA (Miller 1997). To graphically display genetic similarity among populations, I used the unweighted pair group method (UPGMA) of average linkage clustering based on Nei’s original distance (1978) conducted with POPGENE (Yeh et al.
To assign conservation priorities based on genetic diversity, I ranked populations using an approach modified from Neel and Ellstrand (2003) that included the following parameters per population: $H_e$, $P$, $N_a$, Nei’s unbiased (1978) genetic distance, private alleles (those that are unique to a population), and $F_{IS}$ values. For each parameter, populations with the greatest values ranked highest except for $F_{IS}$ for which the lowest values ranked highest. Populations were ranked from one to eleven unless there were ties which resulted in more than one population with the same rank. All six parameters were weighed equally for the overall conservation ranking for each population and high conservation value was assigned to those that had the highest average ranking across all categories. The program TFPGA (Miller 1997) was used to calculate Nei’s unbiased (1978) genetic distance and FSTAT (Goudet 1995) was used to calculate $F_{IS}$ per population.

Results

Species-wide study

Fourteen (61%) of the 23 loci scored for *M. caroliniana* were polymorphic. Within populations, the mean percentage of polymorphic loci was 32.5% (ranging from 8.7% in JUN to 45.5% in HOW, ECC and EDB; Table 5.1). The average number of alleles per polymorphic locus ($AP$) was 2.43 for the species and 2.09 within populations. The effective number of alleles per locus ($A_e$) was 1.3 for the species with a mean of 1.16 within populations. Even though more than half of the loci were polymorphic, genetic diversity was somewhat low ($H_{es} = 0.172$ for the species and $H_{ep} = 0.095$ for the population mean). Gene diversity ranged from 0.010 for JUN to 0.147 for ASP.
The *M. caroliniana* populations located near the center of the species range generally exhibited the highest levels of genetic variability (Table 5.1, Figure 5.1). ASP and EDB had the highest mean expected heterozygosities ($H_{ep} = 0.147$ and $0.145$ respectively). ECC and HOW had the most alleles (33 and 34, respectively). Populations ASP and EDB had the highest mean effective number of alleles per locus ($A_e = 1.27$ and 1.25 respectively).

Of the 73 tests of polymorphic loci for deviations from Hardy-Weinberg expectations, 28 (58%) indicated no significant differences from expected. Fifteen of the 22 statistically significant tests were positive (68%), indicating heterozygote deficiency and seven were negative (31%), indicating an excess of heterozygotes. Average $F_{IS}$ for all loci was slightly positive (0.053), showing that overall the populations are close to Hardy-Weinberg equilibrium.

A large amount of the variation was found among populations ($G_{ST} = 0.333$). However, three of the polymorphic loci (Fe-1, Mdh-1 and Aco-2) had $H_T$ values <0.04 and very low $G_{ST}$ values (<0.07) relative to the other loci. When these three loci are omitted from the calculations, overall $G_{ST}$ increased to 0.409 and is probably a more accurate representation of actual genetic differentiation among populations. Due to the hierarchical nature of my sampling design, the total among population $G_{ST}$ could be partitioned into an among watershed component ($G_{SW} = 0.323$) and among population within watershed component ($G_{SP} = 0.080$). Thus, 79% of the total genetic differentiation among populations occurs among river basins with very little partitioning of variation among populations within watersheds.
Population relationships are illustrated in the UPGMA phenogram (Figure 5.3) which indicates that populations occurring within the same river system cluster more closely to one another than to other populations. However, populations from different drainage systems were not clustered with the nearest geographical populations. For example, populations in the Santee River basin clustered with the POS population (128 km) and HOW (287 km), but not with ASP (68 km).

Pair-wise comparisons of $F_{ST}$ ranged from 0.00 (between BMS and MMP) to 0.89 (MOC and JUN), with a mean pairwise $F_{ST} = 0.48$ for the species. The Mantel test indicated a significant correlation between genetic and geographical distances ($r = 0.38$, $P < 0.05$). The correlation was considerably stronger when the outlier population pair (MOC-JUN) was removed from the calculations ($r = 0.58$, $P < 0.001$).

All eleven populations ranked in the top five for at least one category when prioritizing the populations for conservation (see Table 5.2). ECC and EDB tied for first as the highest ranked population because they had the lowest mean rankings for the six genetic parameters included in the assessment. HOW, SRS, and ASP were the 3rd, 4th and 5th most highly ranked populations for conservation.

Canopy gap and closed canopy patches compared

Contrary to my expectations that more flowering in gaps would lead to more sexual reproduction and greater genetic diversity, the mean genetic diversity for sub-populations was not greater for patches occurring in canopy gaps than those of closed canopies; it was approximately the same ($H_e = 0.110$, and 0.107 respectively; Table 4.3). However, how genetic diversity is distributed among gap versus closed canopy sub-
populations differs ($G_{ST} = 0.0445$ and $0.199$ respectively). In other words, sub-populations from gaps are genetically more like each other than sub-populations of closed canopies. The sub-population relationships are illustrated in the UPGMA phenogram (Figure 5.4) which indicates that sub-populations occurring in gaps cluster more closely to one another than do those of closed canopy sub-populations. This result is not due to geographic distance as gap sub-populations were not closer to each other than closed canopy sub-populations (Figure 5.2). Additionally, the Mantel test indicated no strong correlation between geographic distance and genetic similarity ($r = -0.102$).

**Discussion**

**Species-wide study**

Levels of genetic diversity and structure have been shown to be associated with various species characteristics, including geographic range and breeding system (Hamrick and Godt 1989). Genetic diversity in *M. caroliniana* mostly conforms to these expectations. The species is restricted to wetland associated habitats in the coastal plain of three southern states. It has a much larger range than its only congener, *M. alba*, and greater genetic diversity values (Table 5.4), consistent with the general observation that species with wider geographical ranges generally have more genetic diversity than species with more limited ranges (Hamrick and Godt 1989). Among a handful of other mints reported in the literature, *M. caroliniana* has some of the highest genetic diversity values (Table 5.4). However, compared to a small group of southeastern mint species that have had their allozymes studied, genetic diversity measures for both *Macbridea* species are low. While both *Macbridea* species have relatively small ranges, these other
southeastern mints are very narrowly endemic and their growth habit is woody, suggesting greater longevity. Whether herbaceous or woody, long lived perennials are associated with greater genetic diversity compared to short lived perennials (Hamrick and Godt, 1989).

Population level genetic diversity is mostly influenced by the combination of breeding system and geographic range (Hamrick and Godt 1989). *Macbridea caroliniana* has a mixed breeding system, insects are necessary for fruit set, and flowers are self compatible (Chapter 4). *Macbridea caroliniana* has almost the equivalent population level genetic diversity ($H_{ep} = 0.095$) as the mean of 85 other plant species with a mixed breeding system ($H_{ep} = 0.090$ for animal-mixed; Hamrick and Godt 1989). Genetic diversity within *M. caroliniana* populations is also very similar to the mean of 115 other narrowly distributed species’ population level genetic diversity levels ($H_{ep} = 0.105$; Hamrick and Godt 1989).

The breeding system is the single most important characteristic determining population differentiation (Hamrick and Godt 1989), with selfing and mixed mating species having more of their genetic diversity among their populations compared to highly outcrossing species. Reduced gene flow can increase population differentiation, which can result from lack of pollen and seed movement between populations as well as a disjunctive spatial distribution.

Based on casual field observations over three growing seasons, the primary pollinators of *M. caroliniana* are bumblebees (Chapter 4). The maximum flight distance for a bumblebee species is about two km (for *Bombus terrestris*; Kreyer et al. 2004),
which is less than the distance separating the two closest populations making inter-
population pollination unlikely. Seeds are thought to be dispersed by gravity and/or
water (LeBlond and Sorrie, 2002) as they are buoyant and *M. caroliniana* frequently
occurs in flooded habitats. Therefore, seed dispersal is possible between populations
within the same river basin, but is improbable between populations of different river
basins. As a result, *M. caroliniana* has much higher population differentiation ($G_{ST} = 0.41$) than *M. alba* ($G_{ST} = 0.10$; Godt et al., 2004), most of which occurs between
watersheds ($G_{sw} = 0.32$). The low level of population structure found in *M. alba* is
probably a function of the close proximity of its populations (mean distance = 6.9 km;
Godt et al. 2004). More than half of the 63 occurrences of *M. alba* are within the
Apalachicola National Forest (U.S. Fish and Wildlife Service 1994) as are nine of the ten
sampled populations (Godt et al. 2004). In comparison, *M. caroliniana* populations are
separated by a mean distance of 276 km, ranging from 3 km (between ECC and EDB, the
two populations at the Congaree National Park) to 670 km (between MOC and HOW).
Further, Hamrick (2004) and Gonzales and Hamrick (2005) have argued that species with
naturally disjunct ranges have greater genetic structure than species with more continuous
ranges.

**Canopy gap and closed canopy patches of ECC**

Although the mechanism is unknown, genetic diversity is maintained under
different conditions at the Congaree National Park’s ECC population including closed
canopy conditions where patches do not experience reduced levels of genetic diversity
compared to patches in light gaps. I suspect these sub-populations may have become
established and/or increased in size following the formation of canopy gaps many years ago. Over time, as canopy gaps closed in with new trees, levels of genetic diversity were maintained perhaps by rare seedling recruitment. Even infrequent seedling establishment will suffice in maintaining genetic diversity in long-lived clonal plants (Watkinson and Powell 1993). Considering my gap-disturbance scenario, the results concur with Pluess and Stöcklin (2004), who found no indication of genetic depletion in late-succession populations of *Geum reptans*, a clonal plant in the Rosaceae family.

This is a different scenario than the one proposed by Kudoh et al. (1999) who found no diversity in the closed canopy sub-populations of their study species (*Uvularia perfoliata*, a temperate woodland herb). They suggested a “waiting” strategy for plants of closed canopies whereby single individuals reproduce asexually until gap formation creates more optimal conditions for genet recruitment by seeds. It is possible that *M. caroliniana* also uses this waiting strategy, assuming the sub-populations I sampled occurred in areas that were past the gap-formation phase.

Although much greater flowering in gap sub-populations did not result in greater genetic diversity, it suggests a reasonable explanation for the differences in how the genetic diversity is partitioned. Presumably, more flowering results in more visitations from pollinators and gene flow via pollinator foraging can explain the result of less genetic differentiation between gap sub-populations compared to closed canopy sub-populations. Because the closed canopy sub-populations have little or no flowering, gene flow via pollen is unlikely so these sub-populations would be more different from each other than the sub-populations experiencing more frequent pollinator visitation.
Conservation Implications

The most highly ranked populations were EDB, ECC, SRS, HOW and ASP (Table 5.2). If only these five populations are protected, 40 of the 44 alleles (90%) would be captured. Leblond and Sorrie (2002) considered all of these except HOW to be essential habitat for *M. caroliniana*. My conclusions, based on genetic criteria, agreed with their more subjective assessment that was based on habitat characteristics including location in a swamp forest of a river or stream floodplain that is saturated or intermittently flooded and where herbs instead of shrubs dominate. I caution that my assessment was limited to 11 of the 36 known populations and that I did not sample across its entire geographic range. I made no effort to assess the conservation value of the remaining 25 populations, and acknowledge that un-sampled populations, (e.g., on unique or marginal habitat types) are likely to also have conservation value.

As acknowledged, my prioritization approach assigned the highest value to populations that tended to conserve genetic diversity in the species. However, in some cases, it may be important to consider populations with low genetic diversity for conservation priority because they may be at greater risk of extinction. Low genetic diversity alone does not necessarily put populations at risk, but for those with increased levels of inbreeding, population extinction may follow. The three populations ranking the lowest in my assessment JUN, POS, and MOC, are very small with fewer than 100 stems observed. Compared to large populations, small populations have a greater chance of inbreeding depression and consequently the likelihood of population extinction is greater (Frankham et al. 2002).
For small, genetically depauperate populations, augmentation using pollen or seeds may be a wise option for conservation management. The phenogram can be used to determine source populations based on their genetic similarity to each of the depaupapercate populations in my study. For example, the best source population for MMP would likely be BMS (Figure 5.3); for POS the Congaree National Park populations (ECC or EDB); and for FGA, SRS would be the best source. It would be more complicated for JUN and for MOC. MOC is the most southern and genetically divergent population. The best strategy for this population may not be augmentation, but protection from physical degradation (e.g., runoff, development), protection of the pollinators, and perhaps the implementation of controlled hand pollinations within the population.

Other genetic factors besides within population genetic diversity should be considered for conservation planning. Because of the high degree of population differentiation observed ($G_{ST} = 0.41$), if more populations are protected then there will be a greater chance of protecting more of the overall genetic biodiversity of the species. It would also be important to protect populations from several different watersheds because of the high level of among watershed differentiation ($G_{ST} = 0.323$).

In summary, population differentiation must be addressed when the goal is preservation of species level genetic diversity. If more populations are conserved with consideration given to differentiation among groups of populations, more of the genetic variation will likely be captured. Populations that ranked highly for conservation priority
should not be the only ones given management protection. Special attention may already be needed for the small populations with low genetic diversity.

References


Figure 5.1. Locations of eleven *Macbridea caroliniana* populations sampled in North Carolina, South Carolina and Georgia. The river sub-basins of three river basins are highlighted in white. I sampled three pairs of populations (circled) from the same river basin and two of these pairs were from the same sub-basin.
Figure 5.2. Map of *Macbridea caroliniana* patches sampled at Congaree National Park in areas with canopy gaps (G) and closed canopies (C).
Table 5.1. Genetic diversity statistics$^a$ for *Macbridea caroliniana*

<table>
<thead>
<tr>
<th>Population</th>
<th>$P$</th>
<th>$AP$</th>
<th>$N_a$</th>
<th>$A_e$</th>
<th>$H_o$ (SD)</th>
<th>$H_e$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRS</td>
<td>27.8</td>
<td>2.20</td>
<td>24</td>
<td>1.18</td>
<td>0.134 (0.031)</td>
<td>0.105 (0.044)</td>
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<tr>
<td>FGA</td>
<td>31.6</td>
<td>2.00</td>
<td>25</td>
<td>1.13</td>
<td>0.081 (0.033)</td>
<td>0.076 (0.037)</td>
</tr>
<tr>
<td>ASP</td>
<td>33.3</td>
<td>2.00</td>
<td>24</td>
<td>1.27</td>
<td>0.122 (0.051)</td>
<td>0.147 (0.050)</td>
</tr>
<tr>
<td>MOC</td>
<td>26.1</td>
<td>2.00</td>
<td>29</td>
<td>1.06</td>
<td>0.013 (0.018)</td>
<td>0.043 (0.021)</td>
</tr>
<tr>
<td>MMP</td>
<td>28.6</td>
<td>2.17</td>
<td>28</td>
<td>1.15</td>
<td>0.073 (0.051)</td>
<td>0.087 (0.041)</td>
</tr>
<tr>
<td>BMS</td>
<td>36.4</td>
<td>2.13</td>
<td>30</td>
<td>1.23</td>
<td>0.115 (0.060)</td>
<td>0.126 (0.044)</td>
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<tr>
<td>POS</td>
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<td>2.17</td>
<td>28</td>
<td>1.16</td>
<td>0.080 (0.056)</td>
<td>0.087 (0.042)</td>
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<td>JUN</td>
<td>8.7</td>
<td>2.00</td>
<td>25</td>
<td>1.01</td>
<td>0.008 (0.025)</td>
<td>0.010 (0.016)</td>
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<tr>
<td>HOW</td>
<td>45.5</td>
<td>2.20</td>
<td>34</td>
<td>1.14</td>
<td>0.077 (0.036)</td>
<td>0.092 (0.034)</td>
</tr>
<tr>
<td>ECC</td>
<td>45.5</td>
<td>2.10</td>
<td>33</td>
<td>1.22</td>
<td>0.127 (0.037)</td>
<td>0.123 (0.042)</td>
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<tr>
<td>EDB</td>
<td>45.5</td>
<td>2.00</td>
<td>32</td>
<td>1.25</td>
<td>0.147 (0.036)</td>
<td>0.145 (0.043)</td>
</tr>
<tr>
<td>Mean</td>
<td>32.5</td>
<td>2.09</td>
<td>28.4</td>
<td>1.16</td>
<td>0.089</td>
<td>0.095</td>
</tr>
<tr>
<td>SD</td>
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<td>0.09</td>
<td>3.6</td>
<td>0.08</td>
<td>0.013</td>
<td>0.012</td>
</tr>
<tr>
<td>Pooled Species</td>
<td>60.9</td>
<td>2.43</td>
<td>44.0</td>
<td>1.30</td>
<td>&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;&quot;</td>
<td>0.172</td>
</tr>
</tbody>
</table>

$^a$ $P$ is the percentage of polymorphic loci, $AP$ is the mean number of alleles per polymorphic locus, $N_a$ is the total number of alleles observed in a population, $A_e$ is the effective number of alleles per locus, $H_e$ is genetic diversity (expected heterozygosity), and $H_o$ is observed heterozygosity. SD is standard deviation.
Figure 5.3. UPGMA phenogram of *Macbridea caroliniana* populations based on Nei’s (1978) genetic distance. River basins are indicated as SV = Savannah River, CF = Cape Fear and ST = Santee River. Numerals indicate the relative genetic identity of the populations.
Table 5.2: Rankings for population prioritization of eleven *Macbridea caroliniana* populations. EDB and ECC were tied for highest ranking overall, while JUN ranked lowest. $H_e$ is genetic diversity (expected heterozygosity), $N_a$ is the total number of alleles observed in a population, $P$ is the percentage of polymorphic loci, Gen. dist. is Nei’s (1978) genetic distance, and $F_{IS}$ is the inbreeding coefficient.

<table>
<thead>
<tr>
<th>Pop.</th>
<th>He</th>
<th>Na</th>
<th>P</th>
<th>Gen. dist</th>
<th>Lowest FIS</th>
<th>Private alleles</th>
<th>Average Rank</th>
<th>Overall Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECC</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td>3.83</td>
<td>1</td>
</tr>
<tr>
<td>EDB</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>6</td>
<td>3.83</td>
<td>1</td>
</tr>
<tr>
<td>HOW</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>SRS</td>
<td>5</td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4.67</td>
<td>4</td>
</tr>
<tr>
<td>ASP</td>
<td>1</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>6</td>
<td>5.33</td>
<td>5</td>
</tr>
<tr>
<td>MMP</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>5.67</td>
<td>6</td>
</tr>
<tr>
<td>BMS</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>5.83</td>
<td>7</td>
</tr>
<tr>
<td>FGA</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>5.83</td>
<td>7</td>
</tr>
<tr>
<td>MOC</td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>6.33</td>
<td>9</td>
</tr>
<tr>
<td>POS</td>
<td>7</td>
<td>6</td>
<td>11</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>JUN</td>
<td>11</td>
<td>8</td>
<td>11</td>
<td>5</td>
<td>11</td>
<td>6</td>
<td>8.67</td>
<td>11</td>
</tr>
</tbody>
</table>
Table 5.3. Genetic diversity statistics\(^a\) for *Macbridea caroliniana* patches at Congaree National Park.

<table>
<thead>
<tr>
<th>Population</th>
<th>(P)</th>
<th>(AP)</th>
<th>(N_a)</th>
<th>(A_e)</th>
<th>(H_o) (SD)</th>
<th>(H_e) (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gap1</td>
<td>31.82</td>
<td>2.14</td>
<td>1.36</td>
<td>1.22</td>
<td>0.135 (0.048)</td>
<td>0.119 (0.044)</td>
</tr>
<tr>
<td>Gap2</td>
<td>31.82</td>
<td>2.00</td>
<td>1.32</td>
<td>1.16</td>
<td>0.084 (0.044)</td>
<td>0.095 (0.039)</td>
</tr>
<tr>
<td>Gap3</td>
<td>33.33</td>
<td>2.00</td>
<td>1.33</td>
<td>1.21</td>
<td>0.127 (0.045)</td>
<td>0.120 (0.043)</td>
</tr>
<tr>
<td>Gap4</td>
<td>36.36</td>
<td>2.13</td>
<td>1.41</td>
<td>1.18</td>
<td>0.092 (0.047)</td>
<td>0.105 (0.039)</td>
</tr>
<tr>
<td>Mean</td>
<td>33.33</td>
<td>2.07</td>
<td>1.36</td>
<td>1.19</td>
<td>0.110</td>
<td>0.110</td>
</tr>
<tr>
<td>SD</td>
<td>2.14</td>
<td>0.08</td>
<td>0.04</td>
<td>0.03</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Clo1</td>
<td>27.27</td>
<td>2.00</td>
<td>1.27</td>
<td>1.16</td>
<td>0.110 (0.037)</td>
<td>0.091 (0.036)</td>
</tr>
<tr>
<td>Clo2</td>
<td>33.33</td>
<td>2.00</td>
<td>1.33</td>
<td>1.19</td>
<td>0.116 (0.049)</td>
<td>0.110 (0.040)</td>
</tr>
<tr>
<td>Clo3</td>
<td>27.27</td>
<td>2.00</td>
<td>1.27</td>
<td>1.22</td>
<td>0.127 (0.048)</td>
<td>0.120 (0.043)</td>
</tr>
<tr>
<td>Clo4</td>
<td>36.36</td>
<td>2.00</td>
<td>1.36</td>
<td>1.18</td>
<td>0.126 (0.046)</td>
<td>0.106 (0.037)</td>
</tr>
<tr>
<td>Mean</td>
<td>31.06</td>
<td>2.00</td>
<td>1.31</td>
<td>1.19</td>
<td>0.120</td>
<td>0.107</td>
</tr>
<tr>
<td>SD</td>
<td>4.54</td>
<td>0</td>
<td>0.05</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(a\) \(P\) is the percentage of polymorphic loci, \(AP\) is the mean number of alleles per polymorphic locus, \(N_a\) is the total number of alleles observed in a population, \(A_e\) is the effective number of alleles per locus, \(H_e\) is genetic diversity (expected heterozygosity), and \(H_o\) is observed heterozygosity. SD is standard deviation.
Figure 5.4. UPGMA phenogram of *Macbridea caroliniana* sub-populations tested from open canopy (Gap) areas and closed canopy (Clo) areas. Phenogram was based on Nei’s (1978) unbiased genetic distance measures conducted with POPGENE (Yeh et al., 1997).
Table 5.4. Comparisons of genetic diversity between *Macbridea caroliniana*, *M. alba*, other mint species, plants with similar life history traits, and other narrowly distributed species.

<table>
<thead>
<tr>
<th>Species</th>
<th>$H_{es}$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. caroliniana</em></td>
<td>0.172</td>
<td>Present study</td>
</tr>
<tr>
<td><em>M. alba</em></td>
<td>0.121</td>
<td>Godt et al. (2004)</td>
</tr>
</tbody>
</table>

**Other southeastern Lamiaceae**

- *Conradina brevifolia* 0.349 Crook (1998)
- *C. canescens* 0.365 Crook (1998)
- *C. grandiflora* 0.364 Crook (1998)
- *C. verticillata* 0.227 Crook (1998)
- *Dicerandra christmanii* 0.170 McDonald and Hamrick (1996)
- *D. cornutissima* 0.206 McDonald and Hamrick (1996)
- *D. frutescens* 0.243 McDonald and Hamrick (1996)
- *D. immaculata* 0.116 0.255 (mean) McDonald and Hamrick (1996)

**Other Lamiaceae species**

- *Mentha pulegium* (N. Africa) 0.229 Ben and Boussaid (2004)
- *Thymus quinquecostatus* (Korea) 0.066 Chung et al. (1998)
- *Phlomis purpurea* (Spain) 0.085 Aparicio et al. (2000)
- *P. composita* (Spain) 0.079 Aparicio et al. (2000)
- *Scutellaria angustifolia* complex 0.067 Olmstead (1990)
  (10 species from western U.S.A.)
- *Stachys maritima* (Iberian peninsula) 0.066 López-Pujol et al. (2003)
- *Salvia pratensis* (Netherlands) 0.136 0.099 (mean) Van Treuren et al. (1991)

**Short lived herbaceous perennials**

- 0.116 (mean) Hamrick and Godt (1989)

**Narrow geographic range**

- 0.137 (mean) Hamrick and Godt (1989)
CHAPTER SIX

*M. CAROLINIANA* AS A MODEL FOR CONSERVATION: LESSONS LEARNED AND INFORMATION NEEDS

The scarcity of information about the ecology of floodplain herbs and the declining numbers of forest herbs in general, emphasizes the need for knowledge of individual species’ biology in order to protect and conserve species (Chapter 1). However, the acquired knowledge of individual species becomes more valuable for understanding ecosystem function and restoration if the applicability to other species or a group of other species can be established. Adaptive strategies (*sensu* Grime 1979) and functional traits (e.g., McGill et al. 2006) represent approaches to seeking generalities in the structure and composition of plant communities. Grime (1979) defined adaptive strategies as “groupings of similar genetic characteristics which recur widely among species or populations and cause them to exhibit similarities in ecology.” Grime’s adaptive strategy classification based on the ways plant species respond to environmental stress and disturbance gradients provides a starting point for identifying the suite of species that may be ecologically equivalent or similar to *Macbridea caroliniana*.

Based on combinations of high and low levels of environmental stress and disturbance, Grime identified three feasible combinations that would select for distinct suites of character states he defined as adaptive strategies: competitive (C), stress-tolerant (S), and ruderal (R). Conditions with both low stress and low disturbance would select for species with competitive ability (C-strategists). ‘S-selection’ would bring about adaptations for endurance in unproductive environments at the expense of vegetative and
reproductive vigor, and ‘R-selection’ in severely disturbed environments would result in species generally with a short life span and high seed production (Grime 1979). He further refined his classification to include four secondary strategies which have evolved in habitats experiencing intermediate intensities of competition, stress, and disturbance. CR species, competitive ruderals, are adapted to low stress where competition is restricted to moderate intensity by disturbance; SR, stress-tolerant ruderals, are adapted to lightly disturbed unproductive habitats; CS, competitive stress-tolerators, adapted to relatively undisturbed conditions with moderate stress; and CSR, competitive stress-tolerant ruderals, adapted to habitats in which the level of competition is restricted by moderate intensities of stress and disturbance.

The floodplain habitat of *M. caroliniana* is infrequently disturbed with annual flooding and not extremely stressful in the sense of limiting plant productivity. According to Grime, these conditions would select for competitive stress-tolerant species, CS strategists. Indeed *M. caroliniana* along with many common bottomland ground layer species share some predicted characteristics (Table 6.1). In particular, many are perennials with lateral growth from underground stems, they overwinter as rosettes, and develop peak biomass in the summer. Stress comes from limited oxygen in the root zone as a result of wet soil conditions and is a major limiting factor in floodplains (Sharitz and Mitsch 1993). *M. caroliniana* and other herbs of this habitat must compete for light as the canopy is usually dense in floodplains and blocks light from reaching the forest floor (Kellison et al. 1998).
While *M. caroliniana* shares growth morphology and phenology with many bottomland understory species, its dependence on insect pollinators differs from most. *M. caroliniana* is associated with 75 other flowering species across its range (Leblond and Sorrie 2002). Of those, only seven have conspicuous flowers likely to require insect pollination (Table 6.3). For this comparatively rare group of species, lessons learned from studies of *M. caroliniana* are applicable. Significantly, concerns for genetic diversity must be considered; members of this group may be limited by low pollinator visitation and have similar patterns of genetic diversity structure. If conservation management is planned for any of these species, it would be wise to protect the insect pollinators and to manage plant populations from as many different watersheds as possible to conserve the species’ genetic diversity. Besides floodplain species, conservation strategies for *M. caroliniana* may apply to insect dependent CS-strategists from other habitats that are naturally fragmented or disjunct, such as isolated wetlands.

Even among the restricted group of bottomland herbs, *Macbridea caroliniana* stands out by being more narrowly distributed, occurring in parts of three states as opposed to the entire southeast region (Table 6.2). Additionally, it is one of two species in a small genus of mints. Its congener *M. alba*, Federally listed as threatened, is narrowly endemic and occurs in disjunct flatwoods locations. A narrow distribution and membership in a small taxon may well raise the species’ value in terms of biodiversity conservation.

The strongest recommendations for conservation at the species level come from my study of genetic diversity conducted across the species’ range (Chapter 5).
Implications from studies conducted at a single site, the CNP, are more speculative but helped to identify additional information needs to support *M. caroliniana* conservation. Because I found a high degree of population differentiation (\(G_{ST} = 0.41\), Chapter 5), more populations should be protected for a greater chance of protecting more of the overall genetic biodiversity of the species. These populations should represent as many different watersheds as possible because of the high level of among watershed differentiation (\(G_{ST} = 0.323\), Chapter 5). However, populations that ranked highly for conservation priority should not be the only ones given management protection. Special attention may already be needed for the small populations with low genetic diversity.

Low genetic diversity alone does not necessarily put populations at risk, but for those with increased levels of inbreeding, population extinction may follow. The three populations ranking the lowest in my assessment JUN, POS, and MOC (See Chapter 5), are very small with fewer than 100 stems. For small, genetically depauperate populations, augmentation using pollen or seeds may be a wise option for conservation management. The phenogram can be used to determine source populations based on their genetic similarity to each of the depauperate populations in my study. For example, the best source population for POS would be the Congaree National Park populations (ECC or EDB). It would be more complicated for JUN and for MOC. For JUN, the best sources may be MMP or HOW. MOC is the most southern and genetically divergent population and the best strategy for this population may not be augmentation, but protection from physical degradation (e.g., runoff, development), protection of the pollinators, and perhaps the implementation of controlled hand pollinations within the
population. Lastly, protecting the native pollinator guild or improving the habitat and
resource availability for pollinators must be considered (Chapter 4).

Before the genetic diversity and structure study was conducted, it was known that
the Congaree National Park population at ECC was very important to the species’
conservation because it is large (Chapter 2). The EDB population is also relatively large
(Chapter 2). However, the findings of the genetic diversity and structure study underline
the importance of both Congaree populations to the conservation of the species (Chapter
5). ECC and EDB tied the number one spot for conservation priority based on multiple
genetic measures (Table 5.2). Compared to the rest of the populations studied (including
the next largest populations), the Congaree populations are doing extremely well and are
at the lowest risk of requiring any management intervention for genetic reasons.
However, these populations could potentially be used for augmenting other populations
that require additional gene flow and/or individuals. For example, if the HOW
population was in need of augmentation, ECC and EDB would be good potential source
populations because they are genetically closest (Figure 5.3). Additionally, ECC would
be the natural source population for management to consider if a catastrophic event leads
to a sharp decline in the population size and genetic diversity at EDB (and vice-versa).

Population sizes varied widely from year to year. While adverse effects of
cclimate related variability may be buffered in large populations, it may result in the
extirpation of small populations. Monitoring selected small populations along with the
large populations at Congaree National Park would provide a much needed better
understanding about threats and resilience of this rare species.
The physiological limits of *Macbridea caroliniana* were not studied, but the habitat models constructed in my study indicate that it is abundant in areas where other ground layer herbs are abundant, a phenomenon that may be related to soil variations (K and P concentrations) and the amount of light reaching the forest floor. It is not known how these findings apply to other potential *M. caroliniana* habitats. A related question to be addressed experimentally is to evaluate the effects of competition with other herbs, particularly with *Murdannia keisak*. Results of the current work did not indicate any clearly adverse effects of *Murdannia keisak* on *M. caroliniana*, but careful experiments would resolve the uncertainty.

The vigor and size of *M. caroliniana* populations in large gaps as well as the finding that genetic diversity was partitioned differently in gap patches compared to closed forest patches indicate that the persistence of *M. caroliniana* in the dynamic floodplain landscape may depend on gap dynamics through time. Large flowering patches of *M. caroliniana* are positively correlated with large canopy openings due to tree-fall gaps, but I don’t know what happens to the size of the patches as the canopy gaps close over time. Tracking these variables over long periods of time would provide data to create extinction curves of patches, that is, graphs that show the relationships between gap size and shrub encroachment to patch size over time until there are no stems in the patches. Tracking patches starting at the beginning of the extinction curve (when gaps first appear) would provide an even greater understanding of how *M. caroliniana* persists on the landscape. This would involve monitoring new tree-fall gaps for *M. caroliniana* stems through time. Coupled with knowledge about the rate of gap
formation at the landscape level, understanding the colonization of gaps and how time since gap formation affects patch size may influence management strategies in the future.

Hog disturbed areas had few if any stems that were left standing upright. It seems clear that hog disturbance is detrimental to patches at least in the short term; however, it is not known if *M. caroliniana* patches are capable of recovering after hog disturbance. In general, it would be helpful to understand the structure and dynamics of local populations, for example, to find out how fast populations can increase, how important is sexual reproduction compared to vegetative increases. A detailed demographic study would be very informative; however, the logistics of implementing this would be formidable.

References


Table 6.1. Selected species characteristics of *Macbridea caroliniana*, common herbs of alluvial floodplain bottoms\(^a\), and the most abundant range-wide associates of *M. caroliniana*\(^b\).

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth habit</th>
<th>Flowers</th>
<th>Habitats</th>
<th>Southeastern Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Macbridea caroliniana</em></td>
<td>Perennial with underground stolons</td>
<td>Thyrses of flowers with pink to lavender 2-4 cm long corollas</td>
<td>Bogs, marshes and alluvial woods</td>
<td>NC, SC, and GA</td>
</tr>
<tr>
<td><em>Arundinaria tecta</em>(^a)</td>
<td>Rhizomatous, woody perennial</td>
<td>Spikelets 1-5 cm long of 8-12 small flowers</td>
<td>Bogs, low woods, savannas and dry woods</td>
<td>Throughout</td>
</tr>
<tr>
<td><em>Boehmeria cylindrica</em>(^ab)</td>
<td>Perennial with stems from ligneous crown</td>
<td>Inconspicuous green flowers lacking corollas</td>
<td>Swamp forests, bogs, marshes and alluvial woods</td>
<td>Common throughout</td>
</tr>
<tr>
<td><em>Carex spp.</em> (e.g., <em>C. bromoides</em>)(^a)</td>
<td>Rhizomatous perennial</td>
<td>Flowers w/o perianths on spikes</td>
<td>Low woods</td>
<td>NC, SC, VA, GA, FL, TN, KY, and WV</td>
</tr>
<tr>
<td><em>Carex folliculata var. australis</em>(^b)</td>
<td>Rhizomatous perennial</td>
<td>Flowers w/o perianths on spikes</td>
<td>Bogs</td>
<td>NC, SC, FL, GA, MS, and AL</td>
</tr>
<tr>
<td><em>Chasmanthium laxum</em>(^ab)</td>
<td>Rhizomatous perennial</td>
<td>Spikelets w/5 or 6 flowers 6-9 mm long</td>
<td>Savannas, low woods and ditches</td>
<td>NC, SC, VA, GA, FL, AL, MS, TN, and KY</td>
</tr>
<tr>
<td><em>Hydrocotyl spp.</em> (e.g., <em>H. umbellata</em>)(^a)</td>
<td>Stoloniferous perennial</td>
<td>Umbels w/15-50 inconspicuous flowers</td>
<td>Low or moist areas</td>
<td>NC, SC, VA, GA, FL, AL, and MS</td>
</tr>
<tr>
<td>Knotweed (e.g., <em>Polygonum sagittatum</em>)(^a)</td>
<td>Annuals</td>
<td>Flowers on spikes w/white or pink calyxes 2-3.5 mm long</td>
<td>Wet ground</td>
<td>Throughout</td>
</tr>
<tr>
<td><em>Ludwigia spp.</em> (e.g., <em>L. alternifolia</em>)(^a)</td>
<td>Short-lived perennial</td>
<td>Flowers with 4 petals 6 mm long</td>
<td>Marshes, ditches, savannas and low woods</td>
<td>Throughout</td>
</tr>
<tr>
<td><em>Penstemon spp.</em> (e.g., <em>P. laevisatus</em>)(^a)</td>
<td>Single stemmed to bushy perennial</td>
<td>Panicles of flowers w/purplish corollas 1.5-2.2 cm long</td>
<td>Low meadows and forest edges</td>
<td>NC, SC, GA, FL, AL, and WV</td>
</tr>
<tr>
<td><em>Saururus cernuus</em>(^ab)</td>
<td>Rhizomatous perennial</td>
<td>Racemes of small flowers w/o perianths</td>
<td>Streams, lake margins, marshes, swamps and low woodlands</td>
<td>Throughout</td>
</tr>
<tr>
<td><em>Triadenum walterii</em>(^b)</td>
<td>Rhizomatous perennial</td>
<td>Cymules of flowers with 5 pinkish petals 4-6 mm long</td>
<td>Low woods and marshes</td>
<td>NC, SC, TN, VA, and GA</td>
</tr>
<tr>
<td><em>Viola spp.</em> (e.g., <em>V. primulifolia</em>)(^a)</td>
<td>Stoloniferous perennial</td>
<td>The chasmogamous flowers are 1-2 cm wide with white petals</td>
<td>Pond and stream margins, bogs, savannahs and pocosins</td>
<td>Throughout</td>
</tr>
<tr>
<td><em>Woodwardia areolata</em>(^ab)</td>
<td>Rhizomatous perennial</td>
<td>Not applicable (fern)</td>
<td>Acid swamps, bogs and wet pinelands</td>
<td>Essentially throughout</td>
</tr>
</tbody>
</table>

\(^a\)Plants listed in Kellison et al. (1998) and \(^b\)plants listed in Leblond and Sorrie (2002). Selected species characteristics are from Radford (1968). The example species were chosen because they are common co-occurring species of *M. caroliniana* (Leblond and Sorrie 2002) with the exception of *Penstemon laevisatus* which was chosen because this was the only *Penstemon* species occurring in habitats similar to *M. caroliniana*. 
Table 6.2. Characteristics of flowering herbs associated with *M. caroliniana* with conspicuous flowers (up to and greater than 2 cm) including growth habit, general flower description, habitat and the Southeastern range. Plants are from Leblond and Sorrie (2002) and characteristics are from Radford et al. (1968).

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth Habit</th>
<th>Flower description</th>
<th>Habitat</th>
<th>Southeastern range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Macbridea caroliniana</em></td>
<td>Perennial with underground stolons</td>
<td>Thyrses of flowers with pink to lavender 2-4 cm long corollas</td>
<td>Bogs, marshes and alluvial woods</td>
<td>NC, SC, and GA</td>
</tr>
<tr>
<td><em>Lobelia cardinalis</em></td>
<td>Perennial from basal offshoots</td>
<td>Bright red tubular flowers with 1.7-2.2 cm corollas</td>
<td>Marshes, streambanks, wet meadows, and low woods</td>
<td>Throughout</td>
</tr>
<tr>
<td><em>Oenothera fruticosa</em></td>
<td>Branched perennial</td>
<td>Symmetrical flowers with 4 yellow 1-2 cm petals</td>
<td>Dry woods, roadsides, and meadows</td>
<td>Throughout</td>
</tr>
<tr>
<td><em>Agalinis linifolia</em></td>
<td>Rhizomatous perennial</td>
<td>Pink tubular flowers with 3-4 cm corollas</td>
<td>Pine savannahs, and margins freshwater marshes</td>
<td>NC, SC, GA, FL, AL, and MS</td>
</tr>
<tr>
<td><em>Scutellaria integrifolia</em></td>
<td>Perennial forming clumps, but not stoloniferous</td>
<td>Blue to violet tubular flowers with 1.3-2.5 cm corollas</td>
<td>Savannahs, pine barrens, low meadows, and roadsides</td>
<td>Throughout</td>
</tr>
<tr>
<td><em>Rhexia mariana</em></td>
<td>Perennial frequently colonial from underground roots</td>
<td>Symmetrical flowers with 4 lavender 1-2.5 cm petals</td>
<td>Marshes, meadows, ditches, and savannahs</td>
<td>Throughout</td>
</tr>
<tr>
<td><em>R. nashii</em></td>
<td>Same as above</td>
<td>Same as above</td>
<td>Same as above</td>
<td>NC, SC, VA, GA, FL, and MS</td>
</tr>
<tr>
<td><em>Viola primulifolia</em></td>
<td>Stoloniferous perennial</td>
<td>The chasmogamous flowers are 1-2 cm wide with white petals</td>
<td>Pond and stream margins, bogs, savannahs and pocosins</td>
<td>Throughout</td>
</tr>
</tbody>
</table>
Appendix A

Pearson correlation coefficients of components sampled in patch-area study highlighting those associated with *Macbridea caroliniana* abundance variables (in bold)

|                  | Buffer | Canopy | shrub | sedge | moss | Triad | Woodw | Saur | pH | pH | PHOS | K | CA | MG | ZN | MN | CU | B | NA | OM |
|------------------|--------|--------|-------|-------|------|-------|-------|------|----|----|------|---|----|----|----|----|---|----|----|----|----|----|
| Canopy           | 1.00   | 0.09   | 0.34  | 0.23  | -0.60| 0.52  | 0.18  | -0.28| -0.09| -0.49| 0.00  | -0.17| 0.04  | -0.20| -0.17| -0.22| 0.06| -0.01| 0.16|
| shrub            | 0.09   | 1.00   | 0.21  | -0.20 | -0.18| -0.17 | -0.03 | 0.39 | 0.19 | -0.09| -0.02 | 0.39 | 0.05  | -0.09| -0.29| -0.19 | 0.22| -0.30| -0.33|
| sedge            | 0.34   | 0.21   | 1.00  | 0.24  | 0.09 | 0.51  | -0.05 | 0.20 | 0.15 | -0.12| 0.35  | 0.58 | 0.39  | 0.36 | 0.45 | -0.32 | 0.31| 0.41 | 0.06|
| moss             | 0.23   | -0.20  | 0.24  | 1.00  | 0.14 | 0.44  | 0.37  | 0.36 | -0.17| -0.18 | 0.49  | 0.22 | 0.69  | 0.43 | 0.10 | -0.61 | 0.54| 0.47 | 0.48|
| Triad            | -0.60  | -0.18  | 0.09  | 0.14  | 1.00 | 0.23  | 0.08  | 0.04 | -0.01| 0.48 | 0.27  | 0.27 | 0.26  | 0.34 | -0.11| -0.29 | 0.26| 0.21 | 0.37|
| Woodw            | 0.52   | -0.17  | 0.51  | 0.44  | 0.23 | 1.00  | 0.23  | -0.32| -0.19| -0.14 | 0.52  | 0.29 | 0.53  | 0.36 | 0.02 | -0.55| 0.45| 0.34 | 0.61|
| Saur             | 0.18   | -0.03  | -0.05 | 0.37  | 0.08 | 0.23  | 1.00  | -0.33| -0.26| -0.21 | 0.18  | -0.07 | 0.35  | 0.04 | -0.23| -0.41 | 0.23| 0.28 | 0.40|
| pH               | -0.28  | 0.39   | 0.20  | -0.36 | 0.04 | -0.32 | -0.33 | 1.00 | 0.34 | 0.16  | 0.02  | 0.48 | 0.04  | -0.03| 0.40 | 0.23  | 0.00| -0.07| -0.59|
| Buffer pH        | -0.09  | 0.19   | 0.15  | -0.17 | -0.01| -0.19 | -0.26 | 0.34 | 1.00 | 0.29  | 0.27 | 0.03  | -0.23 | 0.07 | 0.05 | -0.22 | 0.14| -0.14| -0.30|
| Phos.            | -0.49  | -0.09  | -0.12 | -0.18 | 0.48 | -0.14 | -0.21 | 0.16 | 0.29 | 1.00  | 0.12  | 0.01 | 0.03  | 0.00 | -0.24| -0.01 | 0.17| 0.11 | -0.10|
| K                | 0.00   | -0.02  | 0.35  | 0.49  | 0.27 | 0.52  | 0.18  | 0.02 | -0.27| 0.12  | 1.00  | 0.57 | 0.82  | 0.44 | 0.25 | -0.52 | 0.44| 0.63 | 0.45|
| CA               | -0.17  | 0.39   | 0.58  | 0.22  | 0.27 | 0.29  | -0.07 | 0.48 | 0.03 | 0.01  | 0.57  | 1.00 | 0.70  | 0.69 | 0.71 | -0.45 | 0.65| 0.43 | 0.18|
| MG               | 0.04   | 0.05   | 0.39  | 0.69  | 0.26 | 0.53  | 0.35  | 0.04 | -0.23| 0.03  | 0.82  | 0.70 | 1.00  | 0.64 | 0.40 | -0.63 | 0.66| 0.71 | 0.50|
| ZN               | -0.20  | -0.09  | 0.36  | 0.43  | 0.34 | 0.36  | 0.04  | -0.03| 0.07 | 0.00  | 0.44 | 0.69 | 0.64 | 1.00 | 0.57 | -0.50 | 0.65| 0.56 | 0.54|
| MN               | -0.17  | 0.29   | 0.45  | 0.10  | -0.11| 0.02  | -0.23 | 0.40 | 0.05 | -0.24 | 0.25  | 0.71 | 0.40  | 0.57 | 1.00 | -0.01 | 0.23| 0.17 | -0.11|
| CU               | -0.22  | -0.19  | -0.32 | -0.61 | -0.29 | -0.55 | -0.41 | 0.23 | -0.22 | -0.01 | -0.52 | -0.45 | -0.63 | -0.50 | -0.01 | 1.00 | -0.71 | -0.46 | -0.66|
| B                | 0.06   | 0.22   | 0.31  | 0.54  | 0.26 | 0.45  | 0.23  | 0.00 | 0.14 | 0.17  | 0.44  | 0.65 | 0.66  | 0.65 | 0.23 | -0.71 | 1.00| 0.39 | 0.42|
| NA               | -0.01  | -0.30  | 0.41  | 0.47  | 0.21 | 0.34  | 0.28  | -0.07| -0.14| 0.11  | 0.63  | 0.43 | 0.71  | 0.56 | 0.17 | -0.46 | 0.39| 1.00 | 0.43|
| OM               | 0.16   | -0.33  | 0.06  | 0.48  | 0.37 | 0.61  | 0.40  | -0.59| -0.30| -0.10 | 0.45  | 0.18 | 0.50  | 0.54 | -0.11| -0.66 | 0.42| 0.43 | 1.00|
| Stems/Patch      | -0.55  | -0.06  | -0.14 | -0.14 | 0.66 | -0.11 | 0.34  | -0.08| 0.08 | 0.09  | -0.18 | -0.04 | -0.13 | 0.07 | -0.20 | -0.02 | -0.01 | -0.13 | 0.11|
| Density          | -0.36  | -0.11  | 0.31  | 0.04  | 0.69 | 0.35  | 0.24  | -0.03| -0.03 | -0.02 | 0.26  | 0.32 | 0.26  | 0.30 | 0.20 | -0.08 | 0.04| 0.28 | 0.20|
| Total Area       | -0.38  | -0.23  | -0.34 | 0.00  | 0.51 | -0.06 | 0.62  | -0.27 | -0.03 | 0.02  | -0.15 | -0.17 | -0.07 | 0.10 | -0.36 | -0.13 | 0.09| -0.06 | 0.34|