Ecological dynamics of a terrestrial orchid symbiosis

by

Jeffrey M. Diez

(Under the direction of H. Ronald Pulliam)

Abstract

My dissertation research tested elements of niche theory by determining how the population dynamics of a terrestrial orchid, *Goodyera pubescens*, respond to abiotic gradients, and how mycorrhizal fungi influence patterns of orchid recruitment. Long-term study plots were established along a gradient from the southeastern Piedmont to the southern Appalachian Mountains, encompassing a wide range of environmental conditions. I generated predictive models to show how basic vital rates such as survival and reproduction depend on soil moisture and understory light availability using a combination of approaches including abiotic and demographic monitoring, soil analyses, targeted field experiments, genetic analysis, and computer modelling. I then used these findings to compare predicted population growth rates across abiotic gradients from modelling to realized distributions and abundances from field data. I also conducted the first study to jointly characterize the spatial genetic structure of a plant and its mycorrhizal fungi, in order to test hypotheses about how patterns of genetic diversity are shaped by demographic processes of a symbiotic plant-fungal system along environmental gradients. Through field experiments I found strong evidence for fungal limitations on patterns of plant recruitment that were context dependent (i.e. symbiotic recruitment increased with both soil organic content and moisture). A key to these studies has been the use of newly-available Bayesian statistical approaches to integrate multiple

types of data from a range of spatial and temporal scales, in order to build predictive models with reasonable estimates of uncertainty.

INDEX WORDS: demography, *Orchidaceae*, hierarchical Bayes, predictive modeling, niche theory

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Chapter 1

Introduction and Literature Review

As processes such as global climate change and landscape modifications challenge ecologists to make predictions about how species respond to a changing environment, a basic understanding of the processes shaping species distributions is needed (Thomas et al., 2004). The geographic ranges of many species are characterized by strong gradients in abiotic conditions and biotic communities over multiple spatial and temporal scales. Understanding and accounting for the variability in ecological processes across these various scales is important for making realistic predictions of species dynamics and potential responses to environmental change (Davis et al., 1998). A rich theoretical literature exists on niche theory, but sufficiently evaluating competing hypotheses within this literature will require more long-term field studies linking species dynamics to underlying environmental variation, as well as adoption of statistical methods that can properly evaluate variation in these relationships.

My dissertation research tested elements of niche theory by determining how the population dynamics of a terrestrial orchid, *Goodyera pubescens*, respond to abiotic gradients, and how mycorrhizal fungi influence patterns of orchid recruitment. Long-term study plots were established along a gradient from the southeastern Piedmont to the southern Appalachian Mountains, encompassing a wide range of environmental conditions. I generated predictive models to show how basic vital rates such as survival and reproduction depend on soil moisture and understory light availability using a combination of approaches including abiotic and demographic monitoring, soil analyses, targeted field experiments, genetic analysis, and computer modelling. I then used these findings to compare predicted population growth rates across abiotic gradients from modelling to realized distributions and abundances from field

data. I also conducted the first study to jointly characterize the spatial genetic structure of a plant and its associated mycorrhizal fungi, in order to test hypotheses about how patterns of genetic diversity are shaped by demographic processes of a symbiotic plant-fungal system along environmental gradients. Through field experiments I found strong evidence for fungal limitations on patterns of plant recruitment that were context dependent (i.e. symbiotic recruitment increased with both soil organic content and moisture). A key to these studies has been the use of newly-available Bayesian statistical approaches to integrate multiple types of data from a range of spatial and temporal scales, in order to build predictive models with reasonable estimates of uncertainty.

1.0.1 Study system

Terrestrial orchid mycorrhizae

This study's focal species, *Goodyera pubescens*, is a terrestrial, evergreen, clonal orchid distributed throughout eastern North America, from Florida, west to Arkansas, north and east into Canada. Like all orchids, its seeds lack significant nutrient reserves and are therefore dependent on the transfer of carbohydrate energy, water and mineral nutrition from mycorrhizal fungi, at least until reaching photosynthetic stage (Rasmussen and Whigham, 1998*b*). The fungi associated with *G. pubescens* are from the genus *Tulasnella*, a group of saprotrophic basidiomycetes from the order *Tulasnellales*. Little is known about the nature of the relationship beyond the seedling stage, although adults remain heavily colonized by the fungi (Rasmussen, 1995; Rasmussen and Whigham, 1998*b*), and the constraints on germination are potentially large.

Overview of study design

The study used a hierarchical sampling design, consisting of 3 landscapes and 16 study grids distributed across a geographic gradient of approximately 120 km. from Athens, Georgia to the the Coweeta Long-term Ecological Research (LTER) site in the southern Appalachian

Mountains. The 3 landscapes were the Whitehall Forest in Athens, GA, the Nancytown area near Cornelia, GA, and the Coweeta LTER in North Carolina. Within each landscape, we established six study grids (only four in Nancytown) each between 250 and 480 m^2 in size for a total of 16 study grids. For the purpose of abiotic monitoring, these study plots were divided into 4 m^2 cells. These grids were all located in relatively mature (80^+ yrs old) deciduous forest and chosen to reflect a range of elevation, temperature, and precipitation conditions experienced by the forbs under study. Much of the analysis in this work adopts a hierarchical Bayesian framework, which is a newly available tool being rapidly adopted by more ecologists for analyzing complex problems. The approach is briefly introduced at the end of this introduction.

1.0.2 Chapters

Chapter 1: Hierarchical controls on soil moisture

A consistent theme of this study is the importance of accounting for the abiotic gradients across which ecological processes unfold. I thus begin with a brief exploration of the factors that control perhaps the most important abiotic variable - soil moisture - across the geographic gradient of this study. Soil moisture is an important driver of many above and belowground ecological processes (Weltzin and McPherson, 2000; Urban et al., 2000; Churkina et al., 1999; Stephenson, 1998; McKenzie et al., 2003; Xiao and Moody, 2004). Precipitation patterns and available moisture are also likely to change under various climate change scenarios (Harte and Shaw, 1995; Gordon and Famiglietti, 2004). Thus, an ability to predict how soil moisture is related to climate is of interest for explaining observed patterns in ecological processes across current environmental gradients, and for making predictions of how these processes are likely to be influenced by climate change.

It is generally agreed that soil moisture is jointly determined by factors at multiple scales, from regional precipitation patterns, through meso-scale topographic impacts on flow patterns and evaporation, down to micro-site characteristics of the soil, such as texture, that

influence water-holding capacity (Helvey et al., 1972; Yeakley et al., 1998; Lookingbill and Urban, 2004).

I use the overall study's hierarchical sampling design to test the relative importance of microsite and meso-scale variables for determining soil moisture within eastern deciduous forests. A large part of the variance in soil moisture over time can be explained by precipitation in the previous week, however this effect varied across landscapes. I show that the effects of precipitation patterns were mediated by characteristics of both meso-scale topography and micro-site soil characteristics such as soil texture. Together, this work supports the concept that factors at multiple spatial scales are important to determining soil moisture, and sets the stage for understanding plant population responses to abiotically heterogeneous landscapes.

Chapter 2: Spatial patterns of symbiotic germination

Chapter two uses seed packet experiments to test for mycorrhizal constraints on the recruitment patterns of *G. pubescens*, and to see how these patterns may vary across the abiotic gradients. This is a critical step in understanding population dynamics because the recruitment stage of many organisms' life histories can be critical in determining population dynamics, as juveniles often experience high mortality rates (HilleRisLambers et al., 2002). The dependence of orchids on mycorrhizal fungi in order to reach photosynthetic stage introduces a potential bottleneck on orchid recruitment, but very little is known about the factors controlling the distribution of the fungi or how fungal distributions may constrain orchid recruitment and distributions.

I used a series of seed introductions to show strong evidence of decreasing germination success as seeds get further from adult plants, and increased success with increasing soil moisture and organic content in areas greater than 1 meter from adults. I connect this work with the demographic monitoring to show that patterns of germination success are highly correlated with observed recruitment patterns at the population level.

Chapter 3: Hierarchical analysis of terrestrial orchid distributions across environmental gradients

Moving up in scale to population-level processes, I used extensive surveys and abiotic modelling to explore how the distributions and abundances of two terrestrial orchids, *Goodyera pubescens* and *Tipularia discolor*, are related to abiotic variables at different spatial scales.

Species distributions are the result of a combination of responses to ubiquitous environmental gradients and a potentially wide range of biotic interactions. Responses to abiotic variables, such as moisture, temperature, and resource availability, are modified by numerous biotic processes, such as competition, dispersal, mutualisms, and disease. Therefore, models that attempt to explain and predict species distributions must account for both the abiotic template and biotic processes that are likely to shift observed distributions from the optimal.

Here I use hierarchical models to evaluate how two orchids, with different life history characteristics, differ in responses to environmental gradients. Incorporating local spatial effects serves two purposes: 1) estimation of the aggregate impact of local spatial processes on species distributions and performance, relative to the abiotic 'signal', and 2) a more honest evaluation of the impact of environmental gradients on species distributions that accounts for inherent spatial autocorrelation in the data.

I found light availability and soil moisture to be the most influential explanatory variables for both orchid's patterns of presence and abundance, but *Goodyera* was found to have greater spatial effects than *Tipularia*. These differences are consistent with predictions based on life history characteristics.

Chapter 4: Demographic responses of a terrestrial orchid to abiotic gradients

In this chapter I expand on the idea of distribution modeling to look more directly at patterns of performance. I test predictions of niche theory that suggest the possibility of substantial population sizes even in habitat that is not suitable enough to maintain the population.

Although many studies continue to describe demographic variability in natural populations, less progress has been made in linking basic vital rates and overall population growth rates to abiotic driving variables. I again use hierarchical Bayes methods for parameter estimation and prediction across a range of environmental conditions and show how light and soil moisture influence vital rates and overall population growth rates of *G. pubescens*. Comparisons with observed distributions suggest a lack of correspondence between distribution and suitability that changes across spatial scales. Supportive of predictions of source-sink and metapopulation theory, I find this species absent from some substantial areas of suitable habitat and present in some unsuitable habitats. This pattern is most evident at very local spatial scales, while the correspondence between abundance and suitability increases with increasing spatial scale. I also show how the uncertainty estimated by the models influences the evaluation of theory and helps inform the interpretation of predictions of species dynamics.

Chapter 5: Spatial genetic structure of a terrestrial orchid and its symbiotic fungi

I finish the dissertation by examining how some of these key ecological interactions - responses to abiotic gradients and mycorrhizal symbiosis - influence the distribution of genetic diversity within and among populations of both the plant and fungi. Although many studies have described patterns of genetic variability within and among populations of different plant taxa, and some fungal taxa, no studies to date have concurrently studied the spatial genetic structure of any plant and its mycorrhizal symbionts. In this study, I used genetic data from five mapped populations of the terrestrial orchid, *Goodyera pubescens*, and its associated mycorrhizal fungi to show significant spatial autocorrelation in genetic diversity within populations for both the plants and the fungi. However I found most of the genetic variability partitioned within populations compared to among populations, likely reflecting the high dispersal capabilities and clonal growth of both taxa. I also report a limited pattern of

association among plant and fungal genotypes after controlling for geographic distance. Comparisons of genetic diversity with abiotic and demographic variables are limited by sample sizes, but suggestive of causal links between the abiotic environment, demographic rates, and patterns of genetic diversity.

1.0.3 Framework for analysis

I have adopted a hierarchical Bayes (HB) framework for analysis in much of my dissertation work. For researchers unfamiliar with Bayesian methods, some of the terminology will be foreign at first, but its basis in probability theory and close relationships to standard linear models afford ecologists some familiarity. In a brief introduction to these methods here, I explain the basic structure of hierarchical models, what it means to be Bayesian, and what some of the advantages may be for analysis of ecological processes.

Traditional statistical approaches have been largely inadequate for incorporating complex, hierarchical datasets, and too awkward for adequately making predictions that reflect the uncertainties in the system. Developments in statistical approaches in the last decade that have accompanied improved computer technology are now providing exciting alternatives that allow more useful integration across levels of organization (Clark, 2005; McMahon and Diez, 2005). Critical to developing and testing ecological theory, as well as evaluating likely response to such perturbations as climate change, these models allow more robust prediction through proper incorporation of multiple sources of uncertainty.

It is important to recognize that not all hierarchical models are Bayesian, and not all Bayesian analysis is hierarchical. It turns out that for moderately complex hierarchical problems (and even simple ones) it is just more feasible and powerful to implement hierarchical models in a Bayesian framework.

Hierarchical linear models (HLMs)

Hierarchical linear models (HLM), sometimes called 'mixed models', use nested regression equations to investigate associations between variables at different scales. Ecologists are well-aware of simplified forms of HLM, such as nested ANOVA designs. An increase of computational power is now allowing the exploration of more complex models. Begin with a simple regression equation:

$$Y_{ij} = \beta_{0j} + \beta_{1j} X_{ij} + r_{ij}, \tag{1.1}$$

where Y_{ij} is a measured response variable, which has a group-level intercept β_{0j}, and is related to an individual-level predictor variable, X_{ij} by the group-level regression coefficient, β_{1j}. Because the response variable Y is associated not only with the individual $_i$ observations, but is nested within the $_j$ groups, the residuals can not be assumed to be normally distributed. HLM accounts for this. The second level of the hierarchy consists of equations describing the intercept and slope terms from the first level:

$$\beta_{0j} = \gamma_{00} + \gamma_{01} W_j + u_{0j}, \tag{1.2a}$$

and

$$\beta_{1j} = \gamma_{10} + \gamma_{11} W_j + u_{1j}, , \tag{1.2b}$$

where now γ_{00} and γ_{01} are level-2 coefficients for the intercept and slope respectively of these level-2 regression models (in other words, the γ parameters are group-level equivalents of the β parameters at the individual level). W_j is a level-2 predictor, as was X_i in equation (6.1). u_{0j} and u_{1j} are the level-2 random effects that are assumed to be distributed normally with means of zero and variances of τ_{00} and τ_{11} respectively. The covariance between these random effects is τ_{01}. The main difference between this hierarchical specification and single-level models is the treatment of correlated error. The typical ordinary least squares (OLS) approach requires the deviations from the grand mean to be independent, normally distributed, and with constant variance. In fact, if the u_{0j} and u_{1j} terms are zero, this model reduces to a model

similar to an OLS model. The hierarchical model can now be used to infer relationships between predictor variables and the response variable at specific scales.

Bayesian analysis

At the core of Bayesian analysis is Bayes theorem, which simply says that the probability of Y, given X, equals the probability of X, given Y, times the probability of Y, all divided by the probability of X, as such:

$$P(Y|X) = \frac{P(X|Y)P(Y)}{P(X)} \tag{1.3}$$

As mundane as it may seem at first glance, there are books written about the implications of this simple relationship of probability theory for statistical analysis. At its heart though, the significance is the following: while frequentist (classical) statistics asks what the probability of obtaining the data is, given a particular hypothesis (or alternatively, given a particular parameter value), we use Bayes theorem to flip this around and ask what the probability of a hypothesis (or parameter value) is, given the data. For most researchers, this makes intuitive sense: what we know for sure are the data in hand (though we may add in measurement error), and what we want to know is the probability that our hypothesis is correct (or even better: which is the *most* probable hypothesis out of a set of conceivable alternatives). Bayes theorem allows us to do this.

Abstract in this form, it may be easier to think of an example that will appear in my dissertation. Let X above be data on whether or not individuals survived in several populations, and Y is the true probability of survival that we are interested in estimating. Bayes theorem gives us a probability distribution (the posterior) for the true probability of surviving (Y), given that we observed some number to survive (X).

To actually calculate this 'posterior' probability distribution, we need two essential components, which arise again and again in my dissertation: the likelihood (probability of the data, given a hypothesis or parameter value) and a prior (the distribution describing the a priori probability of different parameter values). Together, these allow us to describe the

posterior distribution as proportional to the likelihood times the prior:

$$Posterior \propto Likelihood \times prior \tag{1.4}$$

Because I had no prior data to inform my estimation of parameters in this study, I use 'non-informative' prior distributions (i.e. all values of a parameter, for example survivorship, are equally likely, or nearly so, before considering the data), which allows the data solely to describe the posterior distribution. The likelihood describes the sampling distribution from which the data was drawn, and may be the Normal distribution for many types of data, or the Poisson for count data, or the Binomial for dichotomous 'coin-flipping' type trials such as whether individuals survive or not. The posterior distributions then are actual probability distributions, and contain everything we need to know about the variable of interest. They can thus be summarized in a variety of ways. The most convenient summaries are the means and 95% intervals, which give an indication of the direction of the effect and the uncertainty of that estimate.

Hierarchical Bayes

Combining the HLM and the Bayesian framework, we have hierarchical Bayes models. Whether a researcher is drawn philosophically to Bayesian or frequentist analysis, in practice many of the hierarchical models of even modest complexity are easier to implement in the Bayesian framework (Raudenbush and Bryk, 2002).

Thus, although the preferred approach may vary with the particular problem, a hierarchical Bayesian statistical framework is increasingly turned to by ecologists for pragmatic advantages for dealing with complex ecological datasets (Clark, 2005). Several of these advantages that are relevant to this study include:

- First, it provides a flexible framework capable of accommodating a wide range of study designs including unbalanced designs and use of multiple data sources (Okuyama and Bolker, 2005; Clark and LaDeau, 2005).

- Second, the data is structured, both from sampling design (cells within grids, grids within landscapes), and from levels of biological organization (individuals within populations, within species), and hierarchical models enable hypotheses to be tested at each of these levels explicitly (Raudenbush and Bryk 2002, McMahon and Diez 2005).

- Third, and particularly important for applications in which prediction is of interest, the full Bayesian framework enables a full accounting for all sources of variability that inevitably contribute to the overall uncertainty of model inference. Inference of every parameter in a model is inherently dependent on the uncertainty of other parameters because of the structure of the model and process of conditional MCMC updating (Raudenbush and Bryk, 2002).

- Finally, the interpretation of posterior distributions as direct summaries of the probability of model unknowns, given what is known, is both philosophically appealing and practically convenient for communicating results (Ellison, 1996; Wade, 2000).

Typically cited as the strongest argument against Bayesian methods, the inappropriateness of having to assign prior beliefs to parameter values, is largely a non-issue given modern computation. As some authors point out, it can still be difficult or impossible to assign fully non-informative priors in multi-level models, but the effects are small compared to the increased range of model structures that become available.

There are a small but growing number of examples in the ecological literature in which hierarchical Bayes models are used for applications similar to my work. These include spatial distribution models of the generalized linear mixed model form (GLMM) with explicit spatial effects allowing for otherwise unexplained autocorrelation of responses or explanatory variables (Fleishman et al., 2001; Hooten et al., 2003; Gelfand et al., 2005), species demography (Clark, 2003; Clark et al., 2005), population spread rates (Wikle, 2003*a*).

Chapter 2

Multi-scale Controls of Soil Moisture: A hierarchical Bayes model of soil moisture [1]

[1]Diez, J.M. To be Submitted as *Brief Report* to *Landscape Ecology*

ABSTRACT

Soil moisture is a major driver of many aboveground and belowground ecological processes, so it is important to understand how it varies across different spatial scales. A hierarchical set of factors is thought to be important in determining soil moisture, from regional precipitation patterns down to meso-scale topography and micro-site soil characteristics. However, despite an extensive literature on modeling soil moisture, there have been surprisingly few tests of this idea across nested spatial scales, and none within a hierarchical statistical framework. Moreover, precipitation patterns are predicted to change with changing climate, so it is important to build predictive models that incorporate precipitation and key explanatory variables from different scales.

In this study I used a hierarchical study design to test whether microsite and meso-scale variables are important for soil moisture relative to precipitation patterns across a large geographic and environmental gradient within southeastern deciduous forests. I used a hierarchical Bayesian regression framework to show that the influence of precipitation patterns on soil moisture is mediated by characteristics of both meso-scale topography, most notably aspect, and microsite soil characteristics such as soil texture and organic content. The precipitation in the previous week significantly affected soil moisture readings, but this effect varied across landscapes. Together, this work supports the concept that factors at multiple spatial scales are important to determining soil moisture, and provides an example of the utility of hierarchical Bayes models for estimating the contributions of multi-level explanatory variables and building predictive models that realistically reflect the variability and uncertainties in the system.

Introduction

Soil moisture is important for many ecological processes, including individual plant recruitment and growth (Knapp and Canham, 2000), plant species distributions and community

composition (Weltzin and McPherson, 2000; Urban et al., 2000; McKenzie et al., 2003; Stephenson, 1998; Zinko et al., 2005), soil community composition (Ettema et al., 2000), nutrient cycling and decomposition rates (Knoepp and Swank, 2002; Hackl et al., 2004; Pavao-Zuckerman and Coleman, 2005), and overall ecosystem productivity (Churkina et al., 1999; Xiao and Moody, 2004; Webb et al., 1983; Rosenzweig, 1968). Even extremely local variation in edaphic factors such as soil moisture can be critical for individual plant performance, competition dynamics, and determining local community composition (Hutchings et al., 2003). Root foraging has been shown to respond to local microsite variation in nutrient availability, which is strongly influenced by soil moisture. Moreover, many organisms and ecosystem processes may respond to the variation in moisture patterns as much as the mean values (Knapp et al., 2002).

It is also likely that precipitation patterns and available moisture may change under various climate change scenarios (Harte and Shaw, 1995; Gordon and Famiglietti, 2004). The magnitude of change is uncertain, and there is likely to be significant variation across regions, but some change is likely (IPCC 2001). Thus, an ability to predict how soil moisture is related to precipitation is of interest for explaining observed patterns in ecological processes across existing environmental gradients, and for making predictions of how these processes are likely to be influenced by climate change.

It is generally agreed that soil moisture is jointly determined by factors at multiple scales, including regional precipitation patterns, meso-scale topographic impacts on flow patterns and evaporation, and micro-site soil characteristics that influence water-holding capacity (Helvey et al., 1972; Yeakley et al., 1998; Lookingbill and Urban, 2004). This hierarchical set of controls appears to be the case in the highly heterogeneous environments of eastern deciduous forests (Helvey et al., 1972; Yeakley et al., 1998), where this study takes place. In particular, previous studies have identified the important influences on soil moisture to be local soil properties (Helvey et al., 1972; Boyer et al., 1990), and intermediate scale topography (Lookingbill and Urban, 2004).

Despite the acknowledgement of this hierarchical structure, and a substantial amount of soil hydrologic process and statistical modeling (reviewed by Mahmood, 1996; Western et al., 2002) there has often been a gap between the conceptual model of how these processes operate, and the actual analysis. Regression models that incorporate multiple scales can include variables at one scale that significantly influence the estimation and interpretation of effects of variables at other scales (Raudenbush and Bryk, 2002). Therefore, although individual studies investigating the importance of explanatory factors at a given level have been important for identifying a suite of relevant controllers of soil moisture, efforts are needed to integrate data from multiple scales within single coherent multi-level models to distinguish their relative importance.

Fortunately, explicitly hierarchical models suited to this task are becoming more widely available with advancements in computational statistics (Raudenbush and Bryk, 2002), and hierarchical models implemented with a Bayesian framework specifically are beginning to be used more frequently by ecologists for a variety of complex, multi-level problems (Wikle, 2003*b*; Clark, 2005; McMahon and Diez, 2005). In this study, I show how such hierarchical models may be useful for explaining soil moisture patterns as a function of key explanatory variables at different scales. Through simultaneous estimates of factors at multiple scales, while including associated variability at each level, these methods also provide straightforward translation of parameter estimates to predictions with appropriate amount of estimated uncertainty. Overall, it is argued that such methods may provide a useful probabilistic link between local ecological processes and climatological changes across broader landscapes.

This study focuses on two essential questions for understanding and predicting patterns of soil moisture based on key controlling variables:

(1) At what spatial scales does soil moisture vary most, from local microsites to large latitudinal gradients?

(2) What are the relative importance of key explanatory variables, such as topographic features and soil properties, for mediating the effects of regional precipitation patterns on soil moisture in forested landscapes?

Materials and Methods

Study Design

A hierarchical sampling design was used to capture a range of environmental conditions across a geographic gradient of approximately 120 km. from the Piedmont of Georgia to the Southern Appalachian mountains (Figure 3.2). At the largest scale, I sampled three landscapes along this gradient: Whitehall Forest in Athens-Clarke County, GA (33°92' N latitude, 150-240 m elevation), the Nancytown area (34°31' N latitude, 315-450 m elevation) Habersham County, GA, and the Coweeta Hydrologic Laboratory (35°03' N latitude, 750 - 1,500 m elevation) in Macon County, N.C. Within each landscape, I established six study grids (four in Nancytown) between 250 and 480 m^2 in size for a total of 16 study grids, which were divided into 4 m^2 'cells.' All sites were placed in relatively mature ($\sim$ 80 yrs old) deciduous forest, and chosen to reflect the range of elevation, topographic, and precipitation conditions in the region.

Soil Moisture

The handheld Soil Water Content Measurement System from Hydrosense was used to measure soil moisture in the top 12 cm of soil in 16 2 x 2 meter cells within each of sixteen grids every two weeks during 2003. These 16 cells were distributed evenly across the grid, for a total of 256 cells measured on 23 sampling dates. Daily precipitation data were obtained from three sources for the different landscapes: Coweeta Hydrologic Lab (35°03'N / 83°26'W), the NCDC climate station in Cornelia, GA (34°31'N / 83°32'W), and the Georgia Forestry Commission station at Whitehall Forest in Athens, GA (33°92'N / 83°30'W).

Soil Analyses

Three 12 cm soil cores were taken within a 0.5m radius from the center of each of the 16 "intensive" cells within each of the 16 study grids for analysis of soil texture and organic content. The three soil cores from each cell were bulked and sifted through a 2 mm sieve to obtain sufficient quantity for all soil analyses, for a total of 256 samples. In a few circumstances, course rocky topsoil prohibited three complete 12 cm cores and 4 more shallow cores within the 0.5 m radius were used. Organic content was determined by percent weight lost after combustion in a muffle oven. Soil texture was determined using the hydrometer method (Gee and Bauder, 1986). Because percent sand and percent clay were highly correlated, percent sand was used as the index of soil texture in all analyses.

GIS Analyses

For all topographic analyses, NED 1/3 arc second DEMs (digital elevation models) with 10 meter resolution were obtained for each study landscape from the USGS (Data available from U.S. Geological Survey, EROS Data Center, Sioux Falls, SD). A variety of topographic variables were derived from this DEM using ESRI's ArcGIS 9.0 and Arc GRID software, including slope, aspect, flow accumulation, distance to ridge, and distance to nearest stream. Aspect was cosine transformed to vary between 1 and -1 via $-(cos(aspect - 180))$, similar to Urban et al. (2000) but here using a 180 degree offset, giving North facing slopes a value of 1 and South facing slopes a -1. The basic topographic data were used to model two derivative measures of potential topographic wetness. First the commonly used Topographic Convergence Index (TCI) was calculated as $Ln(flowaccumulation/(tan(slope))$ (Beven and Kirkby, 1979). A second topographic index was calculated to reflect the combination of the relative slope position of site within a watershed, and the overall size of the sub-watershed of which any given site is a part. This index, Relative Slope Position and Watershed Size

("RSPWS"), was calculated as

$$RSPWS = \frac{distance\ to\ ridge}{distance\ to\ ridge + distance\ to\ stream} * FA$$

where FA is the flow accumulation of the nearest downslope stream. This measure therefore weights the size of the nearest stream by the relative slope position.

Statistical modeling

Hierarchical Bayesian models were used for all analysis, including partitioning variance in soil moisture measurements and estimating the effects of predictor variables from multiple spatial scales. The model consists of a nested set of regression equations, with 4 levels and covariates at 3 of these levels. The level-1, temporal model, is the level at which the soil moisture data are collected: twenty-three measurement dates in each of 256 cells. The covariate of interest at this level is the amount of precipitation in the preceding week. The first spatial level is the microsite (cell), and is the scale at which I hypothesize soil properties (soil texture and organic content) to be important determinants of soil moisture. The second spatial level is the grid (250 - 480 m^2), where topographic variables such as slope, aspect, and flow accumulation are hypothesized to be important. At the largest scale, landscapes, any regional differences that impact soil moisture are accounted for, but covariates are not explicitly included at this level (although the temporal differences in precipitation also account for variation at the landscape level, as described later). So, the first level of the model relates individual soil moisture measurements to precipitation as follows:

$$Y_{cglt} = \alpha_c + \psi_l * precip_{lt} + \epsilon_t \tag{2.1}$$

where Y_{cglt} is the soil moisture in cell c, grid g, landscape l, at time t, and is a function of a cell-specific intercept α_c and the precipitation in the preceding week in that landscape, $precip_{lt}$. The precipitation coefficient ψ_l represents the effect of precipitation on soil moisture, and is estimated for each landscape, but is modeled hierarchically such that $\psi_l \sim Normal(\mu, \tau)$.

The 'hyperpriors' on μ and τ are specified as non-informative. The temporal variation is considered $Normally$ distributed as $\epsilon_t \sim Normal(0, \tau_{temp})$.

Forming the hierarchical link to higher levels, the cell-level intercepts are considered random effects drawn from a distribution as follows:

$$\alpha_c = \gamma_g + \beta_1 sand_c + \beta_2 light_c + \beta_3 organic_c + \epsilon_c \tag{2.2}$$

where γ_g is a grid-level intercept, β's are coefficients describing the effects of cell-level covariates, and the error term is again $Normally$ distributed as $\epsilon_c \sim Normal(0, \tau_{cell})$. The estimated coefficients for the covariates again at this level are assumed to be random variables but invariant across sites and years, as there is no a priori reason to assume otherwise. Each coefficient is given a non-informative prior distribution: $\beta_{1-3} \sim Normal(0, 1000)$

The grid-level intercepts are modeled similarly, with landscape level intercepts and grid-level covariates:

$$\gamma_g = \lambda_l + \delta_1 RSPWS_g + \delta_2 TCI_g + \delta_3 Elev_g + \delta_4 Aspect_g + \epsilon_g \tag{2.3}$$

where λ_l is a landscape-level intercept, δ's are coefficients describing the effects of topographic covariates, and the error term is again $Normally$ distributed as $\epsilon_g \sim Normal(0, \tau_{grid})$. The estimated coefficients for the covariates again at this level are assumed to be random variables but invariant across sites and years. Each coefficient is given a non-informative prior distribution: $\delta_{1-3} \sim Normal(0, 1000)$. The landscape level intercepts are modeled hierarchically with a global mean and landscape level variance: $\lambda_l \sim Normal(\mu, \tau_{land})$. The global mean is given a prior $\mu_{global} \sim Normal(0, 1000)$ and the priors for all variance parameters are uniform distributions on the standard deviations Gelman et al. (2004). Variance partitioning was performed by fitting an unconditional model and calculating intraclass correlation coefficients (Raudenbush and Bryk, 2002).

All Bayesian models were fit using WinBugs 1.4, which uses a variety of Markov Chain Monte Carlo (MCMC) sampling methods, depending on the demands of the model, to describe the posterior distributions of model parameters (Spiegelhalter et al., 2002; Gilks

et al., 1996). I simulate three independent chains, and convergence was assessed via visual inspection of chain histories and the Gelman-Rubin statistic. Although convergence occurred quickly with these models, a burn-in period of 5,000 iterations and slight thinning rate were used to assure satisfactory sampling of posterior distributions.

Results

Scales of Variability

Significant variability in soil moisture existed at multiple spatial and temporal scales across the sampled gradient. There was a significant gradient in precipitation from Athens to Coweeta (Figure 2.2), and a high degree of correlation in precipitation among landscapes across years (correlation coefficients: 0.94 between coweeta-nancytown, 0.98 between nancytown-whitehall, and 0.98 between coweeta-whitehall). This correlation decreases however when landscapes are compared at a monthly time frame (correlation coefficients: 0.70 between coweeta-nancytown, 0.73 between nancytown-whitehall, and 0.57 between coweeta-whitehall).

I also found significant temporal variability in soil moisture, including a periodicity related to seasons. Patterns of soil moisture are quite consistent spatially at different scales. For example, Figure 2.3 shows the parallel patterns in soil moisture across grids within the Coweeta landscape, and within one of those grids, 36A. Partitioning the variance in soil moisture using an unconditional model, I found that the majority of the variation was found at the landscape scale (85%), while only 5%, 3%, and 6% was found at the grid, cell, and temporal scales (Table 2.2).

Interactions among precipitation, topography, and soil characteristics.

The relative consistency of soil moisture among grids, as well as consistent responses to precipitation events can be seen in Figure 2.4. The hierarchical regression models indicated an influence of factors at multiple spatial scales (Figure 5.3, Table 2.1). At the landscape scale,

soil moisture values are significantly related to the amount of precipitation in the previous 6 days, with the most significant effect occurring in the Whitehall landscape. However, regression analysis using longer time periods preceding the sample dates showed that precipitation appears to have an impact for a longer period of time at Coweeta. At the within-watershed level, soil moisture was most related to aspect, and microsite soil organic content and texture were significantly correlated with soil moisture values. Table 2.1 gives the exact values of the parameter estimates.

The intraclass correlation coefficients in Table 2.2 describe the effect that adding explanatory variables at each level of organization has on the variance partitioning, and the proportion of variance explained relative to the unconditional model can be calculated. Adding precipitation (in the "Time" model) explains 13% of the variation at the level-1 of the model and 54% of the variation at the landscape level. Adding cell-level covariates (sand and % organic) explains 7% of the cell variance and 26% of the grid-level variance, suggesting that soil texture differences measured at the microsite level also vary considerably among grids. Finally, adding aspect at the grid level does not reduce the variance any more, suggesting that the influence of aspect is masked by differences in soil texture.

Discussion

This study used hierarchical statistical modeling to quantify the importance of variables at each of three spatial scales for determining soil moisture across a latitudinal and environmental gradient, and partition the overall variance among these levels. The relative ranking of wet and dry sites, both among grids and within grids, remained very consistent, supporting the idea that there are site-specific characteristics that determine moisture supply and retention. The consistent effect of recent precipitation on measured soil moisture is perhaps not surprising, but suggests that patterns of precipitation at the scale of latitudinal gradients - more so than topographic or soil properties - tend to determine relative soil moisture. This link between precipitation and soil moisture is important to quantify for its

implications for the responses of terrestrial systems to changes under climate change scenarios. After accounting for the effects of precipitation, sandier soils at the microsite scale (4 m^2) and south-facing slopes at the meso-scale ($\sim$ 300 - 500 m^2) had negative impacts on soil moisture. The 'microsite' soil texture also explains much of the variation at the grid level, however, demonstrating the importance of explicitly partitioning the variance in a hierarchical model framework. Although topographic variables may be found to be 'significant' explanatory variables, their effects may be swamped out by variables at other scales.

The results of this study support the findings of other studies, although most studies have focused on individual scales or variables in isolation, so the relative contributions to soil moisture are difficult to compare. A number of studies have found support for the importance of topographic variables (van Asch et al., 2001; Lookingbill and Urban, 2004) and the combination of topography and soil storage properties (Helvey et al., 1972; Boyer et al., 1990). In a study conducted within one of the watersheds used in this study, the Coweeta basin, Yeakley et al. (1998) found evidence of both topographic and soil texture controls of soil moisture, and interestingly found that the relative impact of these factors varies with soil depth and time since rain. (Boyer et al., 1990)

Scale, prediction, and uncertainty

The centrality of soil moisture to so many ecological processes, and the likelihood of changes under climate change scenarios, make it imperative to begin building predictive models of the implications of changes in precipitation patterns. Realistic prediction requires careful attention to scale and adequate estimation of associated process variability and uncertainty (Turner et al., 1989; Clark et al., 2001). Hierarchical views of soil ecology and landscapes have been around for some time now (Allen and Starr, 1982; Coleman et al., 1992), and scale remains a central organizing principle in ecological research today (Keitt and Urban, 2005; Harte et al., 2005). This attention to scale has carried over to both conceptual and empirical studies of soil moisture and microclimate more generally (Chen et al., 1999).

Conceptual models of how hierarchical processes interact at different scales become implemented via the modeling framework chosen in each study. Western et al. (2002) describe two broad modeling approaches for addressing the scaling characteristics of soil moisture: 'behavioral' techniques based on statistical relationships at different scales, and 'process-based' techniques which typically involve deterministic distributed water balance models. Both sets of approaches have advantages and disadvantages that must be balanced given the goals of a study, and both may address scale issues in different ways. The appeal of process models for elucidating underlying physical processes determining water and biogeochemical distributions across a landscape (Abbott and Refsgaard, 1996; Grayson and Bloschl, 2000; Band et al., 2001), is tempered by limitations of intensive data requirements and computational demands that force researchers to focus on certain spatial or temporal scales (Western et al., 2002). Distributed process models can struggle with both the incorporation of scale and estimation of uncertainty (Beven, 2001).

A variety of statistical approaches have also been used to explore scale relationships of soil moisture, including wavelet analysis (Chen et al., 1999), probabilistic models (Ridolfi et al., 2003), structural equation modeling (Benayas et al., 2004), correlation analysis (moving windows of different scales) and variograms and cross-variograms (Urban et al., 2000; Lookingbill and Urban, 2004; Xu et al., 2004). Although each of these techniques provides useful properties for some applications, there are several nice properties of the hierarchical Bayes approach described here for applications such as this, including: (1) flexibility to build models that integrate complex datasets with multiple sources of information, often from unbalanced sampling designs (Clark and Bjornstad, 2004; Clark and LaDeau, 2005); (2) propagation of known process variability and measurement uncertainties to prediction uncertainty (Clark, 2005); (3) explicit consideration of explanatory variables at a range of scales simultaneously (Raudenbush and Bryk, 2002; McMahon and Diez, 2005); (4) conceptual similarities to regression analysis (Raudenbush and Bryk, 2002).

The key to the ability of this hierarchical Bayesian modeling to transfer uncertainty about parameters across levels of the model lies in the Markov chain Monte Carlo (MCMC) sampling procedure that estimates every parameter in the model as conditional on all others simultaneously (Gilks et al., 1996). The possibilities of hierarchical Bayes models for prediction offer significant potential for modeling of soil moisture. Although not performed in this study, one could map this model back onto landscape, similar to that done by others (Lookingbill and Urban, 2004). Obtaining quality soil property data over large spatial scales is difficult, but it is still informative to put into such a model, when available, for what is learned about relationships at all levels. Inclusion or exclusion of explanatory variables at one level of a multi-level model can influence the interpretation of variables at other levels (Raudenbush and Bryk, 2002). Prediction can still proceed in applications with no knowledge of the soil characteristics. The inference about unknown places will in such cases be based on data from other scales (e.g. topography) alone, while incorporating the uncertainty from the unknown, but important, microsite soil characteristics.

A useful approach for many questions may in fact be an integration of statistical models and process-based models, and an important role for the hierarchical framework described in this paper is identification of key explanatory variables and scales that are most important to include. And by partitioning variance of a full model into different components, we can learn how important variables are at different scales and weigh their inclusion against the difficulties of obtaining them.

Conclusions

Ecologists, and perhaps landscape ecologists in particular, are very accustomed to thinking about processes as scale-dependent. The distribution of soil moisture is a critical process for many biological processes in ecological systems and is determined by factors at multiple scales. Hierarchical Bayesian models are now providing a useful framework for integrating information from different spatial scales to build predictive models with associated estimates

of uncertainty (Banerjee et al., 2004; Gelman et al., 2004). This study has provided an example of the utility of using models that reflect the hierarchical process of interest.

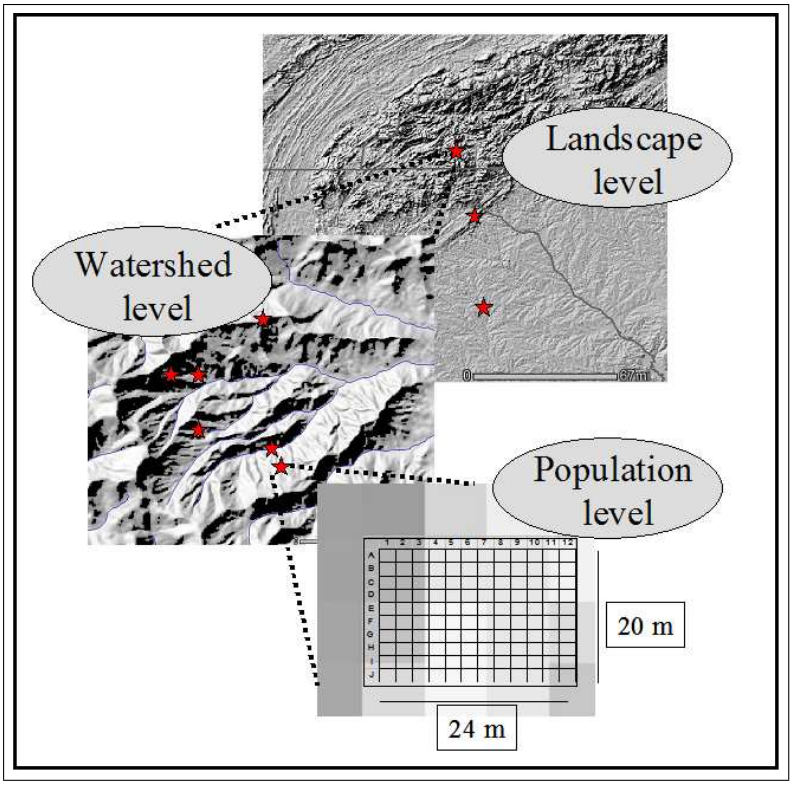
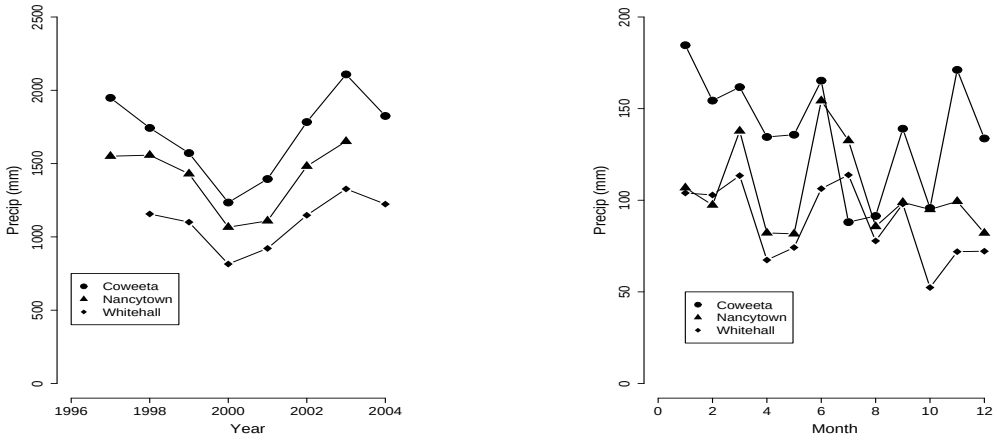


Figure 2.1: **Hierarchical Study Design.** A hierarchial sampling design was used to explicitly investigate scale-specific controls on soil moisture. At the largest scale, three landscapes were sampled across a gradient stretching from the Piedmont of Georgia to the southern Appalachian mountains. Within each landscape 4 to 6 population-level sites (grids) were selected to represent a range of topographic conditions. Each site was then divided into 2 x 2 meter cells, sixteen of which were regularly monitored for soil moisture and characterized for soil properties.



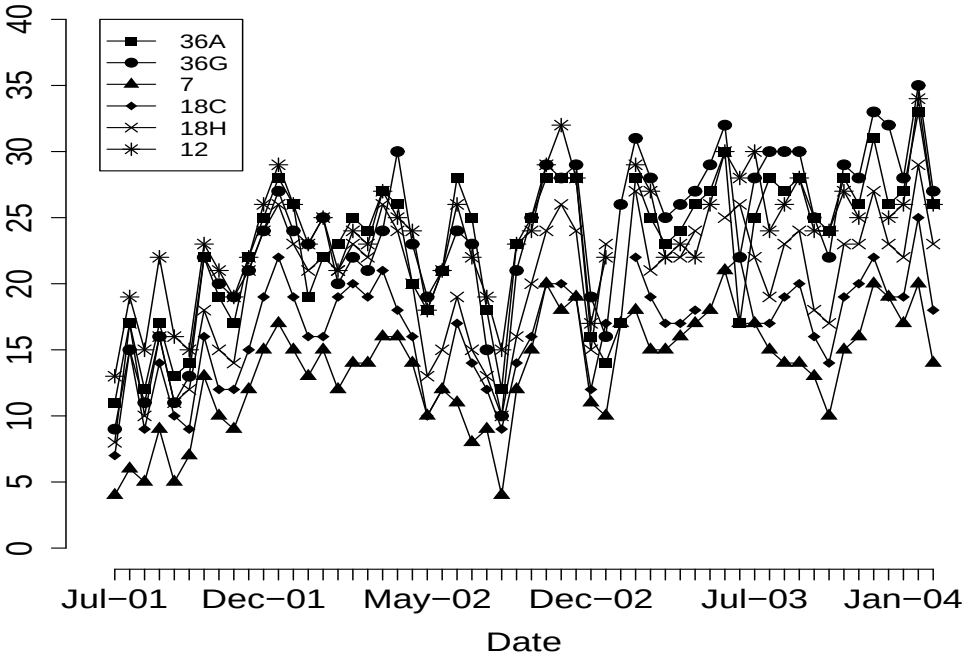
(a) Annual Totals at Landscape level

(b) Monthly precipitation averages during study

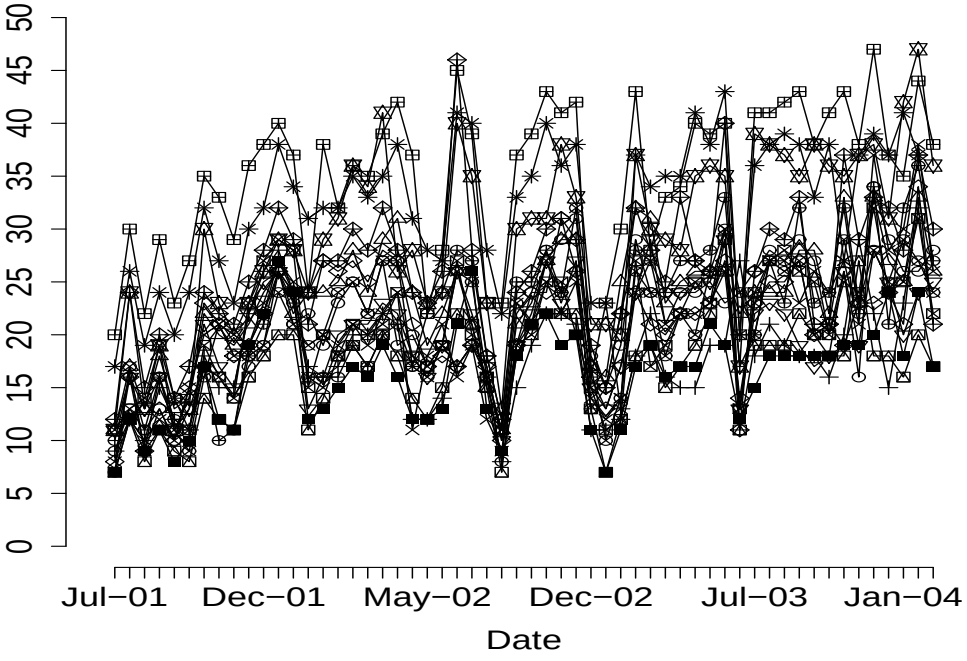
Figure 2.2: **Large-scale precipitation patterns.** At both yearly (a) and monthly (b) time scales, there are consistent patterns of precipitation differences at the largest spatial scale of the study

Table 2.1: **Regression Coefficient Estimates:** Given are summaries of the posterior distributions for model parameters. In bold are coefficients with posteriors not substantially overlapping zero.

Parameter	Mean	SD	2.50%	97.50%
Microsite				
sand	**-0.27**	**0.06**	**-0.40**	**-0.15**
% Organic	**-0.09**	**0.05**	**-0.19**	**-0.002**
Summer light (cell)	0.04	0.04	-0.05	0.12
Fall light (cell)	-0.03	0.05	-0.13	0.07
Population				
RSPWS	0.35	0.35	-0.34	1.05
TCI	-0.02	0.17	-0.35	0.31
Aspect	**0.30**	**0.19**	**-0.07**	**0.68**
Elevation	0.34	0.60	-0.76	1.69
Slope	-0.18	0.40	-0.98	0.63
Summer light (grid)	0.25	0.48	-0.71	1.20
Fall light (grid)	0.13	0.19	-0.25	0.51
Landscape				
Cow Precip	**0.27**	**0.02**	**0.23**	**0.30**
Nancy Precip	**0.20**	**0.02**	**0.17**	**0.24**
WH Precip	**0.67**	**0.04**	**0.60**	**0.74**

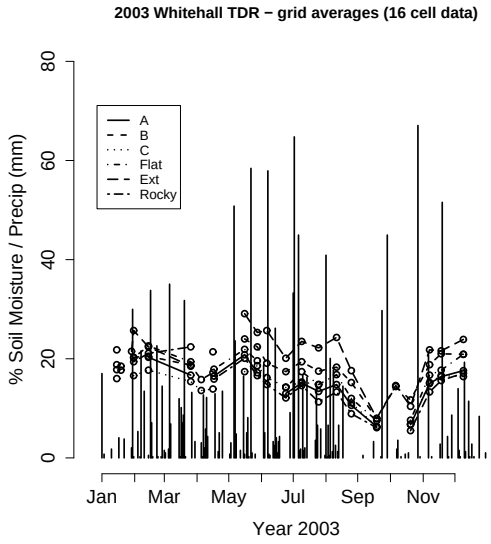


(a) Grid-level patterns

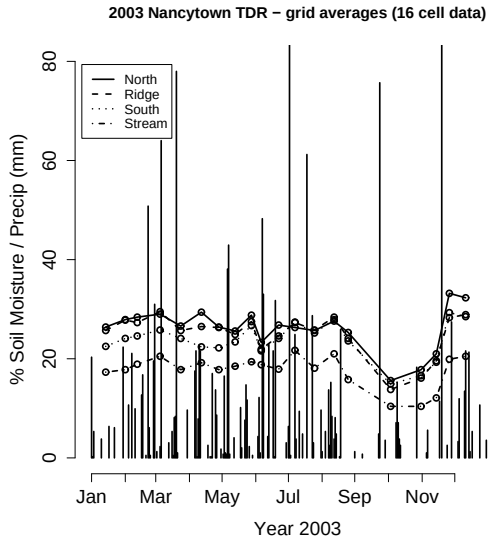


(b) Within-grid: 16 cells

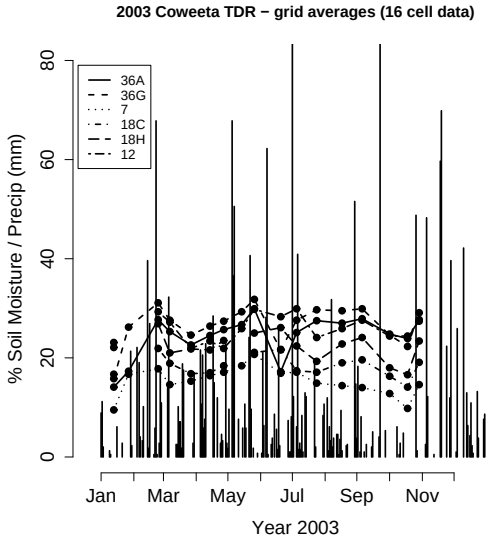
Figure 2.3: **Spatial Patterns consistent across spatial scales** Patterns of measured soil moisture remain consistent across spatial scales, from (a) the among population soil moisture values in the Coweeta landscape, and (b) for example, within the 16 measured cells in one of the study grids at Coweeta



(a) Whitehall



(b) Nancytown



(c) Coweeta

Figure 2.4: **Relationship between precipitation events and soil moisture** Histograms show the rainfall events throughout the year, with mm on the y-axis. Overlaid lines represent the biweekly grid-level average of 16 TDR (percent soil moisture) readings on each grid. Data is shown for the three different landscapes, from south to north: (a) Whitehall forest, Athens, GA; (b) Nancytown forest, Cornelia, GA; and (c) Coweeta forest, Otto, NC

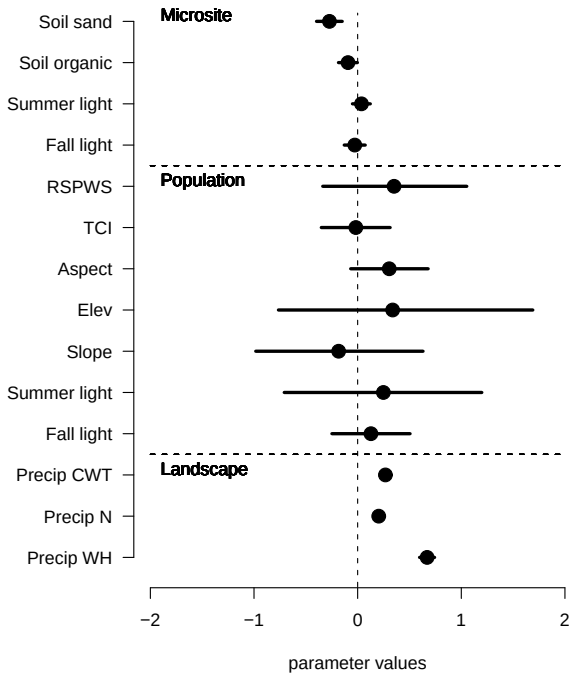


Figure 2.5: **Coefficient Estimates:** Lines represent 95% credible intervals of posterior distributions of regression coefficients. Those not overlapping zero may be considered 'significant'

Table 2.2: **Variance partitioning:** Reported are the variance components for each level of the model, the intra-class correlation coefficients, and the proportion of variance explained by adding covariates.

Level of model:	Time	Cell	Grid	Landscape
Variance (σ^2)				
Unconditional	0.43	0.20	0.36	5.70
Time	0.37	0.20	0.36	2.6
Time and cell	0.37	0.18	0.26	2.09
Time, cell, grid	0.37	0.18	0.27	2.79
Intra-class correlation coefficients				
Unconditional	0.06	0.03	0.05	0.85
Time	0.11	0.06	0.10	0.74
Time and cell	0.13	0.06	0.09	0.72
Time, cell, grid	0.10	0.05	0.07	0.77
Proportion explained relative to unconditional				
Time	0.13	-0.02	0.00	0.54
Time and cell	0.13	0.07	0.26	0.63
Time, cell, grid	0.13	0.07	0.24	0.51

Chapter 3

Spatial Patterns of Symbiotic Germination in a Terrestrial Orchid [1]

[1] Diez, J.M. To be Submitted to *Oecologia*

ABSTRACT

The recruitment stage of many organisms' life histories can be critical in determining population dynamics, as juveniles often experience high mortality rates. The offspring of plants are subject to the abiotic and biotic environments where they happen to land after dispersal, with little recourse for moving to another habitat. Terrestrial orchids differ from most vascular plants in their obligate dependence on mycorrhizal fungi in order to reach photosynthetic stage. Despite this important potential bottleneck on orchid recruitment, very little is known about the factors controlling the distribution of the fungi or how fungal distributions may constrain orchid recruitment and distributions. In this study, I use seed introduction experiments to test for relationships between symbiotic germination success and both proximity to adult orchids, and soil abiotic conditions, for the terrestrial orchid *Goodyera pubescens*. By conducting these experiments in natural populations of individually mapped plants whose growth, recruitment and reproduction have been monitored for five years, I am also able to place the patterns of symbiotic germination rates in the context of observed, longer-term demography across populations. I find strong evidence of decreasing germination success as seeds get further from adult plants, and increased success with increasing soil moisture and organic content in areas greater than 1 meter from adults. Moreover, the demographic monitoring shows that patterns of germination success are highly correlated with observed recruitment patterns at the population level. By linking the hypothesis testing in this study to predictive model building, an effort is made to begin coupling our growing understanding of the basic biology of these species with probabilistic models that can be used for robust prediction.

Introduction

The recruitment of new individuals into a population can be an important process for determining population dynamics and community structure (HilleRisLambers et al., 2002).

Restrictions on fecundity and dispersal can limit the number of propagules available for subsequent development, and juveniles often experience high mortality rates. Thus, an understanding of patterns of recruitment, particularly when linked to underlying relationships with abiotic factors, is important for inferring many aspects of population dynamics, including population spread rates and responses to changing climate and land use (Clark et al., 1999; Silvertown et al., 1993; Uriarte et al., 2005; Munzbergova and Herben, 2005).

Although forest herbs constitute most of the vascular plant diversity in eastern deciduous forests, little is known about their long-term population dynamics. In a recent review of woodland herb ecology, Whigham (2004) highlights the fact that although much is known about the basic biology of many of these species, there have been few detailed studies that allow the prediction of population dynamics over larger spatial and temporal scales. One step toward building an understanding sufficient for predicting population dynamics is to design studies that link important demographic stages to underlying driving variables and explicitly quantify the uncertainties of predictions (Franklin et al., 2000; Clark, 2003).

Most, if not all, perennial forest herbs are either obligate or facultative associates with mycorrhizal fungi (Whigham, 2004). While most mycorrhizal relationships are thought to be mutualistic, with the fungus providing phosphorus or nitrogen and the plant providing carbohydrates derived from photosynthesis, there appear to be many variations on this relationship, both between taxa and for the same taxa in different environments (van der Heijden and Sanders, 2002). While the majority are likely associated with arbuscular mycorrhizal (AM) fungi from the Glomales order (Zygomycotina), orchids associate with saprotrophic basidiomycete genera traditionally placed in the form-genus Rhizoctonia. The symbiotic relationships of non-photosynthetic orchids ("myco-heterotrophs") have been found to be quite specific and also connected to neighboring ectomycorrhizal trees (Taylor and Bruns, 1997, 1999). Although it is traditionally assumed that autotrophic species would associate with a wider group of symbionts, recent molecular work has provided surprising evidence of relative specificity even among the autotrophic orchids (McCormick et al., 2004). The

fungal symbionts vary across species, but appear to be more saprotrophic than ectomycorrhizal (Rasmussen, 2002). The fungi associated with the current study species, *Goodyera pubescens*, appear to be one or two species within the predominately saprotrophic genus *Tulasnella* (McCormick et al., 2004).

The orchids' obligatory dependence on the fungi in order to germinate and grow to photosynthetic stage sets them apart from most other photosynthetic plants. Although some orchid species can be induced to germinate on artificially high nutrient media, in nature orchids are dependent on fungi for early nutrition (Rasmussen and Whigham, 1998*b*). Adult plants typically remain heavily colonized by fungi, although the importance of the symbiosis as adults is less certain. Recent stable isotope evidence has documented that even autotrophic orchids gain carbon and nitrogen from their fungal symbionts, but all attempts to document a transfer of carbon from the orchid to the fungus have proven unsuccessful (Gebauer and Meyer, 2003; Smith and Read, 1997). This suggests the interesting possibility of a parasitism of the fungi by the plants at least through their non-photosynthetic seedling stage, although a non-trophic benefit to the fungi may also be possible.

The obligate nature of the relationship suggests a potential for a strong influence on orchid recruitment, however very little is known about the distribution of these fungi in the environment. Previous studies have suggested that the mycorrhizal fungi of another terrestrial orchid, *Tipularia discolor*, are associated with decayed logs (Rasmussen and Whigham, 1998*a*), and a study of an Austrailian orchid, *Caladenia arenicola*, found increased germination success with proximity to adult orchids and correlated with some soil factors, such as potassium levels (Batty et al., 2001). While non-photosynthetic, and even some photosynthetic orchids can be associated with ecto-mycorrhizal fungi, and thus expected to have increased recruitment near host trees, the *Tulasnella* associated with *G. pubescens* are saprotrophic. Therefore, factors that influence the abundance of their saprotrophic fungi, such as substrate availability and suitable abiotic conditions, may be important for their recruitment.

In this study I use a series of seed introduction experiments to explore patterns of symbiotic germination of the terrestrial orchid *Goodyera pubescens*. Specifically, I use experiments along with concurrent abiotic and demographic monitoring, to test three hypotheses:
(1) I test the hypothesis that symbiotic germination of the orchid will be more likely in close proximity to adult plants because the same fungi needed for germination are also used by adults. (2) I test the hypothesis that patterns of successful germination are related to specific edaphic factors that contribute to the site suitability for saprotrophic fungi, namely soil organic content, pH and moisture. Finally, (3) I use concurrent demographic monitoring of these populations to test the hypothesis that differences in the probability of symbiotic germination translate into differences in seedling recruitment.

A general goal of this study was to begin to bridge the gap between what is known about the unique biology of these plants, and the implications for population dynamics. In the course of testing these specific hypotheses, I use hierarchical Bayes methods to show how hypothesis testing can be usefully placed within the context of building predictive models that translate experimental data into predictions across observed and potentially unobserved conditions. A critical component of realistic prediction, facilitated by the hierarchical Bayes methods, is proper incorporation of associated process variability and parameter uncertainty. I demonstrate how these methods are useful for understanding how the mycorrhizal relationships of terrestrial orchids may constrain patterns of seed germination across environmental gradients.

Materials and Methods

Study Species

Goodyera pubescens is a terrestrial, evergreen, clonal orchid distributed throughout mixed forests of eastern North America, from Florida, west to Arkansas, north and east into Canada. The main growing period for roots and leaves is from summer to fall, although the mycotrophic period during which nutrition is gained from the fungi may be much longer

(Rasmussen, 1995). Both roots and leaves can be long-lived, and are replaced on a continual basis - not in a single flush (Rasmussen and Whigham, 2002). Flowers develop in July and August, which if pollinated form capsules containing thousands of minute seeds (< 2 mm length, < 0.5 mm width). Gravity and wind dispersal of the seeds begins with dehiscence of the capsules in September, but can be spread over several months, with some seeds occasionally still remaining in the capsules by early Spring (J.D. personal obs.). Like all orchids, *G. pubescens'* seeds lack significant nutrient reserves and are therefore dependent upon colonization by the appropriate fungi for the transfer of carbohydrate energy, water and mineral nutrition at least until reaching photosynthetic stage. Upon wetting, seeds imbibe and if encountering a suitable fungus a symbiotic protocorm is formed, which can develop into a more advanced corm structure and in two years a seedling. If not colonized by fungi, seeds do not survive, so there is no persistent seed bank. For the purposes of this report, I refer to protocorm formation in the first year as germination although some may technically refer to the imbibing as germination. In the context of recruitment for plant population dynamics, only the symbiotic germination will contribute individuals that can continue to grow.

The fungi associated with *G. pubescens* have been placed in the genus *Tulasnella* by McCormick et al. (2004) using DNA sequencing of the ITS region. While difficult to make species assignments with these fungi, sequence data suggest isolates from *G. pubescens* from Georgia to Michigan, including samples from the current study's populations, most closely align with one or two species of *Tulasnella*, with *T. bifrons* being the closest match in GenBank (McCormick et al., 2004, 2005). These saprotrophic fungi use dead leaves and decayed wood in the soil as a substrate for growth, and are capable of decomposing cellulose but not lignin (Rasmussen, 1995). Reproduction can be clonal via hyphal fragmentation or sexually via difficult to observe resupinate (flat) fruiting bodies.

Study Design

Study sites, as part of a larger study of the demography of forest herbs, were selected across a geographic gradient of approximately 120 km from the Piedmont of Georgia to the Southern Appalachian Mountains. At the largest scale, referred to as landscapes, I used three areas along this gradient: Whitehall Forest in Athens-Clarke County, GA (33°92' N latitude, 150 - 240 m elevation), the Nancytown area of northern Georgia, Habersham County (34°31' N latitude, 315 - 450 m elevation), and the Coweeta Hydrologic Laboratory in Macon County, North Carolina (35°03' N latitude, 750 - 1,500 m elevation). Sixteen study grids were established in relatively mature ($\sim$ 80 yrs old) deciduous forest, and chosen to reflect the range of elevation, temperature, and precipitation conditions experienced by the forbs under study.

Seeds were collected from wild adult plants near the study sites within the Coweeta basin. Seed packets, using a modified design from Rasmussen and Whigham (1993), were constructed by placing approximately 150 seeds within a square of 53 μm plankton netting, enclosed within a Polaroid slide mount. In late Fall, these seed packets were placed just under the top soil layer, marked with a flag, and retrieved the following Summer. Protocorms were identified in the field using a hand lens or field-worthy dissecting scope. Seed packets were placed in the field in two experimental designs.

Experiment 1 - Distance relationships:

To test for the effect of proximity to adult plants on protocorm formation, seed packets were placed at 4 distances from adult plants: 5, 10, 20, and 100 cm. Twenty-eight separate adult plants were selected from 5 of the study grids. Three transects of seed packets were placed around each adult plant (Fig. 3.1). The criteria used for selection were that the adult plants contain ≥ 6 leaves and the locations were such that none of the seed packets ended up within 1 meter of another *Goodyera* plant. In some cases, small patches of adult plants were used in place of a single adult, with the transects placed to start on the outside of the patch. All together, 336 seed packets were used, with 84 at each distance class.

Experiment 2 - Abiotic-relationships:

In addition to the packets placed at defined distances to adult plants, packets were introduced at 16 locations on each of 16 study grids where abiotic data has been collected, including soil moisture, organic content, carbon:nitrogen, NO_3 and pH. Packets were placed around the area of abiotic sampling in a square design (Fig. 3.2). The same sites were used in two consecutive years, and the data were pooled for analysis to increase samples size, which is justified given that the explanatory variables of interest tend to be more spatially variable than temporally. Care was taken to avoid placement of packets within 1 meter of adult *G. pubescens*.

Soil sampling and analysis

A handheld Soil Water Content Measurement System from Hydrosense was used to measure percent soil moisture in the top 12 cm of soil at the same 16 cells in each grid every two weeks between the years 2003-2004. For the purposes of these analyses, these readings were averaged within each cell to give a measure of relative soil moisture over the course of a year. The spatial patterns of relative soil moisture remain fairly consistent over time (Diez, in prep).

For characterization of the edaphic environment, three 12 cm soil cores were taken within a 0.5 m radius from the center of each of the 16 cells within each of the 16 study grids. The three cores from each cell were bulked and sifted through a 2 mm sieve, for a total of 256 samples. In a few circumstances, course rocky topsoil prohibited three complete 12 cm cores and 4 more shallow cores within the 0.5 m radius were used. Total carbon, nitrogen, and carbon:nitrogen ratios were determined using dry Micro-Dumas combustion and analysis by gas chromatography. A 1:1 suspension of fresh soil in deionized water was measured for pH. Soil organic content was determined by percent weight lost after combustion in a muffle oven. Nitrate and phosphate availability were measured using in situ anion-exchange resin bags. Resin bags were placed at a depth of 5 cm under the soil surface and retrieved after 1

month in the field. In the lab, ions adhered to the resin were extracted using KCl, and NO_3 and PO_4 ion concentrations of the solution were determined via continuous flow colorimetry. Soil texture was determined using the hydrometer method (Gee and Bauder, 1986). Because percent sand and percent clay were highly correlated, percent sand was used as the index of soil texture in all analyses.

Statistical Analysis

To assess the effect of proximity to adult plants on germination, I used the R computing package (R Development Core Team, 2005) to fit a binomial generalized linear model with a logit link function to assess whether distance has a significant effect on the probability of germination. Then, to infer the shape of the relationship between germination and distance, I fit four different possible models to the distance data using Bayesian nonlinear model fitting and the Deviance Information Criteria (DIC) to select the best model. The DIC is an information criteria developed by Spiegelhalter et al. (2002) and like AIC is a function of model fit penalized for increasing model complexity. The models consider each packet as one trial of a Binomial process within each cell, such that

$$Y_d \sim Binomial(\phi_d, n_d) \tag{3.1}$$

where Y_d is the raw number of seed packets at distance d that contained protocorms, ϕ_d is the probability of successful germination at distance d, and n_d is the number of seed packets, or 'trials' at that distance. The probability of germination ϕ_d is then modeled with several competing models chosen to reflect a range of possible forms that could realistically describe the relationship between distance and germination success:

1) negative exponential

$$\phi_d = \alpha e^{-\beta d} \tag{3.2}$$

2) logistic

$$\phi_d = 1 - \left(\frac{e^{\beta d}}{1 + e^{\beta d}} \right)^m \tag{3.3}$$

3) mixed exponential and power function

$$\phi_d = \alpha e^{-\beta d} + \tau d^{-\gamma} \tag{3.4}$$

4) uniform (null)

$$\phi_d = \mu \tag{3.5}$$

To assess the effect of soil abiotic variables on germination success, a step-wise logistic regression approach was used to select important explanatory variables from the handful that were hypothesized to be important for determining suitability of a site for germination. A hierarchical Bayesian framework was used for more robust inference and quantification of uncertainty given an unbalanced sampling design. As discussed later, the Bayesian models also allow robust prediction at unobserved environmental states, while incorporating uncertainty from each level of the model. With data at the individual packet level, I consider the germination of seeds in each packet as a Binomial process within each cell, such that

$$Y_{ij} \sim Binomial(\phi_{ij}, N_{ij}), \tag{3.6}$$

where N_{ij} is the number of packets placed in a given cell i and landscape j and Y_{ij} is the number of packets in that cell in which at least one protocorm was found. The probability of germination, ϕ_{ij}, is modeled as a function of cell-level abiotic covariates via the logit link function as follows:

$$logit(\phi_{ij}) = log\left(\frac{\phi_{ij}}{1 - \phi_{ij}} \right) = \alpha_j + \beta_j X_{ij} \tag{3.7}$$

The α_j are landscape-level intercepts, β_j is a vector of regression coefficients, and X_{ij} is the design matrix containing covariates of interest. Following the logic of hierarchical modeling, the landscape-level intercepts α_j are modeled as random effects drawn from a global distribution,

$$\alpha_j \sim Normal(\mu, \tau), \tag{3.8}$$

with the global mean μ and variance τ given non-informative hyperpriors $\mu \sim N(0, 1000)$ and $\tau \sim InverseGamma(.01, .01)$. Likewise, the effects of abiotic conditions within each landscape are drawn from a global distribution: $\beta_j \sim Normal(\mu^\beta, \tau^\beta)$, again with non-informative hyperpriors describing the global mean and variance distributions. The hierarchical formulation allows for landscape-level differences due to unmeasured site conditions, but a pooling of data across sites to reflect a common process across sites and allow greater inferential power (Gelman et al., 2004).

Prediction

The output of Bayesian models is in the form of posterior distributions which are interpreted as direct probabilities, given the data, that the parameters of interest take on the given values. One significant benefit to the Bayesian framework is the ability to use the estimated posterior distributions directly for predictions of interest related to those parameters. Because the estimated posteriors contain all information about the uncertainty of our parameter estimates, subsequent predictions made within this framework also conveniently reflect those uncertainties. I use this property of the models to construct predictive surfaces of germination probabilities across a range of distances from adult plants, and across a range of abiotic conditions I expect the species to experience in this region.

All Bayesian models were fit using WinBugs 1.4, which uses Markov chain Monte Carlo (MCMC) sampling methods to characterize the posterior distributions of model parameters (Spiegelhalter and Best, 2000; Gilks et al., 1996). Convergence of three independent chains was assessed via the Gelman-Rubin statistic, and sufficient burn-in periods and thinning rates were used to assure satisfactory sampling of the posterior distributions.

Results

Distance

I found evidence for a sharp decline in germination probability with distance within 1 meter from adult plants. The maximum likelihood estimates of the probabilities of germination at 4 distances within this 1 meter from adults showed a declining probability with distance (Fig. 3.3), which when fit with a binomial generalized linear model showed a significant effect of distance on probability of germination (coefficient $= -0.017; p = 0.0187$).

Out of the four models fit to the distance data, the best fitting model as judged by the DIC was the mixed exponential and power function (Table 3.1). The selection of this model over the others, and particularly over the uniform model, is another way to confirm the rejection of the null hypothesis of no effect of distance on germination.

The parameter estimates of the mixture model were used within the MCMC routine to construct a kernel of predictions over the range of distances from 1 to 100 centimeters (Figure 3.4). While probabilities are low at all distances, the model predicts a decline in germination probability as a function of distance. One way to summarize the posterior predictive distributions from the Bayesian analysis is with 95% credible intervals plotted as dashed lines in the figure. If one were interested in using predictions for subsequent models of population dynamics, the entire posterior distributions could be used.

Abiotic factors

Symbiotic germination was positively correlated with soil organic content and average soil moisture, and negatively correlated with pH (Figure 5.3). I again report the 95% credible intervals of the posterior distributions for each parameter as a convenient way to view the probability that a parameter is different from zero. Because they are direct probabilities of parameter values, one could integrate the portion of the posterior distribution that is above or below zero for a direct probability that the value is greater than or less than zero. I found

that the 95% intervals of the relationship with percent organic content and soil moisture are not overlapping zero only in the Coweeta landscape but highly positively skewed in the other landscapes. Likewise, the relationship with pH is clearly negative in the Whitehall landscape, strongly skewed negative in Nancytown and broadly overlapping zero in Coweeta.

Given the significance of soil moisture and organic content, predictive models were constructed using those two variables, and germination probabilities were predicted across a range of abiotic values that could be expected across the region (Fig. 3.6). These distributions were estimated directly within the model MCMC iterations, thus simultaneously incorporating associated prediction uncertainty. Although only the mean predicted response is shown in the figures (for clarity of presentation), the full posterior distribution could be used to characterize the uncertainty of those predictions across the range of abiotic conditions.

I also found a significant positive relationship between seed germination rates and observed demography within populations (Figure 3.7). The Pearson's product-moment correlation was 0.87 with a ($p \ll .001$). Six of the study sites had no adult *Goodyera*, and thus zero probability of producing flowers and seedlings. These sites also had no successful germination in seed packets. Two other sites with adult plants had zero seedling production but did have some successful protocorm formation in seed packets.

Discussion

This study has quantified a strong pattern of declining probability of symbiotic germination within a meter from adult plants, and increased germination in sites with higher soil moisture and organic content and lower pH. The estimated effects of the abiotic environment vary across landscapes, but the direction of the effect is consistent, suggesting a general pattern applicable to the wider region.

Implications for plant population dynamics

Much of the research performed on patterns of plant recruitment as a function of distance from adults has been with tree species in the context of testing the Janzen-Connell hypothesis (Janzen, 1970). This hypothesis suggests that negative distance and density-dependent recruitment (arising from a number of potential mechanisms, such as accumulated species-specific herbivore or pathogen loads near parent plants) can contribute to the maintenance of species diversity. This hypothesis continues being tested, but evidence for negative density-dependent recruitment has been found in a number of studies of tree species (e.g. Augspurger and Kelly, 1984; Packer and Clay, 2000; Tomita et al., 2002; Packer and Clay, 2003).

Previous studies with herbs or orchids are fewer, and have reported mixed results with regard to the importance of proximity to adult for recruitment. While some studies have found a positive association between germination and adult locations for myco-heterotrophic orchids (McKendrick et al., 2000*b*, 2002; Leake et al., 2004), and at least one study with photosynthetic orchids (Batty et al., 2001), others have found no relationship between protocorm formation and distance to adults, even within a similar 1 meter spatial scale used in this study (Masuhara and Katsuya, 1994; McKendrick et al., 2000*a*).

The declining probability of germination with distance from adults found in this study suggests a positive density dependence for recruitment of these orchids. While germination is possible further away from adults, and increases with greater moisture and organic content of the soil, the probabilities are still significantly lower than within 10 centimeters of adults. The implications of these patterns for population dynamics will depend on subsequent protocorm and seedling survival rates. If there is negative density dependence in growth rates at any stage of the early plant's growth, then these positive density-dependent germination patterns could be neutralized.

Although the use of 4 discrete distance classes in this study has limitations for fitting a continuous recruitment function, the approach is justified in this case for two reasons. One, the goal was not to make predictions at larger scales than for which there are data.

This study used model-fitting and model-selection as a test of whether there is an effect of distance at all, and then to infer what we can about the form of the very local probability decay within the active first meter from adult plants. Second, analysis within a model-building framework provides a foundation upon which future studies can build. Selection of the mixed model is conditional on the available data in this study, and this understanding may be updated by further studies, including data collection from different sites and over a wider range of distances. Whether formally through a Bayesian updating framework or more informal incorporation into experimental designs and/or choice of models to evaluate, the model selection in this study can help inform future research.

The demographic monitoring of the plants has helped to put the seed packet experiments in the context of how these early germination rates may translate to population-level recruitment rates. The high correlation between seed germination rates and seedling recruitment rates may not be surprising given that seeds must germinate in order to eventually produce seedlings, but it suggests that the presence of suitable conditions, and perhaps most importantly the appropriate fungi, may act as a bottleneck on seedling recruitment. The demographic surveys also revealed that seedlings were much more commonly found in close proximity to adult plants (Diez, unpublished data). Even when accounting for decreased detection probabilities further from adult plants, it remains likely that seedling recruitment is most common within existing patches of adults.

The lack of germination in sites that had no adult *G. pubescens* is consistent with the idea that population establishment at some sites is limited at this early stage of symbiotic protocorm formation. Also, a couple of sites with adult plants and some protocorm formation in seed packets, have zero observed natural recruitment, suggesting the existence of factors that are limiting at seed production and/or seedling stages.

Fungal ecology

Because successful symbiotic germination depends on both the presence of the fungus and suitability of abiotic conditions, experimental seed packet results may best be interpreted as a measure of inoculum potential, which reflects both the abundance of the fungi and the suitability of conditions for growth and colonization. As such, seed packet experiments are a useful 'phytometer' type of method for mycorrhizal research, but are likely to underestimate the actual distribution of fungi in the soil.

However, the increased probability of germination with increased soil organic matter and moisture suggests that the suitability of these sites is higher for these saprotrophic *Tulasnella*. The measure of organic content of the soil used in this study is a coarse measure of resource availability for these fungi, but may serve as a proxy for the specific conditions necessary. The fungi within the genus *Tulasnella* are able to utilize cellulose, but not lignin, and in lab studies perform well on a range of raw wood and leaf substrates, as well as other basic media (McCormick et al., 2005, Diez personal observation). But inferring fine scale habitat suitability for fungi in natural systems is a challenge, in part due to difficulties associated with following individual fungal mycelial growth in a natural setting. However, a number of laboratory studies utilizing non-sterile soils have shown the tendency for the growth and density of saprotrophic taxa to track soil resources such as organic matter, pH, nutrient status, or moisture (reviewed by Cairney, 2005). Forest soils can be very heterogeneous both abiotically (Ettema and Wardle, 2002; Fraterrigo et al., 2005, Diez and Pulliam unpublished data) and biotically (Ettema et al., 1998, 2000), so it is possible that substrate availability for the fungi also varies on very local scales. This study's findings of links between germination success and abiotic conditions are promising for translating controlled laboratory studies into field-based ecological realism.

An intriguing alternative explanation to this 'habitat suitability' hypothesis, but not mutually-exclusive, is that declining abundance away from adult plants may reflect decreased fungal access to those sites. The frequency and abundance with which these fungi produce

spores in nature are unknown, as well as their potential to spread clonally into new micro-habitats. Because adult plants remain heavily colonized by fungi, and patches of plants often contain the same fungal genet (Diez and McCormick, in prep), it is possible that the clonal spread of plants across sites provides a significant mode of spread for the fungi as well. The costs and benefits of mycorrhizal association for the fungi are largely unexplored for saprotrophic fungi. Although no benefit has ever been found for these fungi, the higher densities near adult plants could reflect some benefit conferred on the fungi by the presence of the plants. More targeted experiments are clearly needed to separate the effects of potentially overlapping habitat suitabilities.

Large genet sizes found in other mycorrhizal fungal species, primarily ectomycorrhizal species, may contribute to a vision of large contiguous mycelial networks (e.g. Dahlberg, 1997). As many of the ectomycorrhizal species are obligately associated with plants, it might be reasonable to presume that saprotrophic species could be even more widely distributed. It appears, however, that a more heterogeneous distribution may be more likely for these fungi at least in this part of their range. Molecular studies have begun answering many previously unanswerable questions about the distribution of fungal species in nature (Fierer et al., 2005; O'Brien et al., 2005), but fungal biogeography from local to global scales is still largely unknown (Fitter, 2005). However, direct DNA extraction from the soil may actually overestimate the abundance of viable fungi, from the plant's perspective, as fungi are known to exhibit periods of fungistasis in which growth is stopped or limited due to any number of chemical constraints or inhibitions (Lockwood, 1977). As molecular techniques are further developed, important extensions to this work will involve quantitative assessments of the abundance of fungi in the soil, as opposed to simply occurrence, and use of molecular and 'phytometer' methods in conjunction to illuminate how microbial distributions and ecological functions interact.

It is also probable that soil biota could also be influencing plant distributions via effects on mycelial growth (Gange and Brown, 2003). For example, collembola fungal grazers may

play a role under some conditions in shaping the growth patterns of ectomycorrhizal (Ek et al., 1994) and saprotrophic fungi (Kampichler et al., 2004). Fungal competition is also well known to limit the growth of some species at local scales (e.g. Boddy, 2000), with some evidence that interactions are mediated by substrate composition (Wells and Boddy, 2002), but the dynamics of fungal-fungal interactions within the complex 3-dimensional matrix of intact forest soils is not well understood.

Conclusions

Plant ecologists are increasingly incorporating below-ground organisms and processes in their studies of above-ground dynamics. Methodological and conceptual advances in soil ecology and mycorrhizal research are moving these fields along rapidly, and will further facilitate our understanding of linkages between belowground organisms and aboveground dynamics. This study describes within-population patterns of symbiotic germination probabilities, and how these translate into population-level differences in seedling recruitment. These patterns are described using methods that allow full quantification of the variability in the system and uncertainty of our predictions. As ecologists learn more about the basic ecology of above and belowground interactions, incorporation of observational and experimental data into a framework that allows realistic prediction over unobserved states will facilitate meta-analysis and anticipation of species responses to environmental change.

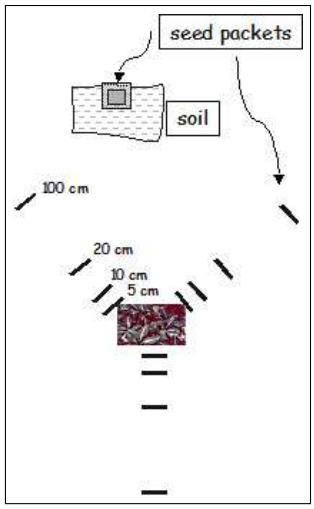


Figure 3.1: **Experiment 1, Basic seed packet design.** Seed packets were placed at four distance classes from clusters of adult plants.

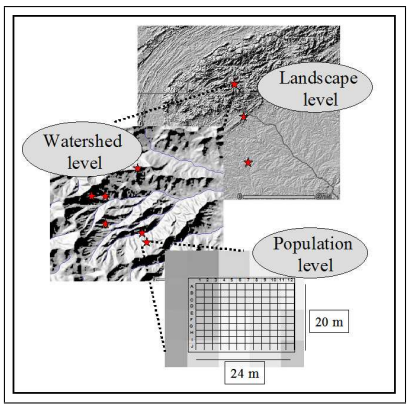


Figure 3.2: **Experiment 2, Basic seed packet design.** Eight seed packets were placed at each of 16 cells within all 16 study grids within .5 meters of the abiotic sampling.

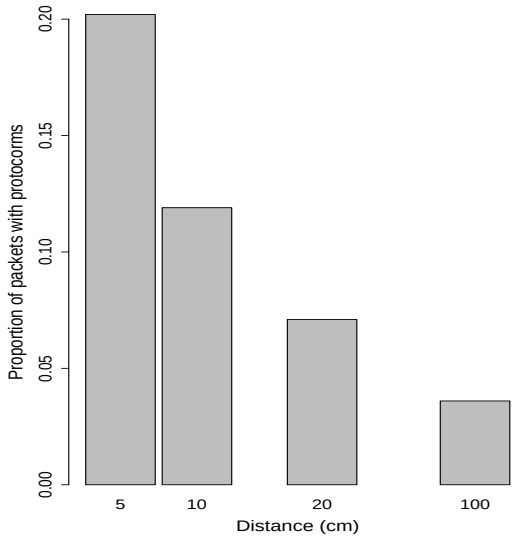


Figure 3.3: **Goodyera germination probabilities.** Shown is a declining trend of fewer successful seed germinations at further distance classes from adult plants.

Table 3.1: **Model fitting:** Model selection for germination success versus distance. Models were fit in WinBugs using 40,000 MCMC iterations, checking for convergence using Gelman-Rubin statistics. The DIC is a measure of model fit, penalized for model complexity, with lower scores preferable. The 'best' model given these data is the mixed exponential and power function.

Model	Dbar	Dhat	pD	DIC
Logistic	28.82	23.29	5.54	34.36
Neg. Exponential	44.51	43.57	.94	45.45
Uniform	33.09	32.13	.96	34.04
Mixed exp/power	**20.12**	**19.58**	**.54**	**20.67**

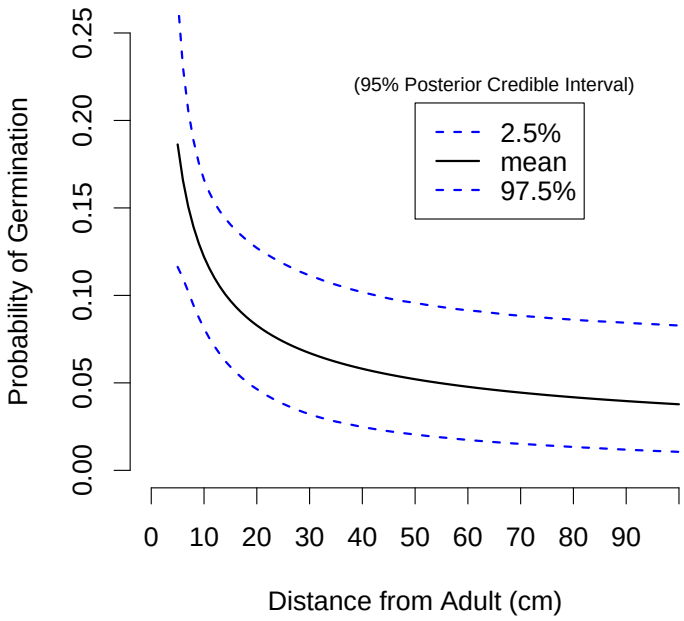


Figure 3.4: **Predicted germination probabilities as a function of distance.** Using estimated parameters of the most well-supported model (the mixed model), predictions are made within the MCMC statistical routine across the entire first meter from adult plants. Dotted lines indicate the 95% credible interval for the model predictions.

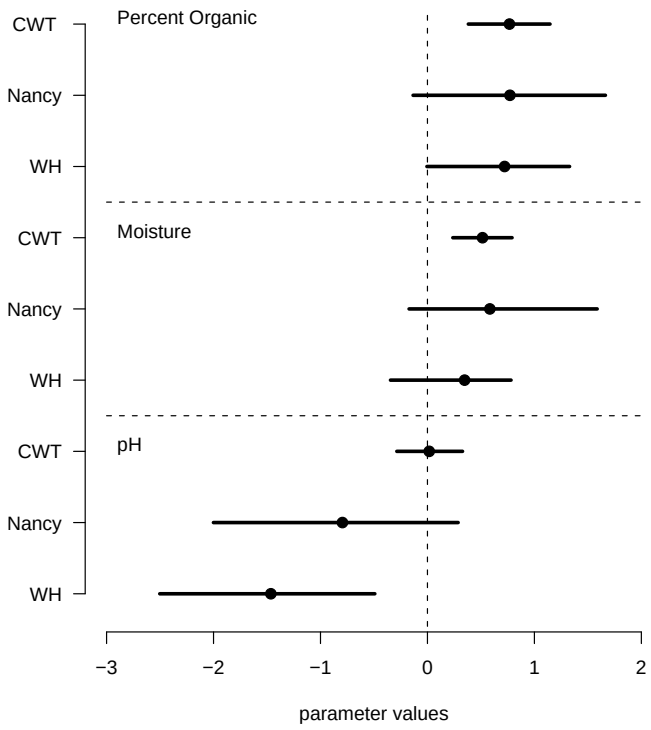
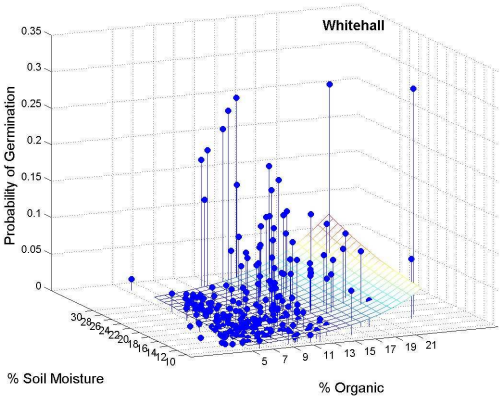
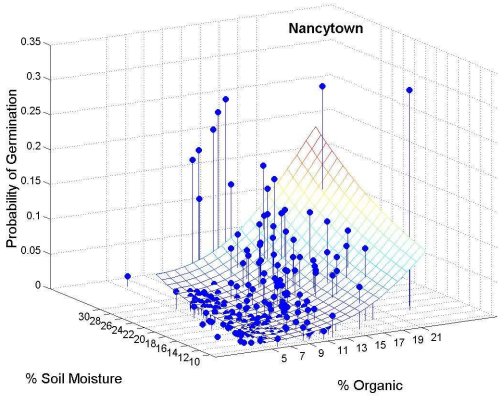


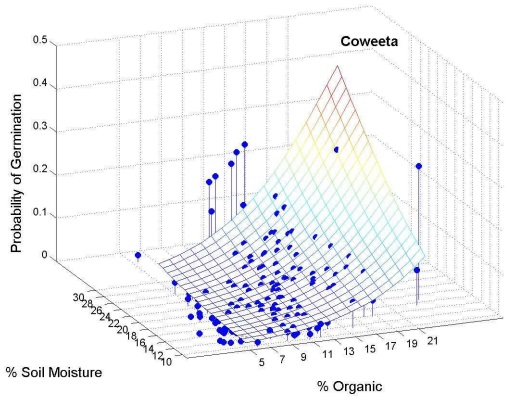
Figure 3.5: **Coefficient Estimates.** Lines represent 95% posterior credible intervals for estimated effects of % organic content, soil moisture, and pH in the three landscapes. Soil moisture values are averages across a year, representing relative soil moisture across the sites.



(a) Whitehall



(b) Nancytown



(c) Coweeta

Figure 3.6: **Predictive Recruitment Surfaces:** Predicted germination success as a function of organic content of the soil and percent soil moisture. The surfaces represent the mean of the posterior distributions when predictions are made at regular intervals across the abiotic space. The individual points are places with actual data; the height of these points represent model predictions at those data points. Differences in the response surfaces among landscapes arise from the landscape-level intercepts.

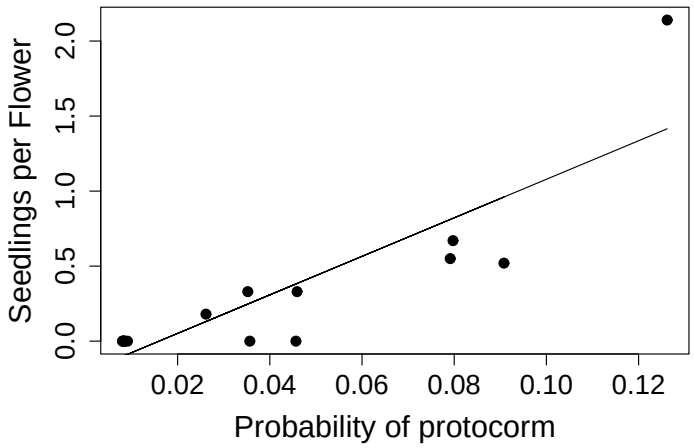


Figure 3.7: **Observed seedlings.** The observed probability of seedling production is significantly related to germination probability in seed packets at the population level. The y-axis represents the total number of seedlings observed in each of the 16 populations between 2001-2004, divided by the number of observed flowering stalks. The x-axis is the predicted population-level seed packet success rate (finding a protocorm in a packet).

Chapter 4

Hierarchical Analysis of Terrestrial Orchid Distributions Across Environmental Gradients[1]

[1]Diez, J.M. To be Submitted to *Landscape Ecology*

ABSTRACT

Abiotic and biotic factors operate at multiple spatial scales to shape species distributions and performance. While physiological tolerances may constrain distributions at large scales, abiotic patchiness and local biotic processes often contribute to spatial autocorrelation in observed patterns of occurrence and performance. In this study, we use surveys of the distribution and abundance of two terrestrial orchids and data on a suite of abiotic factors to test hypotheses regarding the relative influence of major abiotic gradients and local biotic processes on species distributions. We use a hierarchical modeling framework to test explicitly the relative significance of abiotic factors at different spatial scales, and the hypothesis that local spatial effects overlay responses to the abiotic environment to yield observed distributions of two terrestrial orchids, *Goodyera pubescens* and *Tipularia discolor*. Differences in key life history characteristics, including dependence on different groups of fungi for germination, allow a comparison of how abiotic gradients and local biotic processes may influence species differently. Overall, we found significant effects of light availability and soil moisture on patterns of presence and abundance, but also significant local spatial effects. These local spatial effects differed between species in a way that is consistent with the hypothesis that fungal controls over germination influence patterns of recruitment and overall species distributions. Results support the general ideas that species distributions can be explained by a combination of abiotic and biotic factors at multiple spatial scales, and hierarchical models provide a useful tool for uncovering these relationships.

Introduction

Explaining patterns of species distributions has been a focus of ecological work for many years (Andrewartha and Birch, 1954). Species-specific physiological constraints provide a useful expectation for how species may be distributed in relation to abiotic variables, such as moisture and temperature, and form the basis for many ideas about the niche. Since the

early formulation of the niche concept, however, much work has been directed at deconstructing why species observed in nature are not distributed according to their fundamental requirements. Numerous biotic processes, such as competition, dispersal, mutualisms, and disease, as well as various disturbances, keep species out of equilibrium with what might be an optimal distribution with regard to physiological constraints, leading to niche theory that has evolved to incorporate various forms of stochasticity (Pulliam, 2000; Tilman, 2004).

The interest in quantitatively modeling species distributions has taken on new life in the form of what Whittaker et al. (2005) call conservation biogeography - broadly the goal of using biogeographical concepts and methods for conservation goals. One large component of such work involves the general approach of using observed climate-abundance relationships to build predictive models of how species distributions will change under future climatic scenarios (e.g. Skov and Svenning, 2004; Thomas et al., 2004). Such distribution models (recently reviewed by Guisan and Thuiller, 2005) as a whole have been criticized on a number of grounds, including failure to incorporate biotic interactions, dispersal limitation, and evolutionary change (Pearson and Dawson, 2003; Hampe, 2004). Although it is widely understood that presence and abundance can be limited measures for describing species dynamics, this is often the only feasible data to collect, and the degree to which these limitations are problematic will likely depend on the intended goals and spatial scales of the study (Pearson and Dawson, 2003).

Various improvements can be made to the basic form of climate-envelope distribution modeling to minimize these deficiencies. In the absence of more detailed information about dispersal or biotic effects, one significant improvement has been the introduction of spatially correlated errors (Hooten et al., 2003; Gelfand et al., 2003; Latimer et al., 2005). This approach has the powerful appeal of differentiating species responses to measurable abiotic gradients and other unmeasured or unmeasurable processes that generate spatial structure in species distributions. Even if the focus is on the effects of measured covariates (e.g. temperature or precipitation), including spatial structure in such models helps improve

prediction and reduce the possibility of spurious correlations with abiotic variables due to spatial autocorrelation (Lichstein et al., 2002).

The little work thus far incorporating spatial errors into distribution models has typically focused on large spatial scales on the order of kilometers, but spatial effects may also be relevant to understanding species life histories and abiotic relationships at a within-population scale. Although most species distribution models have niche theory at their roots, few studies explicitly incorporate it as part of the design or interpretation (Guisan and Thuiller, 2005). Niche theory might prove useful however for generating expectations about distribution models, and specifically the strength of responses to abiotic gradients and additional local spatial effects. For example, life history characteristics that favor clumped over diffuse distributions, such as limited seed availability and/or dispersal, clonal growth form, and high survivorship, may be expected to increase the importance of spatial effects relative to effects of abiotic gradients. Thus, estimation of spatial effects in addition to abiotic effects in distribution models may help elucidate how different life history characteristics affect species distributions.

Although there is much discussion of the utility of a hierarchical perspective on species' niches and distributions (Maurer and Taper, 2002; Parmesan et al., 2005), there has been little use of explicitly hierarchical models for exploring these relationships. Having undergone more significant development in some social sciences (Raudenbush and Bryk, 2002), hierarchical models are rapidly becoming more widely used by ecologists for understanding complex relationships at different scales (Wikle, 2003*b*; Clark, 2005; McMahon and Diez, 2005). Indeed, spatial errors implemented within a mixed-effects model are one element of a larger class of hierarchical models, but the full potential of models to explore scale-specific relationships with covariates has been under-utilized.

Forest herbs, with a range of life history characteristics and size that's amenable to measurement of key abiotic variables, offer an interesting opportunity to evaluate the importance of life history traits for determining responses to abiotic gradients at different scales. In this

study, we analyze the patterns of presence and abundance of two terrestrial orchids, *Goodyera pubescens* and *Tipularia discolor*, with different key life history characteristics. These species require different groups of fungi in order to germinate, leading to quite different expectations for patterns of recruitment. *G. pubescens* associates with saprotrophic soil fungi from basidiomycete genus *Tulasnella*, while *T. discolor* requires fungi closely associated with decaying logs (Rasmussen and Whigham, 1998*a*). Symbiotic germination success has been found to increase sharply in close proximity to adult *G. pubescens*, and in patches of higher soil moisture and organic content (Diez, 2005), while the pattern of *T. discolor* germination appears strictly associated with decaying logs (Rasmussen and Whigham, 1998*a*, Geffen, unpublished data). These patterns also reflect the fact that seeds of *G. pubescens* can germinate with the same fungi as in the roots of adults, whereas *T. discolor* seeds require different fungi than are associated with adults (McCormick et al., 2005). Thus, while both species are capable of some amount of clonal growth, it is expected from these differences that *G. pubescens* is more capable of spreading laterally once established on a site due to positive density-dependent recruitment.

We use a combination of plant surveys and abiotic monitoring to test the following specific hypotheses:

(1) Given the importance of soil moisture in southeastern North America, and the commonly low light levels beneath forest canopies, we expect the distributions and abundances of these orchids to reflect both local and regional patterns of variation in these variables.

(2) The clonal growth form of these orchids, together with obligate dependence on mycorrhizal fungi in order to geminate, leads me to expect strong spatial effects on both presence and abundance of both orchids.

(3) Given the specificity of substrate requirements for the fungi needed for germination of *T. discolor*, its distribution is expected to be less dependent on the abiotic environment per se, and not display as strong of spatial effects as *G. pubescens*.

A further goal of this paper is to provide an example of some of the under-utilized possibilities for hierarchical models to more thoroughly and explicitly investigate multi-scale controls on species distributions. There are additional benefits stemming from the Bayesian framework for these models that are relevant for predicting species' responses to changing environmental conditions.

Materials and Methods

Study species

Goodyera pubescens is a perennial, evergreen orchid distributed throughout eastern North America, from Florida, west to Arkansas, north and east into Canada. Although growth can occur throughout the year, leaves are formed primarily during the summer season. Clonal reproduction occurs via branching growth of rhizomes on the soil surface. Flowers develop in July and August, which if pollinated form pods containing thousands of minute seeds (< 2mm length, < .5mm across). Gravity and wind dispersal of the seeds begins with dehiscence of the pods in September, but can be spread over several months, with some seeds occasionally remaining in the pods by early spring. Like most orchids, *G. pubescens'* seeds lack significant nutrient reserves and are therefore dependent on colonization by the appropriate fungi for germination. Upon wetting, seeds imbibe and if a suitable fungus is encountered a symbiotic protocorm is formed, which can develop into a more advanced corm structure and in two years a seedling.

G. pubescens associates with mycorrhizal fungi from the genus *Tulasnella*, a group of saprotrophic basidiomycetes from the order *Tulasnellales*. McCormick et al. (2004) determined through ITS sequencing that the *Tulasnella* associating with *G. pubescens* likely represent only one or two species, most closely aligning with *T. bifrons*. Isolates from *Goodyera* roots have been shown to utilize cellulose but not lignin (e.g. from dead leaves and wood) as substrates. Moveover, using isolations from multiple roots from single adults McCormick et al. (2005) have shown that individual plants associate with only one fungal strain at a

time. Little is known about the nature of the relationship beyond the seedling stage, although adults remain heavily colonized by the fungi (Rasmussen, 1995; Rasmussen and Whigham, 1998*b*). While achlorophyllous, 'myco-heterotrophic' plants have been known to depend on fungal associates for nutrition and energy (Leake, 1994), recent stable isotope evidence suggests that even autotrophic terrestrial orchids continue to gain both carbon and nitrogen from their associated fungi (Gebauer and Meyer, 2003).

Tipularia discolor is a wintergreen terrestrial orchid found in mixed deciduous forests of eastern North America. In *T. discolor*'s southern range, a plant's single green leaf emerges above ground in late fall (end of September) and remains until spring (March-April). Flowers, if produced, are found on a single flowering stalk that that emerges in August, before the leaf emerges. Seed pods, containing thousands of seeds each, become dry and dehisce over the course of the fall and winter, with the seeds being predominately wind/gravity dispersed. *T. discolor* is capable of clonal growth via belowground corms (Whigham and ONeill, 1991)), although the importance relative to sexual recruitment is unknown.

T. discolor has been found to associate with a variety of tulasnelloid fungi (McCormick et al., 2005) as adults, but rely on a different group of fungi as protocorms, most likely from the Auriculariales, and at least some of those being most closely aligned with *Tomentella* species (McCormick et al., 2005). In field germination studies, *T. discolor* symbiotic germination has been found to be tightly associated with decaying logs (Rasmussen and Whigham, 1998*a*).

Study Design and Sampling regime

A hierarchical sampling design was used to capture a range of environmental conditions across a geographic gradient of approximately 120 km. from the Piedmont of Georgia to the Southern Appalachian Mountains. Three areas along this gradient were sampled: Whitehall Forest in Athens-Clarke County, GA (33°92' N latitude, 150-240 m elevation), the Nancytown area (34°31' N latitude, 315-450 m elevation) Habersham County, GA, and the Coweeta

Hydrologic Laboratory (35°03' N latitude, 750 - 1,500 m elevation) in Macon County, N.C. These sites were all placed in relatively mature ($\sim$ 80 yrs old) deciduous forest, and chosen to reflect the range of elevation, temperature, and precipitation conditions experienced by the forbs under study. Within each landscape, we established six study grids (four in Nancytown) between 250 and 480 square meters in size for a total of 16 study grids. These study plots were divided into 2 square meter cells. Ten of the grids in which *G. pubescens* was found were used for analysis, while 9 of the grids were used for *T. discolor* analyses. Although these sites are used for repeated demographic measurements, only the 2003 plant surveys and abiotic monitoring are used in this study.

Abiotic measurements

Time domain reflectometry (TDR) methods were used to estimate soil moisture content. To characterize the relatively fine-scale spatial variability, the handheld Soil Water Content Measurement System from Hydrosense was used to measure soil moisture in the top 12 cm of soil at 80 points within each of sixteen grids. These 80 measurements were distributed across the grids in a hierarchical design enabling adequate numbers of pairs of measurements at different distance classes. Geostatistical methods were used to make predictions at each of the 120 cells within each grid, using exponential variograms and linear detrending to account for directional trends in a couple of highly sloped grids. This 80-pt sampling was performed roughly four times a year for three years to capture seasonal changes in the spatial structure.

Direct comparison of measured soil moisture among landscapes is hampered by different times since the last rain event, so an index of moisture was calculated that combines the relatively consistent spatial patterns measured by TDR and the landscape differences in precipitation. As such, the TDR readings are considered good measures of the relative moisture potential of a given site, and these relative values are scaled by the amount of precipitation received during the September to August year preceeding fall plant surveys.

Understory light availability, as estimated by photosynthetic photon flux densities (PPFD), was measured at the level of forest ground cover using a handheld AccuPAR ceptometer. Eighty ceptometer readings were taken on each grid at the same 80 locations as moisture, with concurrent measurements taken in nearby open clearings that allowed calculation of percent transmittance of incident canopy PAR to the forest floor. Measurements were taken between 10 AM and 2 PM on overcast days, thought to better represent overall light availability characteristics for a site (Gendron et al., 1998, 2001; Beaudet et al., 2004, but see Fladeland et al., 2003).

Soil sampling and analysis

Three 12 cm soil cores were taken within a .5 m radius from the center of each of the 16 intensive cells within each of the 16 study grids. The three soil cores from each cell were bulked and sifted through a 2 mm sieve to obtain sufficient quantity for all soil analyses, for a total of 256 samples. In a few circumstances, course rocky topsoil prohibited three complete 12 cm cores and 4 more shallow cores within the .5 m radius were used. Total carbon, nitrogen, and carbon:nitrogen ratios were determined using dry Micro-Dumas combustion and immediate analysis by gas chromatography. A pH meter was used to determine pH of a 1:1 suspension of fresh soil to deionized water. Organic content was determined by percent weight loss after combustion in a muffle oven. Soil texture was determined using the hydrometer method (Gee and Bauder, 1986). Because percent sand and percent clay were highly correlated, percent sand was used as the index of soil texture in all analyses.

Statistical Analysis

Standard generalized linear models (GLM) were used to first identify the most important variables, including soil characteristics, affecting species presence and abundance on intensive cells. Then Bayesian hierarchical models were used for general partitioning of variance of

presence and absence of each species, evaluation of the effect of different abiotic variables at multiple scales, and quantifying the influence of local spatial effects on these relationships.

Generalized Linear Models

The occurrence of a plant is each cell is modeled as a Bernoulli process, with probabilities of occurrence related to linear predictors via the logit link function as follows:

$$Y_{ij} \sim Bernoulli(\phi_{ij}), \tag{4.1}$$

where Y_{ij} is the raw binary occurrence data for each cell i and grid j, and ϕ_{ij} are the estimated probabilities of occurrence on in those cells. The logit transformation is used to relate those probabilities to a linear combination of predictors:

$$log\left(\frac{\phi_{ij}}{1-\phi_{ij}}\right) = \beta_{0j} + \beta X_{ij} \tag{4.2}$$

where β_{0j} is a grid-level intercept term, β is a vector of regression coefficients, and X_{ij} is the design matrix of explanatory variables at the cell level. In this first case, using data from the 16 intensive cells, our full model contains all predictor variables: light and moisture, their squared terms (allowing for unimodal responses), and all measured soil properties. Stepwise regression and model selection using AICs were used to evaluate the importance of individual predictors.

Abundance is modeled as a Poisson process, with the mean value for each cell related to abiotic explanatory variables via a log link function

$$A_i \sim Poisson(\mu_i) \tag{4.3}$$

where

$$log(\mu_i) = \beta_{0j} + \beta X_{ij} \tag{4.4}$$

where as before β_{0j} is a grid-level intercept term, β is a vector of regression coefficients, and X_{ij} is the design matrix of explanatory variables at the cell level.

Hierarchical Bayesian GLMMs

For explicit consideration of processes at multiple spatial scales, including the influence of local spatial processes, the standard GLMs described above were extended to a hierarchical Bayesian framework. To do so, prior distributions were assigned to each model parameter, spatially correlated errors were defined, and Markov chain Monte Carlo (MCMC) methods were used to construct posterior distributions for parameters of interest.

The *Bernoulli* occurrence model is extended to include spatially correlated errors, such that the log-odds is now

$$\eta_{ik} = \beta_{0k} + \beta X_{ik} + \rho_i \tag{4.5}$$

where ρ_i are the random effects for each cell. We allow these to be spatially dependent via a conditional auto-regressive (CAR) specification of the prior, as defined by Besag (1974) and recently used by Gelfand et al. (2003) for similar applications. Thus, the distribution of random effects for cell i is made conditional on values from cells j, given as:

$$\rho_i|\rho_j \sim Normal\left(\frac{\sum_{j\neq i} a_{ij}\rho_j}{a_{i+}}, \frac{\sigma_\rho^2}{a_{i+}}\right) \tag{4.6}$$

where $a_{ij} = 1$ if cells i and j are neighbors, and 0 otherwise. The a_{i+} are the total number of neighbors of cell i, as defined by the neighborhood structure of choice. We define neighbors as those cells directly adjacent, including those diagonal.

The grid-level intercept, β_{0k}, provides the link to the higher level through interpretation as a random effect drawn from a global distribution with a population-specific mean determined by a global intercept and covariates as follows:

$$\beta_{0k} = \gamma_{00} + \pi Z_k + \epsilon_k \tag{4.7}$$

Here, Z_k is the vector of covariate data at the grid level (pH, soil texture, etc), and π is the vector of coefficients describing those relationships. The error terms at this level, ϵ_k are normally distributed $\epsilon_k \sim Normal(0, \tau_\epsilon)$, with the variation attributable to the grid level, $\tau_\epsilon \sim InverseGamma(.001, .001)$. The intercept, γ_{00} represents the global mean and is given

a non-informative prior distribution $\gamma_{00} \sim Normal(0, 1000)$. All regression coefficients are given diffuse prior distributions, $beta \sim Normal(0, 1000)$ and $\pi \sim Normal(0, 1000)$, allowing the data to drive their estimation.

Through the specification of priors, the Bayesian framework provides a straight-forward way to explicitly model hierarchical structure and incorporate covariates at appropriate levels. Because our abiotic data are collected at the cell level, abiotic covariates are modeled at the cell level. It's worth noting that if we had information about the measurement accuracy of these covariate readings, we could easily incorporate it here in the form of a prior distribution for the data itself, thus reflecting the uncertainty associated with imperfect measurements. This is the approach taken for the grid-level soil covariates. We model the grid-level expected values of soil texture, pH, and % organic as the mean of the *Normal* distribution from which the 16 measurements were drawn. For pH, as an example: $pH_{jk} \sim Normal(pH_{0k}, tau_{pH})$, where pH_{jk} are each of 16 individual pH measurements per grid, pH_{0k} are the grid means, used above in the regression model, and tau_{pH} is the estimated measurement error associated with pH at the grid level.

The models of abundance use an identical specification of spatial random effects, and hierarchical structure, with the replacement of the *Bernoulli* sampling distribution and logit link function with a *Poisson* distribution and log link. In all cases similar non-informative distributions are used for parameter priors. For both *G. pubescens* and *T. discolor* unconditional models were initially fit to determine the basic partitioning of variance of presence, abundance and survival between the microsite (cell) and population (grid) scales (Raudenbush and Bryk, 2002).

Results

On the 256 intensive cells monitored for soil characteristics along with the plant surveys, we found that light availability and soil moisture have more significant effects on *G. pubescens'* distribution and abundance than any of the soil characteristics. (Table 4.1).

The hierarchical Bayesian models proved useful for incorporating spatially explicit data into the models and estimating effects at different spatial scales. Overall, the variance partitioning in unconditional models showed that most of the variation of *Goodyera* presence was distributed among cells (63 %), compared to among grids (37 %) and similarly for *Tipularia* 69% among cells and 31% among grids.

The two species showed different responses to abiotic variables in the full hierarchical models. Both the presence and abundance of *G. pubescens* showed a significant response to winter light at the cell scale and organic content of the soil at the grid level, while *T. discolor* showed no significant responses to abiotic variables at either the microhabitat or population scales (Figure 4.1). An additional result of the hierarchical structure of the model is greater uncertainty associated with grid-level effects than at the cell level, as can be seen by the width of the 95% intervals in Figure 4.1.

The significance of spatial effects is judged by whether or not 95% of the posterior distribution for an effect overlaps zero. As shown in Figure 4.2, *G. pubescens* exhibited greater spatial structuring than *Tipularia*, with significant spatial error terms for both presence and abundance (90 and 160 cells, respectively, out of a total of 1020 cells; 8.8% and 15.7% of all cells). *T. discolor* on the other hand, had no significant spatial effects in the presence model and 65 in the abundance model (65 out of total possible of 734; 8.9% of all cells).

Within populations, both presence and abundance and patterns of spatial random effects were found to be spatially heterogeneous. As one example, Figure 4.3, shows maps of a single grid in which the heterogeneity of distribution and spatial effects can be seen. It should be noted that each cell is represented by only the mean of the posterior predictive distribution, although a full probability distribution is the true output of the models. Such predictive distributions for presence, abundance and spatial errors are available for all grids and both species.

For understanding the impact of spatial effects, it is also informative to compare models with and without spatial effects. Figure 4.4 shows a comparison of abiotic parameter esti-

mates in models with and without inclusion of spatial effects. In most cases there is a movement of parameter estimates from significant effects without spatial effects to estimates with greater uncertainty and overlapping 0 when spatial effects are included. The notable exception is for *Tipularia* presence models in which estimates are nearly exactly the same. This is the same model for which no spatial effects were estimated to be different from zero.

Discussion

This study used hierarchical Bayesian models to evaluate the responses of terrestrial orchids *Goodyera pubescens* and *Tipularia discolor* to environmental gradients, and additional local processes that mediate this response. We found support for the idea that these plants are responding to abiotic gradients in the environment, and that soil moisture and light are stronger predictors than other edaphic variables that were hypothesized to be important for plant performance. The data also suggests that local biotic or abiotic processes play a significant role in shaping patterns of presence and abundance for this terrestrial orchid.

The finding of stronger spatial effects for *G. pubescens* than *T. discolor* is consistent with the idea that differences in fungal-mediated patterns of germination play a role in determining patterns of distribution. This hypothesis is supported by known differences in substrate utilization by the fungi associating with each species, and the ability of *G. pubescens* seeds to germinate with fungi associated with adults, while *T. discolor* use distinct fungi associated with decaying logs (McCormick et al., 2005). Also, previous work in this system has documented a decline in symbiotic germination probabilities for *G. pubescens* with distance from adults, and increases in patches of higher soil moisture and organic content (Diez, 2005). Germination of *T. discolor*, on the other hand, has been shown to be very dependent on specific substrates which are temporally transient (Rasmussen and Whigham, 1998*a*, Geffen, unpublished data). Although rates of clonal spread of these species has not been directly estimated, it is likely that clonal propagation is a cause of the spatial structure in *Goodyera* given the prolific cloning observed in the 6 years of the associated demographic

study. The clonality via underground clones in *Tipularia* may mean it is less likely than *Goodyera* to produce large patches via cloning, and observed structure may arise from large recruitment events associated with historical tree falls.

Although other studies have used spatially correlated errors to investigate autocorrelation at landscape scales (Hooten et al., 2003; Gelfand et al., 2003), this is the first such study to my knowledge using such methods to investigate spatial processes at a local, within population scale. In addition to allowing more robust prediction of responses to abiotic gradients, this approach offers a unique opportunity for investigating the strength and variation of local biotic processes that may shape distributions. The finding in this study of significant spatial errors is suggestive that autocorrelation at a within-population scale results from processes other than simply patchiness of the two most important abiotic variables - light and soil moisture. In fact, there are several life history traits of these orchids that may contribute to spatial autocorrelation, including clonal growth and dependence on mycorrhizal fungi for germination.

Although spatial errors are useful to include just for the improvements in prediction and avoidance of spurious effects due to autocorrelation, this study suggests they can also be useful for testing a priori hypotheses about the degree to which certain species are likely to display spatial structure. Also, by analyzing the results of spatial errors, new hypotheses may be formed to help explain what is generating the observed structure. This approach may be extended to explore how large-scale environmental gradients affect the observed spatial structure within a single species across its range.

Distribution modeling

Models can be a useful tool for understanding factors that govern the distributions of species, as well as potential responses to environmental change when used carefully. A range of tools have emerged over the last couple decades for modeling species distributions (reviewed by Guisan and Thuiller, 2005). Despite the noted problems, the approach of using observed

climate-abundance relationships to build predictive models of how species distributions will change under future climatic scenarios will likely remain a common and useful approach to coarse-scale understanding of species distributions. As recognized by many researchers, attempts to explain and predict species distributions will typically be improved by accounting for both the abiotic template and those processes that are likely to shift observed distributions from the optimal (Pearson and Dawson, 2003; Hampe, 2004).

This study has taken the approach of trying to relate observed statistical relationships between abundance and environmental variables and local effects, to notable life history differences between species. Such life history characteristics may help explain not only among-species differences, but variation in abiotic responses and local spatial processes across the geographic range of a single species.

Although the focus in this study has been on climate-envelope type of models, there are a range of approaches to understanding species distributions, including more mechanistic, physiologically-based models (Kearney and Porter, 2004), which have the potential to provide greater generality and an understanding of underlying processes. Such approaches also have the potential to more directly describe the fundamental niche, but are data-intensive and likely quite difficult for many taxa. Hierarchical methods may prove useful for an integration of physiological studies and natural gradient studies.

Scale and prediction

Recent reviews of species distribution modeling have appropriately called for increased attention to scale (Pearson and Dawson, 2003; Guisan and Thuiller, 2005), because it is generally understood that different processes are likely to influence species at multiple spatial scales (Maurer and Taper, 2002). This can make identification of driving variables difficult in the field, and prove challenging to estimate and interpret within models. Moreover, an incorporation of such multi-level structure may be quite important for prediction. Recent developments easing the implementation of hierarchical linear models (HLM) are establishing

these approaches as very useful for simultaneously evaluating information from different scales (Raudenbush and Bryk, 2002; McMahon and Diez, 2005). In this study, we provide an example of how simple hierarchical linear models can be used to explicitly estimate the effect of different factors at multiple spatial scales. The finding that grid-level organic content is far more informative than simply finding a 'grid effect', as there is a likely ecological explanation for such a pattern related to the tight relationships with saprotrophic fungi.

Clearly the degree to which not accounting for scale will be problematic will depend on the intended goals and spatial scales of the study. An additional opportunity afforded by hierarchical Bayesian models is the relatively straight-forward transition between parameter estimation and prediction (Gelman et al., 2004; Clark, 2005). Although not pursued in this study, all estimated relationships with abiotic variables can be used to make predictions about likely responses to changing conditions. The full posterior distributions of effects are well-suited for such predictions, and facilitate placement of such predictions within a dynamic model incorporating dispersal distances.

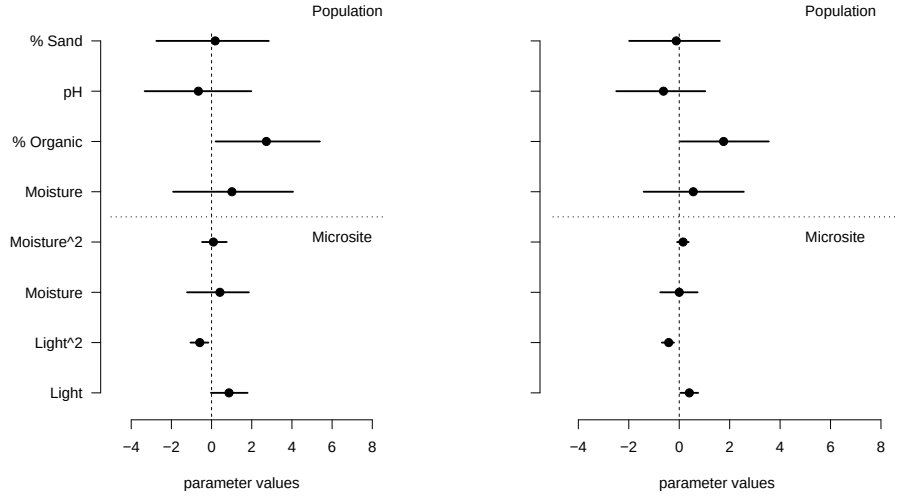
Conclusions

Niche theory has evolved over the years to accommodate what has been learned about how various biotic processes interact to influence species distributions across abiotic gradients (e.g. Pulliam, 2000; Tilman, 2004). Now concepts such as dispersal limitation, source-sink dynamics, and metapopulation dynamics are regularly used as conceptual frameworks for evaluating species' apparent deviations from what might be considered a fundamental niche. To understand the implications of these various processes for inferring a species' dynamics across landscapes and across time, species distribution models will benefit from modern statistical techniques that allow incorporating explicit examination of multi-scale responses to spatial and temporal abiotic variability and estimation of the effects of local spatial interactions.

Table 4.1: **GLM Intensive Cell Results:** Plant presence and performance as a function of soil variables, understory light availability (PAR), and soil moisture (TDR). Only significant coefficients are given. All abiotic readings are Fall 2003

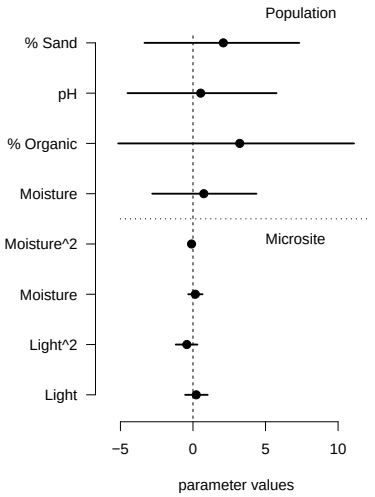
	Response	
Predictor	Presence	Abundance
Light	2.53*	1.05.
Light^2	n.s.	n.s.
Moisture	.864***	.64***
Moisture^2	-.013*	-.0093***
Light*Moisture	-.094*	-.048**
% Organic	n.s.	.11.
pH	n.s.	n.s.
Nitrate	n.s.	n.s.
Phosphate	n.s.	.198***
% Sand	n.s.	n.s.
C:N	n.s.	n.s.

$***p < .001; **p < .01; *p < .05; .p < .1; n.s.p > .1$

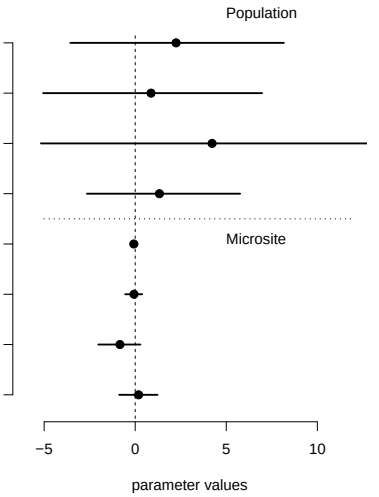


(a) Goodyera Presence

(b) Goodyera Abundance



(c) Tipularia Presence



(d) Tipularia Abundance

Figure 4.1: **Hierarchical parameter estimates:** Shown are 95% credible intervals for estimated coefficients in the hierarchical model.

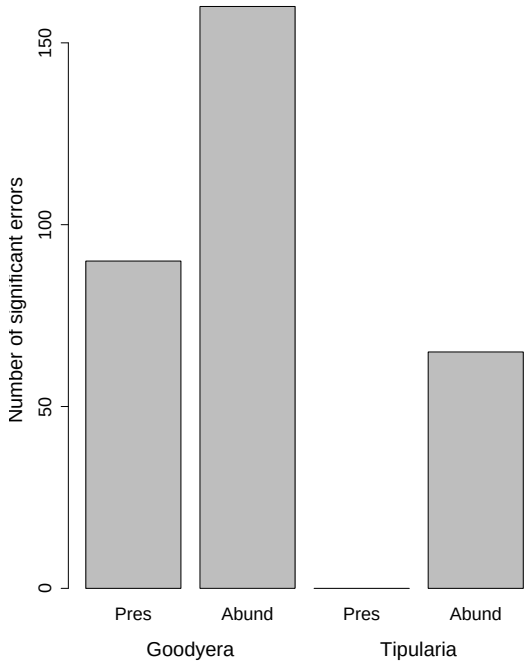
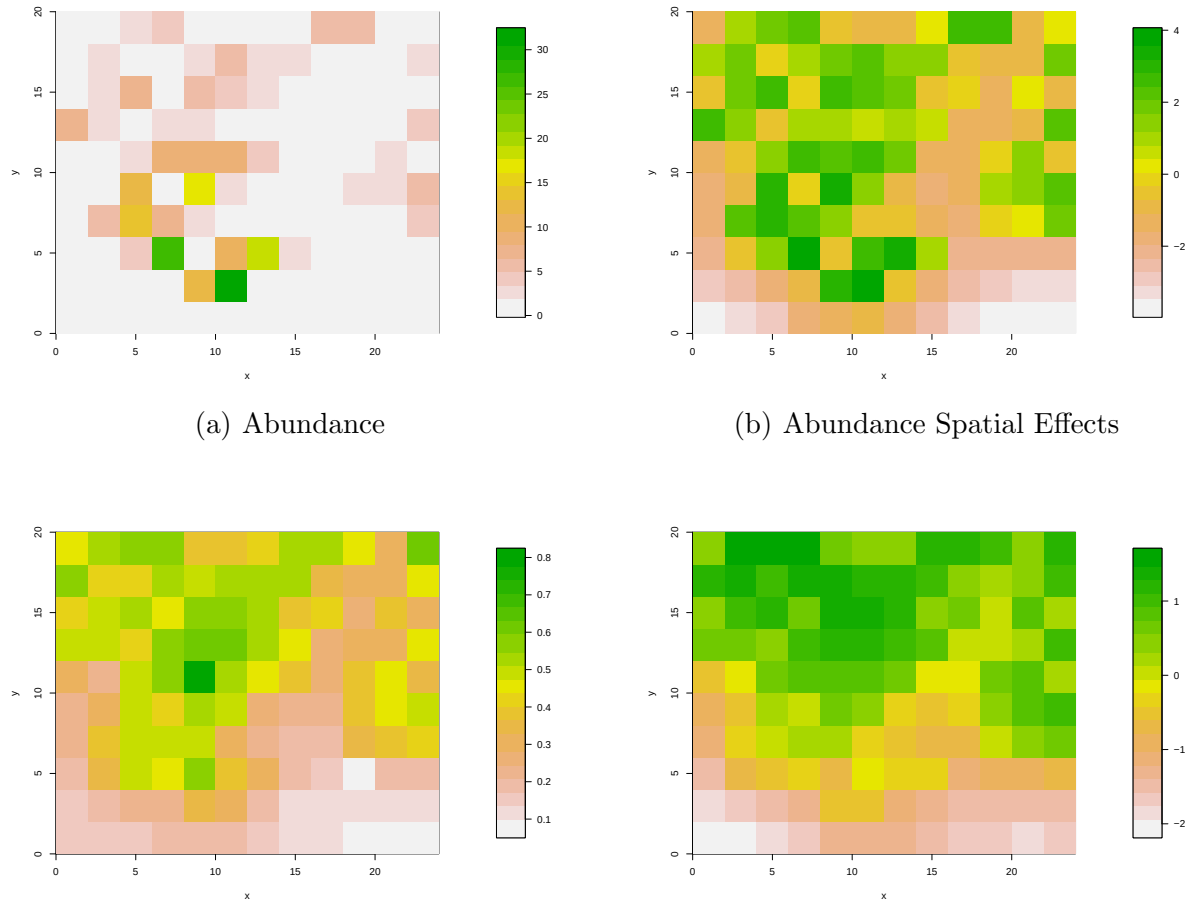


Figure 4.2: **Spatial effects:** Given are the total number of cells within grids that had spatial effects significantly different from zero for each response variable



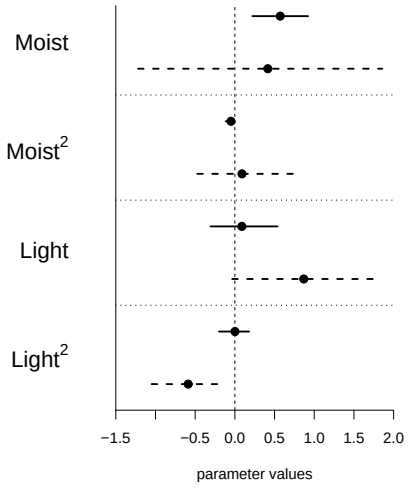
(a) Abundance

(b) Abundance Spatial Effects

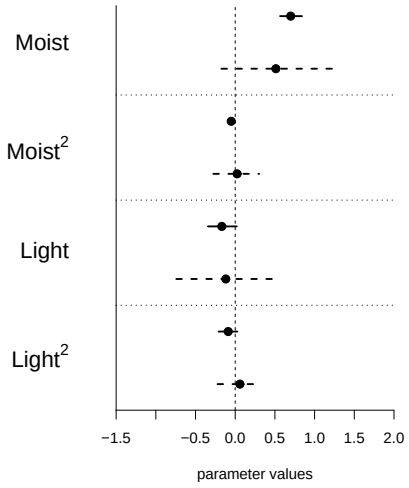
(c) Presence

(d) Presence Spatial Effects

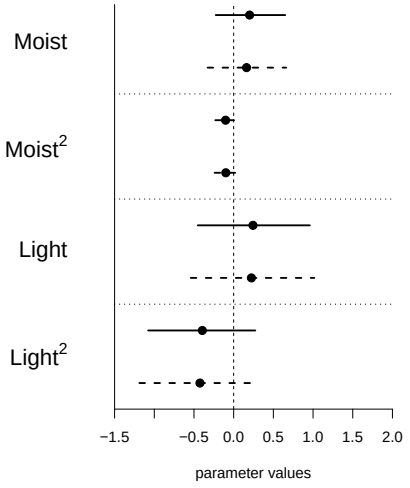
Figure 4.3: **Population CWT 36A:** Map of cell-level predicted abundances (a), spatial effects of abundance model (b), predicted probabilities of occurrence (c), and the spatial effect of the occurrence model (d). Note that displayed for each cell is actually the mean of a full posterior predictive distribution.



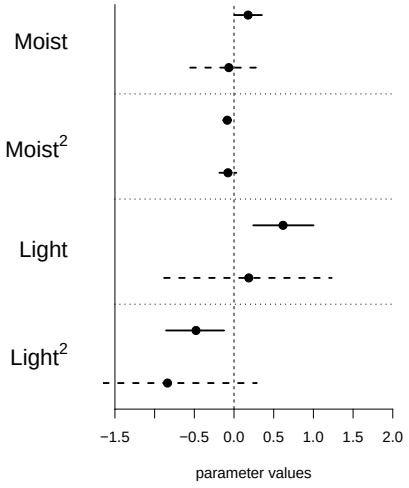
(a) Goodyera Presence



(b) Goodyera Abundance



(c) Tipularia Presence



(d) Tipularia Abundance

Figure 4.4: **Spatial and non-spatial parameter estimates:** Shown are 95% credible intervals for estimated coefficients in the hierarchical model with and without spatial effects included in the models. Solid lines are non-spatial models; dashed lines are models including spatial effects.

Chapter 5

Demographic Responses of a Terrestrial Orchid to Abiotic Gradients [1]

[1] Diez, J.M., H. Ronald Pulliam To be Submitted to *Ecology*

ABSTRACT

Although many studies describe demographic variability in natural populations, very few link basic vital rates and overall population growth rates to abiotic driving variables. Establishing these links to the abiotic environment is crucial, however, for testing niche theory and making predictions of species responses to environmental change. Moreover, a realistic account of the associated variability and uncertainty of these relationships is critical for proper assessment of both theory and predictions. In this study we integrate 6 years of demographic and abiotic monitoring to estimate the responses of vital rates and overall population growth to environmental heterogeneity at different spatial scales. Using hierarchical Bayes methods for parameter estimation and prediction across a range of environmental conditions we show how light and soil moisture influence vital rates and overall population growth rates of a terrestrial orchid, *Goodyera pubescens*, native to eastern North America. Niche theory and the related concepts of metapopulation and source-sink dynamics suggest the possibility of substantial population sizes even in habitat that is not suitable enough to maintain the population, as well as notable absences from suitable habitat. Comparisons with observed distributions suggest a lack of correspondence between distribution and suitability that changes across spatial scales. Supportive of predictions of source-sink and metapopulation theory we find this species absent from some substantial areas of suitable habitat and present in some unsuitable habitats. This pattern is most evident at very local spatial scales, while the correspondence between abundance and suitability increases with increasing spatial scale. We also discuss how the uncertainty estimated by the models influences the evaluation of theory and helps inform the interpretation of predictions of species dynamics.

Introduction

Elements of niche theory combined with source-sink and metapopulation dynamics predict both spatial and temporal variation in habitat suitability and site occupancy (Pulliam, 1988;

Armstrong, 2005; Roy et al., 2005; DeWoody et al., 2005). Populations are posited to exhibit a range of growth rates, some below replacement levels and others above, and these sites may be linked by dispersal (Tilman and Karieva, 1997). Furthermore, both experimental work and theory suggest that these growth rates may be explained in part by abiotic conditions and that species traits may prescribe a range of optimal abiotic conditions, above and below which performance would decline (Thuiller et al., 2004; Gabriel et al., 2005). The degree to which patterns of population growth can be explained by underlying environmental variables remains a fundamental problem in population ecology (Coulson et al., 2004), and for applications of ecological knowledge to conservation measures (Franklin et al., 2000). A number of processes, including various biotic interactions (e.g. mutualisms or competition), and stochastic events (e.g. dispersal or disturbances) have been hypothesized to cause a mismatch between a species' fundamental and realized niche, resulting in observed absences from putatively suitable habitat and presences in unsuitable habitat (Pulliam, 2000).

In population dynamics studies as a whole, there continues to be a great deal of interest in how to balance deterministic modeling of underlying processes with stochastic effects (Coulson et al., 2004). Numerous advances have been made to incorporate stochasticity into population models (Clark and Bjornstad, 2004), and there have been some notable successes in explaining population fluctuations as a function of environmental variables using particularly long-term abundance datasets (Leirs et al., 1997; Stenseth et al., 2002; Coulson et al., 2001). Many population studies, however, are interested in species for which only much shorter time series of data are available, and more often turn to projection matrix approaches instead of trying to explain patterns of abundance directly (Caswell, 2001). While many such studies have documented spatial and temporal variability in population growth rates (Horvitz and Schemske, 1995; Freville et al., 2004; Koop and Horvitz, 2005), there has been surprisingly little work explicitly linking demographic performance of species to local environmental conditions (but see Altwegg et al., 2005; Franklin et al., 2000). Even in studies that document a demographic response to variation in abiotic conditions, an often difficult

and missing next step is understanding how responses translate into estimates of population growth rates under different conditions (Morris and Doak, 2005).

This may be partly due to the difficulty of collecting demographic and environmental data at spatial and temporal scales adequate for capturing a wide range of conditions experienced by the species. Demographic data, even for single species, can be laborious to collect and may require many years to capture temporal variability and document difficult aspects of life history, such as dormancy and clonal reproduction (Caswell, 2001; Kery et al., 2005). Moreover, significant environmental and demographic variation from site to site, even on very local spatial scales, requires substantial monitoring efforts to capture enough of the range of variation to have confidence in resulting predictions. Although identifying large-scale population trends may sometimes only require coarse abiotic data (Hallett et al., 2004), identifying and monitoring the environmental variables on a scale relevant to within-population variation in vital rates is demanding, given high local-scale heterogeneity in many ecosystems.

Another challenge to assessing demographic responses to environmental conditions has been the translation of multiple sources of variability to reasonable prediction uncertainty. While it may be understood that there is inherent process variation and measurement uncertainty, it is important that these translate into reasonable overall estimates of the uncertainty of population-level predictions (Clark, 2003). Traditional inference based on confidence intervals on estimates of vital rates is difficult, if not inappropriate, to translate into a predictive framework and often must discount sources of process variation and measurement uncertainty. Prediction of species and community responses to environmental change can be facilitated by an understanding of niche relationships (Thuiller et al., 2005), but a proper accounting of uncertainty is critical to avoid over-confidence in prediction (Clark et al., 2001, 2003*a*).

In this study we use intensive demographic and abiotic monitoring to explore the relationships between demographic rates of the terrestrial orchid *Goodyera pubescens* and key environmental variables. With 6 years of data on the growth, survival and reproduction of

individually marked plants in 10 populations, and concurrent abiotic monitoring of understory light and soil moisture availability, we use hierarchical Bayes models to ask the following specific questions: (1) How do individual vital rates, including survival, clonality, and fecundity, respond to abiotic gradients? (2) How do these responses of vital rates to abiotic conditions integrate to influence population growth rates across this gradient? And finally, to show how this approach may be used to evaluate elements of niche theory we ask (3) how observed distributions and abundances compare to the distribution of suitable habitat at local, population, and regional spatial scales, and (4) what impact accounting for uncertainty has on interpretation of niche theory and predictions of population dynamics.

Our work provides a concrete example of how hierarchical Bayesian models are flexible enough to accommodate several difficult data aspects of many ecological studies: (*i*) incorporation of both experimental and observational data, (*ii*) necessary use of an unbalanced study design, (*iii*) estimation of difficult to observe life-history stages, and (*iv*) incorporation of multiple sources of variability that should affect resulting prediction certainty. The result is a unique quantitative assessment of the niche relationships of a terrestrial orchid, and a more general example of the importance of process variability and model uncertainty for assessment of ecological theory and predictions of species responses to abiotic gradients.

Methods

Study Design

The study used a hierarchical sampling design, structured to capture a range of environmental conditions experienced by understory herbs across a geographic gradient of approximately 120 km from the Piedmont of Georgia to the southern Appalachian mountains. At the largest scale, referred to as landscapes, we sampled three areas along this gradient: Whitehall Forest in Athens-Clarke County, GA (33°92' N latitude, 150-240 m elevation), the Nancytown area (34°31' N latitude, 315-450 m elevation) in Habersham County, GA, and the Coweeta Hydrologic Laboratory (35°03' N latitude, 750 - 1,500 m elevation) in Macon County, N.C.

These sites were all located in relatively mature (80^+ yrs old) deciduous forest, and chosen to reflect a range of elevation, temperature, and precipitation conditions experienced by the forbs under study. Within each landscape, we established six study grids (four in Nancytown) each between 250 and 480 m^2 in size for a total of 16 study grids, of which the focal study species is found on 10. For the purpose of abiotic monitoring, these study plots were divided into 4 m^2 cells. Overall, more than 1300 plants were individually marked and monitored.

Study Species

Goodyera pubescens is a perennial, evergreen, clonal orchid distributed throughout the East, from Florida, west to Arkansas, north and east into Canada. Although growth can occur throughout the year, leaves are formed primarily during the summer season. Flowers develop in July and August, which if pollinated form pods containing thousands of minute seeds (< 2mm length, <.5mm across). Gravity and wind dispersal of the seeds begins with dehiscence of the pods in September, but can be spread over several months, with some seeds occasionally remaining in the pods by early Spring. Like most orchids, *G pubescens'* seeds lack significant nutrient reserves and are therefore dependent on colonization by the appropriate fungi for germination. Upon wetting, seeds imbibe and if a suitable fungus is encountered a symbiotic protocorm is formed, which can develop into a more advanced corm structure and in two years a seedling.

Plant monitoring

All individuals of *G. pubescens* found within each of 10 populations across these 3 landscapes, were individually marked and monitored for growth, reproduction, and survival over 6 years, from 1998 to 2004. Each individual ramet on the grids was marked with a unique identifier on a flag and revisited at least once a year in the fall to measure growth and reproduction. Three measurements were taken to represent plant size: the number of rosettes (whirls of leaves), the number of leaves, and the width of the largest leaf. Because the rhizomes creep

along the surface of the ground, it is possible to distinguish physiological individuals, or ramets, by following the connections. While most plants have a single rosette, a single ramet may form up to 4 or more rosettes of leaves stemming from connected rhizomes, sometimes resulting in individuals with over twenty leaves.

Abiotic Monitoring

Characterizing the soil moisture availability from local to regional spatial scales, across multiple years, is a significant challenge. In another related study, a suite of abiotic characteristics were measured and used to predict the species distribution and abundance of *G. pubescens*, from which soil moisture and light availability were identified as the most important abiotic variables for this orchid (Diez 2005). Therefore, we focus here on relating demographic performance to these two driving variables. A combination of time domain reflectometry (TDR) methods were used to estimate and validate soil moisture content. To characterize the fine-scale spatial variability, the handheld Soil Water Content Measurement System from Hydrosense was used to measure soil moisture in the top 12 cm of soil at 80 points within each of the sixteen grids. These 80 measurements were distributed across the grids in a hierarchical design enabling adequate numbers of pairs of measurements at different distance classes to allow prediction at all cells with geostatistical methods. Specifically, ordinary kriging was performed using exponential variograms, while in a couple of cases accounting for a directional trend in the spatial autocorrelation. The 80 point sampling was performed four times a year for three years to capture any seasonal changes in the spatial structure of soil moisture.

Because the 80 point data represents a one-time snapshot of soil moisture in each season, in each landscape, we consider them most useful as a measure of relative soil moisture potential. In order to incorporate yearly and regional differences in precipitation, the cell-level TDR values were scaled relative to the mean values and multiplied by precipitation values for a given year and landscape to yield a moisture index at the cell level that is comparable across sites and across years. The summer TDR readings were used, and precipitation from

September to August of the preceding year because our demographic monitoring was done in August of each year. This moisture index thus reflects the combined effects of yearly and regional precipitation differences and within landscape differences in soil moisture potential. Another study on these same sites showed that soil moisture depends most on precipitation, a site's aspect, and soil texture (Diez 2005).

Understory light availability, as measured by photosynthetic photon flux densities (% PPFD), was measured at the level of forest ground cover using a handheld AccuPAR ceptometer. Eighty ceptometer readings were taken on each grid, with concurrent measurements on a second quantum sensor taken in open nearby open clearings, allowing for calculation of percent transmittance of incident canopy PAR to the forest floor. Measurements were taken between 10 AM and 2 PM on overcast days, thought to better represent overall light availability characteristics for a site (Gendron et al., 1998, 2001; Beaudet et al., 2004, but see Fladeland et al., 2003), but does not necessarily reflect any differences in sunfleck exposure among sites (Canham et al., 1990). Because we are working with evergreen plants in deciduous forests, we measured light at four times throughout the year to characterize changes in the seasonal light environment. Because the spring, summer and fall readings were all highly correlated, the fall readings were used as a measure of leaf-on conditions and winter light readings were used as leaf-off conditions. Fall and winter light readings tend not to be well correlated for a given site.

Statistical Methods

Broadly following the approach of Clark (2003) we use hierarchical Bayes methods to characterize demographic rates, estimate effects of light and moisture availability on vital rates, and assemble population growth rates in a projection matrix framework. Fecundity, seedling survivorship, production of clonal ramets, and adult transition probabilities are modeled with sub-models and compiled to calculate population growth rates using eigenanalysis (Clark,

2003; Caswell, 2001). Five demographic stage classes were used, based on their relative homogeneous survival and reproductive rates. These stages are protocorms, seedlings, juveniles (non-reproductive), and two adult stage classes based on the number of leaves.

Fecundity, the production of protocorms by adult plants, is a rather complex process that is divided into parts for estimation (Figure 5.1). Reflecting our ecological understanding of the species' life history, fecundity is modeled as a chain of processes from adults to protocorms in the following year. This process, encompassing flowering, seed production, and protocorm formation, is complicated by the fact that the true numbers of neither seeds nor protocorms are directly observed. These unobserved, or 'latent' variables, must be estimated by the direct observations of the numbers of flowers produced, the number of seedlings observed 2 years later, and ancillary information coming from counts of the number of seed capsules produced per flower, the number of seeds produced per capsule, and rates of protocorm formation derived from seed packet experiments (Figure 5.1). The hierarchical Bayes framework is a useful way here to incorporate these multiple types of information and their associated uncertainties.

The various parts of this fecundity process are modeled as follows. The probability of flowering is modeled as a binomial process

$$Y_{mky} \sim Binomial(\phi_{mky}, n_{mky}), \tag{5.1}$$

where Y_{mky} is the number of flowers in population k and year y produced by adults of size class m, ϕ_{mky} represents the probability of flowering in that population and year, and n_{mky} is the number of adult plants of that size in that cell. ϕ_{mky} is given a beta prior distribution.

The number of seeds produced by each adult plant is modeled as a latent process given what information is observable. Empirical estimates of the number of seeds per seed capsule, and number of capsules per flowering stalk, were used to estimate the number of seeds produced per flowering stalk. Since capsules were not counted for all flowering stalks, particularly in earlier years, the number of capsules per flower is modeled hierarchically as

$capsules_i \sim Poisson(mu)$ with the mean mu estimated from available data across populations. In some cases the length of the flowering stalk is available, and used to estimate the number of capsules in a similar hierarchical manner using data to build the empirical relationship between length and number of capsules. Finally, estimates of the number of seeds per capsule are used to estimate overall seed production per flower, with associated uncertainty given the difficult nature of observing each state.

The probability of protocorm formation from seeds is modeled as a *beta* distribution using data from seed packet experiments to inform the probabilities within each population. Over 120 seed packets placed in each of the populations were used to estimate probabilities of protocorm formation in each population (Diez 2005). Protocorm survival to seedling stage is unobservable given the minute size of protocorms. This latent variable is constrained between two other estimated parameters, protocorm production and seedling emergence, and is thus estimated by default given these two more data-informed parameters. All together, the fecundity sub-model includes estimates of flowering probabilities, protocorm production and seedling recruitment, given all available information and associated uncertainties.

Transition probabilities are estimated from the raw numbers of plants moving among the demographic classes as follows:

$$Y_{ykij} \sim Multinomial(\phi_{ykij}, n_{yki}) \tag{5.2}$$

where Y_{ykij} is the number of plants observed to make the transition from stage class i to class j in population k and year y out of n_{yki} plants in the prior year. ϕ_{ykij} is the probability of a transition from stage j to stage i. Given that these are size classes, individuals can progress or regress among demographic classes, or stay put. The transition probabilities are given Dirichlet prior distributions. The priors are modeled hierarchically, lending the estimation of transition probabilities pooling strength across populations with differing sample sizes. Importantly, the weak inference of some transition probabilities sparsely represented in populations with fewer plants is bolstered by the inference drawn across all populations.

In practice, for software implementation, the Dirichlet distribution is broken into conditional *Gamma* distributions as described in Appendix A.

Although effort was given to find and mark all new plants in each population each year, new non-seedling plants were found each year. These new, unmarked, ramets are potentially the result of two sources: they were either overlooked in previous years as they grew from seedling to adult stages or they resulted from clonal reproduction of an existing adult. Field observations and spatial locations of new individuals were used to differentiate these sources as much as possible. Those plants deemed by proximity likely to be of clonal origin, were used for estimating clonal reproduction at a cell level in a manner similar to the probability of flowering:

$$C_{jky} \sim Binomial(\zeta_{jky}, n_{jky}), \tag{5.3}$$

where C_{jky} is the number of clonal offspring produced in cell j, population k, and year y, and n_{jky} is the number of adults in that cell. The probabilities of producing clonal offspring, ζ_{jky}, are added to the adult survival rates, reflecting an augmentation to the 'production' of new ramets each year.

The posterior distributions for all parameters were used to construct projection matrices and compute population growth rates via eigenanalysis. Direct use of the posterior distributions for subsequent analysis provides a straightforward way of propagating parameter uncertainty through to population-level inferences. For each set of parameter estimates, at each step of the MCMC simulation, a matrix is constructed, the dominant eigenvalue is taken, and a subsequent distribution of population growth rates can be characterized.

Estimating and Predicting Vital Rate Responses to Abiotic Gradients

The approach to modelling demographic responses to abiotic gradients follows the general approach described above, with a few modifications. Demographic rates are estimated on a 2x2 meter cell level instead of the whole population level in order to account for the within

population variability in abiotic conditions and demographic rates. Cells are hierarchically modelled within populations, and population estimates again are drawn from a global distribution. Also, to avoid over-parameterization, adult stage classes are collapsed into a single stage for estimating the effects of the abiotic environment on survival rates and fecundity.

The probability of flowering is modeled as a binomial process with a logit link function, and with abiotic conditions for each cell i specified as covariates as follows:

$$Y_{jky} \sim Binomial(\phi_{jky}, n_{jky}), \tag{5.4}$$

where Y_{jky} is the number of flowers in cell j, population k, and year y, n_{jky} is the number of adults in that cell, and ϕ_{jky} represents the probability of flowering for that cell. The logit transformation allows linearity of parameters, such that

$$\eta_{jky} = log\left(\frac{\phi_{jky}}{1-\phi_{jky}}\right) \tag{5.5}$$

The η_{jky} represents the log-odds of flowering in a given cell, and can now be related directly to explanatory variables at the individual level as follows:

$$\eta_{jky} = \alpha_{0ky} + \beta_1 moist_{jky} + \beta_2 light_{jky} + \beta_3 moist^2_{jky} + \beta_4 light^2_{jky} \tag{5.6}$$

Thus the log-odds of a plant flowering in any given cell is determined by a grid and year-specific intercept α_{0ky} and cell-level abiotic variables. Including the 2nd order terms in the full model allows us to test the hypothesis suggested by niche theory that plants experience an intermediate optimum, above and below which performance declines. Regression coefficients, β_{1-4} are the same across all cells and grids because there is no *a priori* reason to suspect a different inherent relationship between abiotic variables and flowering across sites. Accordingly, each regression coefficient is given a non-informative prior, $\beta_{1-4} \sim Normal(0, 1000)$. The grid-level intercepts α_{0jk} on the other hand are modeled as a random effects, hierarchical in that they are drawn from a common distribution $\alpha_{0jk} \sim Normal(\mu, \tau)$ estimated by the data. Non-informative hyperpriors are specified for the μ and τ parameters as follows $\mu \sim Normal(0, 1000)$ and $\tau \sim InverseGamma(.01, .01)$

The number of protocorms formed per flower was estimated using all of the data, without respect to abiotic conditions. The numbers of protocorms were modeled as a *Normal* distribution, described by the mean and standard deviation estimated from the data. The fecundity parameter used in the projection matrices were the product of this normally distributed number of protocorms multiplied by values drawn from the posterior flowering distribution.

As with flowering, adult and seedling survival rates are modeled as binomial processes and again related to abiotic variables via a logit link function, with the same basic hierarchical structure and specification of priors as the flowering model. Models of clonal reproduction were also extended to the cell-level and effects of abiotic conditions likewise estimated.

Prediction of population growth rates across a range of abiotic conditions proceeds directly within the MCMC framework, as before with the geographic populations. Parameter estimates at each iteration of the sampling routines are used directly for prediction of vital rates at a range of abiotic conditions of interest, thereby translating all uncertainty in parameter estimation to prediction intervals. As before, predicted vital rate responses are assembled within the MCMC framework into projection matrices, and eigenanalysis is used to predict population growth rates.

Results

We found substantial spatial and temporal variation in demographic performance of *G. pubescens* (Figure 5.2). It is clear from the figure that there is a a great deal of variation across all populations and among the different years. The smaller populations also exhibit more uncertainty in their population growth rate estimates. It is instructive to compare the hierarchical analysis to a direct calculation of growth rate using the population-specific observed numbers, which are represented by the X's in Figure 5.2. Many of the means are comparable between the hierarchical analysis and the single matrix estimates, but there are also substantial differences in some cases. The differences reflect both sample sizes in a given population and the degree to which a population is different from others in that year.

Populations with smaller sample sizes and more apparent differences from other populations will be drawn in toward the overall mean via the hierarchical pooling (Gelman et al., 2004).

Individual vital rates differed in how much they could be explained by abiotic variables (Figure 5.3). Model selection was used to test the hypotheses that different seasons of light were important for different vital rates. Winter light availability was found to be the most useful predictor among the seasons for survival. Spring, summer and fall light readings were highly correlated within sites, but were not found to be useful predictors of vital rates, except for fall light having a positive effect on the probability of flowering.

Adult survival was positively related to moisture and negatively related to light availability (Figure 5.4(a)), with a negative second-order moisture term, suggesting a unimodal response function. Seedling survival was also positively related to soil moisture, also showing a unimodal response, but was not significantly related to light. The probability of flowering also followed the unimodal response to moisture, with the 95% intervals slightly overlapping zero on both first and second order terms. Clonal reproduction did not show a significant relationship to either light or moisture.

The predictive distributions derived from these estimates of vital rates are depicted in Figure 5.4. While the uncertainty for all calculations are contained within the posterior distributions, the credible intervals are only depicted for adult survival in Figure 5.4(d) for the ease of viewing the surface shapes.

Vital rate predictions were used to calculate population growth rates as a function of abiotic variables. The surface of predicted growth rates is shown in Figure 5.5. Reflecting the patterns seen in survivorship, we find significant influence of moisture with less of an effect of light availability. Predicted population growth rates increase with increasing moisture and level out above the replacement level of $\lambda = 1$. Were predictions made beyond those levels illustrated in Figure 5.5 the rates would again decline at some point due to the negative second order term.

Comparison of observed distributions and habitat suitability

The comparisons of observed distributions to predicted population growth rate show a mismatch between the observed distribution and habitat suitability (Figure 5.6). At the finest scale, within $4m^2$ cells, a large proportion of predicted suitable habitat appears unoccupied, and to a lesser extent some unsuitable habitat occupied. Plotting the abiotic conditions of populations on these maps suggests a tighter correspondence between occurrence and suitability, and population abundances are broadly increasing with increasing habitat suitability. At the broadest scale, that among landscapes, we see an even tighter relationship between abundance and predicted suitability (Figure 5.6(c)).

Because each predicted vital rate and population growth rate is made up of a full posterior distribution of values, we can summarize the uncertainty of our predicted population growth rates by plotting the range of where positive population growth rates may occur in abiotic space. When we again plot the cell and population-level abundances on these maps of uncertainty, we can see the effect of allowing uncertainty on our inference (Figure 5.7). Although the qualitative direction of the patterns remains unchanged, the proportion of sites within suitable versus unsuitable sites changes substantially.

Discussion

Despite the strong natural history intuition that the abiotic environment is important in shaping species distributions and life histories, surprisingly little work has directly modeled demographic responses of species to environmental gradients. By integrating a combination of intensive demographic and abiotic monitoring within a hierarchical Bayesian statistical framework, this study contributes a unique quantitative assessment of how the demography of the terrestrial orchid, *Goodyera pubescens*, varies across abiotic gradients. We show that a significant component of the spatial and temporal variation in demographic performance can be explained by key abiotic variables, and how the inclusion of estimates of uncertainty influence evaluation of theory and predictions of future dynamics.

Our general finding of substantial spatial and temporal variation of population growth rates supports other studies of forest herb populations, although the degree to which populations fluctuate tends to vary (Jolls, 2003). In general, longer-lived herbs tend to exhibit more stable patterns of population growth, near population replacement levels, while annuals may fluctuate more dramatically (e.g. Kalisz and McPeek, 1992).

Responses to abiotic gradients

Overall, soil moisture had a much stronger influence than light availability on survival, fecundity, and population growth rates. Adult survival responded positively to summer moisture availability and negatively to winter light levels. A negative second order moisture coefficient suggests a saturating effect of moisture within the range of moisture experienced in this region. The probability of flowering was also positively related to moisture, with a negative second order term, but interestingly showed a more significant response to fall light availability than to winter light. Given the high correlation among spring, summer and fall light readings, the response to fall light is taken as a measure of full-canopy conditions rather than anything specific to the fall season.

Moisture availability is a major driver of many ecological processes across ecosystems, including eastern deciduous forests (Stephenson, 1998; Churkina et al., 1999; Xiao and Moody, 2004), but its effects on population dynamics had not been previously quantified. Even in a region with as much rainfall as the southern Appalachian mountains (Coweeta LTER 1950-2003 average $>$ 1800 mm precip/year), where the bulk of this data was collected, soil moisture availability varies considerably across topographic gradients and with soil characteristics (Yeakley et al., 1998, Diez 2005). The importance of water availability for plant growth is well understood physiologically (Lambers et al., 1998), and for determining overall system productivity (Webb et al., 1983; Rosenzweig, 1968), but its role in shaping population dynamics across complex natural landscapes is less well understood. Because precipitation is such an important variable to ecological systems, and predicted to change under

multiple climate change scenarios (Harte and Shaw, 1995; Gordon and Famiglietti, 2004), building explicit links to species dynamics will be critical for assessing likely responses in years to come.

Previous work on understory forest herbs has demonstrated the importance of light gaps, but also highlighted differences in responses depending on species' particular life histories (Collins et al., 1985). Herbs such as *G. pubescens* that are adapted to the very low light environment under full canopies are predicted to have a negative response of survival to light gaps (Collins et al., 1985), while other more sun tolerant species may respond favorably to such openings in the canopy or even depend on such openings for reproduction (Valverde and Silvertown, 1997). The data in our study does not allow an assessment of the effects of tree-fall light gaps, but our finding that increasing winter light has a negative effect on survivorship is consistent with the prediction that shade-tolerant herbs can suffer damage or photo-inhibition under intense light conditions (Oberhuber and Bauer, 1991). However, we find that if a plant survives the high winter light conditions, then greater light availability during the leaf-on season (relative to the overall very low light levels), before fall flowering, contributes positively to the probability of flowering.

We also show how the response of overall population growth rates to light and moisture reflects the dominant responses of individual vital rates. Although several vital rates were significantly affected by both light and moisture, population growth rates are dominated by the effect of moisture (Figure 5.5), highlighting the importance of following how effects on vital rates propagate through to population growth rates. These findings support the findings of other studies that have used elasticity analysis to show that survival of adults is more important than fecundity for determining population growth rates in long-lived perennials (Silvertown et al., 1993).

Niche relationships

As predicted by Pulliam (2000), there is not a one to one relationship between observed distribution and habitat suitability; however, the magnitude of the mismatch is strongly scale dependent. The degree of correspondence between predicted suitability and abundance clearly increased with increasing scale. This scale-dependent lack of correspondence between the observed distribution and the suitability of habitat suggests that the impacts of biotic and/or stochastic constraints on the species distribution occur most significantly at a very local scale. The mismatch between observed distribution and suitable habitat at this scale is likely due to a combination of biotic influence and dispersal limitation. Competition within the sometimes dense, species-rich understory of eastern deciduous forests may be the most obvious explanation for local displacement from suitable habitat. Another strong possibility with this species, however, relates to the obligate mycorrhizal relationships the orchids have with a fairly specialized group of soil fungi (McCormick et al., 2004). Other work in this system has shown how the effect of fungal distributions on recruitment patterns of the orchid can be influenced by small scale soil heterogeneity (Diez, 2005). Additionally, seed and dispersal limitation could lead to local absences if there are simply not enough recruits to occupy all available and suitable habitat.

Lacking information on rates of immigration and emigration at different scales, our assessment of habitat suitability falls short of identifying habitat sources and sinks (Kadmon and Tielborger, 1999), but is supportive of the prediction of source-sink and metapopulation theory that there will be unoccupied but suitable habitat and occupied unsuitable habitat. From such a pattern of site occupancy we can infer the scale at which processes are occurring that keep a species out of equilibrium with the distribution of suitable habitat. This will help in the design of further experiments focusing on important biotic and abiotic effects at the appropriate scale. An informative extension to this work will be to compile such data for multiple species and explore how life-history characteristics, such as seed size or phenology, influence the correspondence between distribution and suitability.

Links to abiotic environment There has been very limited success linking vital rates to measurable aspects of a species' environment. While experimental studies have been able to establish the mechanistic link between moisture and light and plant performance (Muraoka et al., 1997; van Hinsberg and van Tienderen, 1997; Kulheim et al., 2002; Eckstein, 2005; Penuelas et al., 2004; Engelbrecht and Kursar, 2003), it has proven more difficult to characterize demographic responses across heterogeneous environments of natural populations. The rich literature of climate envelope modeling is important for placing broad-scale bounds on factors influencing species distributions (Pearson and Dawson, 2003; Luoto et al., 2005), but to avoid assumptions of equilibrium distributions (Araujo and Pearson, 2005), detailed studies of species demography over natural gradients are essential for predicting how stage-specific responses to abiotic variables translate into population dynamics across complex landscapes. When possible, of course, careful experimental designs embedded within observations across natural gradients can be used to refine our understanding of observed patterns across the complexities of natural landscapes (Dunne et al., 2004).

Complexity and uncertainty. A major challenge for ecologists across sub-disciplines is finding a coherent way to estimate the variability and uncertainty associated with complex processes, and the hierarchical Bayes (HB) framework used here offers new hope (Clark, 2005; Wikle, 2003*b*,*a*). Many studies use data that are structured both from necessarily complex (often unbalanced) study designs (cells within plots, within landscapes), and from levels of biological organization (individuals within populations, within communities). Reflecting the importance of scale to ecological inference, HB methods are enabling hypotheses to be tested at each of these levels explicitly (Raudenbush and Bryk, 2002; McMahon and Diez, 2005). Moreover, multiple data sources, including both experimental and observational, are often available to inform estimation of difficult latent processes, and HB provides a robust framework for integration of disparate data sources (Clark and LaDeau, 2005; Okuyama and Bolker, 2005). Consistent with an ecological understanding of how individuals and groups are

related, hierarchical models allow pooling of information across subgroups (Gelman et al., 2004). This provides more robust inference for smaller sub-groups without having to completely collapse them into the larger groups, and prevents the collapse of variability just due to larger sample sizes by relaxing the unrealistic assumption that all variability stems from estimation error (Clark et al., 2003*b*).

Proper estimation of process variability and model uncertainty is critical for the evaluation of ecological theory (Clark et al., 2003*b*), as well as for management and conservation decisions (Williams, 2001; Brewer and Gross, 2003). It is well-recognized that predictions of population dynamics based on demographic data are limited by the range of variability contained in the period of time and space of data collection (Caswell, 2001). The important role of variability stemming from stochastic environments in predictive analysis of population growth has been recognized for some time (Lewontin and Cohen, 1969), and the incorporation of stochasticity has proven important for both theoretical (Tuljapurkar, 1985) and applied investigations (Fieberg and Ellner, 2001). Although the goal in this study was not prediction of long-term dynamics, but rather current niche relationships, the ability to explain the effects of environmental driving variables on stochasticity will enhance capacity for making future predictions.

A final opportunity afforded by the HB framework, relevant to predictive demographic analysis is ease with which posterior predictive distributions may be interpreted (Wade, 2000; Ellison, 2004). Parameter estimation and prediction are performed simultaneously, and the predictive posterior probability distributions may be more easily incorporated into subsequent dynamic models exploring population behavior. While bootstrapping can be a useful method for constructing confidence intervals for derived statistics such as population growth rates, it alone does not accommodate all sources of variability, does not yield predictive distributions, and does not address the issue of inherent variability among sub-units of a population, so confidence intervals will converge toward zero as sample sizes increase.

Conclusions

Terrestrial orchids such as *G. pubescens* live in complex understory forest environments and have evolved complex life histories. They are long-lived, clonal, produce thousands of uncountable seeds, have obligate relationships with mycorrhizal fungi that are needed for germination, and spend their first year unobservable in a protocorm stage. Complexities in life histories are more the rule than the exception across taxonomic groups and may inspire appreciation for the interesting ecology but also present challenges to predictive demographic modeling. Further, linking the dynamics of ecological systems to key underlying abiotic variables will continue to be a difficult but critical link to understanding and prediction. Tools are increasingly becoming accessible for building models that incorporate sources of variability from multiple levels of organization and provide straight-forward summaries of prediction uncertainties. Approaches that can incorporate multiple sources of information into predictive probability statements that include associated uncertainties will be useful for theoretical and applied areas of ecological research.

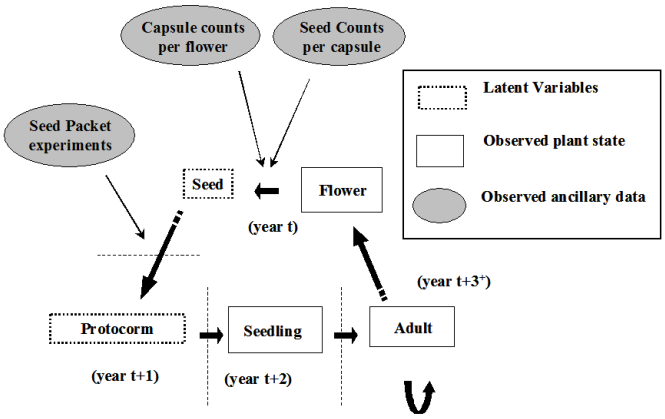


Figure 5.1: **Life history diagram.** Latent variables are those variables of interest for demographic modeling but are not observable. They are estimated using a combination of observed plant states and ancillary data from additional experiments relevant to the transition of interest.

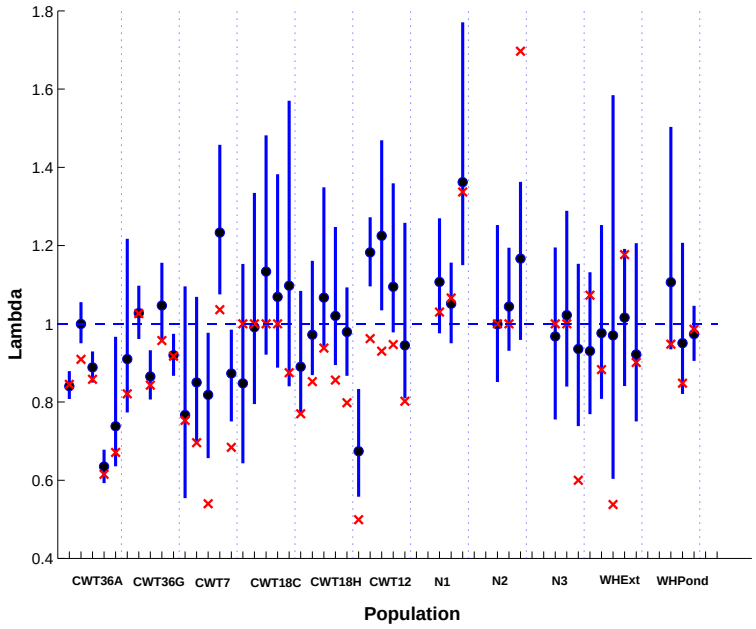
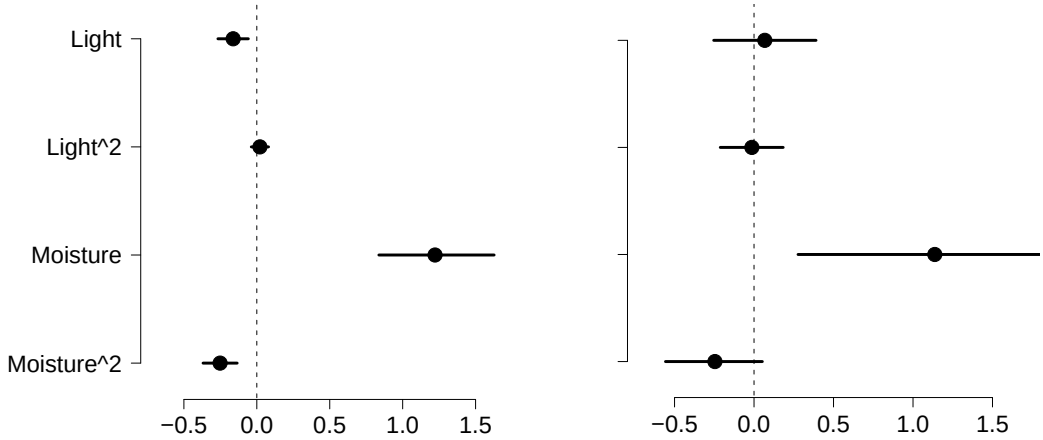
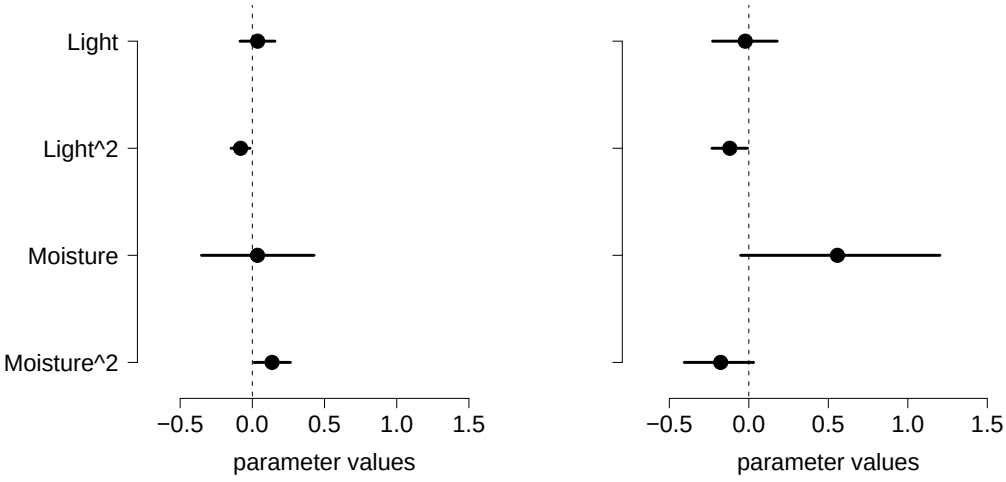


Figure 5.2: **Population Growth Rates.** Plotted are 95% credible intervals for population growth rates over 5 transition periods, summarized from posterior distributions estimated by hierarchical Bayes methods. Five transitions are shown for each population, from 1999-2000 on the left through 2003-2004 on the right. The X's represent a non-Bayesian estimation of growth rate using a Lefkovitch matrix from that population and that time period alone. Differences in the means represent the effects of the hierarchical Bayes approach, typically shifting estimates of those populations with lower sample sizes slightly towards the global mean for a given year. Note that populations N1, N2, N3, and WHPond have no data from the first two years and thus have only 3 transitions represented.



(a) Adult Survival

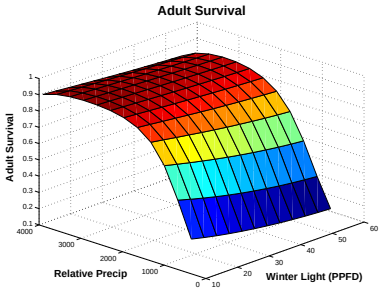
(b) Seedling Survival



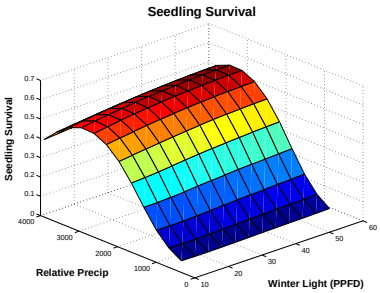
(c) Clonal Reproduction

(d) Probability of Flowering

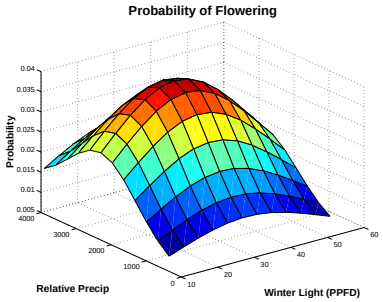
Figure 5.3: **Coefficient Estimates.** Lines represent 95% posterior credible intervals for responses of (a) adult survival, (b) seedling survival, (c) clonal production, and (d) probability of flowering relative to winter light and the moisture. Light refers to winter PAR readings, and moisture variables are the moisture index combination of relative soil moisture potential and precipitation



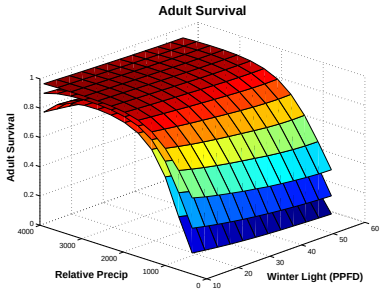
(a) Adult Survival



(b) Seedling Survival

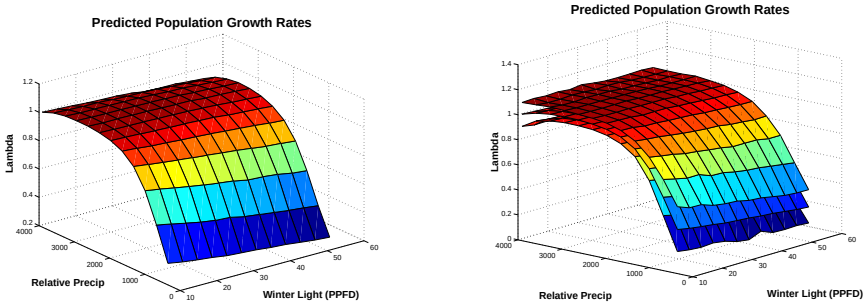


(c) Probability of Flowering



(d) Predicted Adult Survival with associated 95% credible intervals

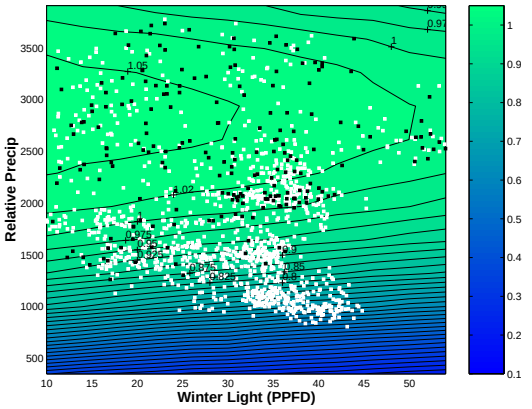
Figure 5.4: **Predictive Distributions of Vital Rates.** Surfaces represent the mean of the posterior distributions for predictions of responses across a grid of abiotic conditions, for a) survival of adults, b) survival of seedlings, and c) probability of flowering. To visualize the fact that all predictive distributions also contain complete estimates of uncertainty within their posterior distributions (d) shows the 95% credible region for the adult survival estimates.



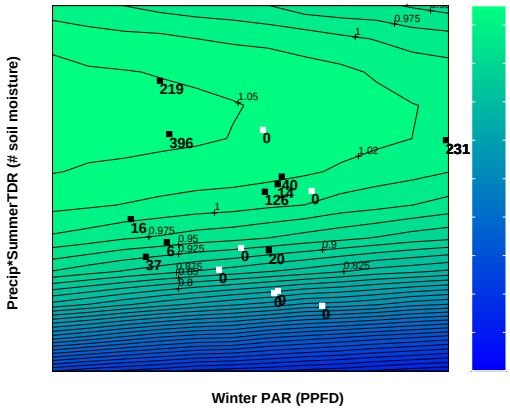
(a) Predicted Lambdas vs Abiotic

(b) Lambdas with 95% intervals

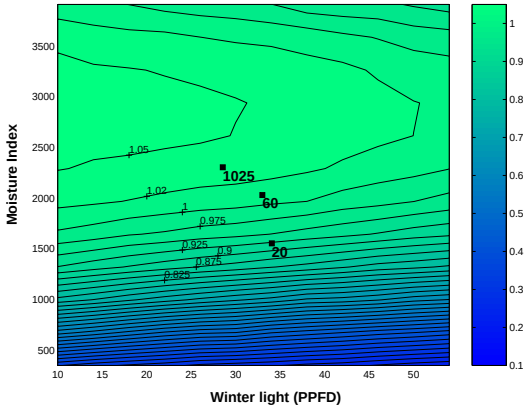
Figure 5.5: **Predicted population growth rates.** (a) Mean predicted population growth rate and (b) 95% credible intervals, as a function of light and moisture availability.



(a) Lambda Contours with cells

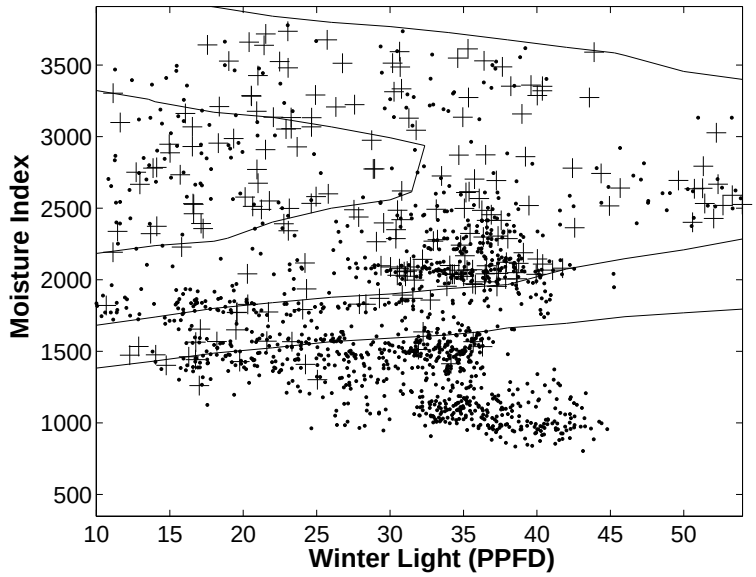


(b) Lambda Contours with grids

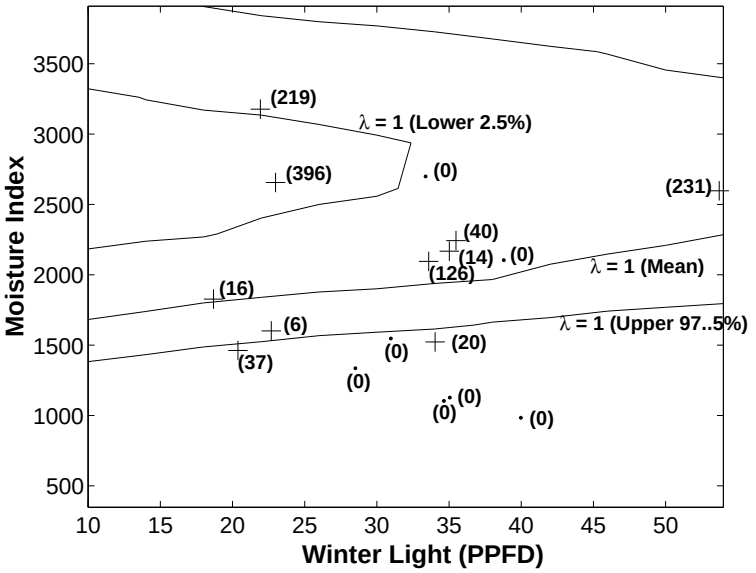


(c) Lambda Contours with landscapes

Figure 5.6: **Observed distributions across scales.** Data shown for 2003 at three spatial scales: (a) Fine scale: Mean predicted population growth rate with individual cells. White cells contain no plants; black cells are occupied. (b) Population scale: abundances given in bold. (c) Landscape scale: : abundances given in bold.



(a) Lambda Contours with cells



(b) Lambda Contours with grids

Figure 5.7: **Measuring uncertainty.** Same data shown for 2003 at (a) cell and (b) population scales. Small circles represent sites where *G. pubescens* is absent and pluses are sites where it is present. The numbers next to the plots in (b) show the abundance on each grid. The middle contour is the mean where lambda=1 (the mean of the posterior distribution). There is a 95% chance that the lambda=1 line falls between the outer two contours.

Chapter 6

Spatial Genetic Structure of a Terrestrial Orchid and its Symbiotic Fungi [1]

[1]Diez, J.M., Melissa McCormick To be Submitted to *Molecular Ecology*

ABSTRACT

Symbiotic relationships are ubiquitous across taxonomic lines and have the potential to influence demographic processes and patterns of genetic variability of associated species. The nearly ubiquitous symbioses between plants and mycorrhizal fungi are known to be important for plant nutrition, with apparently varying levels of specificity for plant and fungal associates. Although many studies have described patterns of genetic variability within and among populations of different plant taxa, and some fungal taxa, no studies to date have concurrently studied the spatial genetic structure of any plant and its mycorrhizal symbionts. In this study, we use genetic data from five mapped populations of the terrestrial orchid, *Goodyera pubescens*, and its associated mycorrhizal fungi from the genus *Tulasnella*, to test hypotheses about the spatial partitioning of genetic variation of both host and symbiont.

We found most of the genetic variability partitioned within populations compared to among, likely reflecting the high dispersal capabilities and clonal growth of both taxa. Within populations, there was significant spatial autocorrelation in genetic diversity for both the plants and the fungi. We also report an interesting pattern of association, in some environments but not others, among plant and fungal genotypes after controlling for geographic distance. Finally, we use extensive abiotic and demographic monitoring on the sites to explore the role the abiotic environment may have in shaping observed patterns of genetic diversity of both the plants and fungal symbionts through effects on demographic processes.

Introduction

Symbioses add an interesting layer of complexity to the question of how genetic variability is partitioned within a species. Tightly interacting species can influence each other's demographic rates and resulting patterns of genetic diversity (Nuismer et al., 1999; Thompson, 2005). Typically not static relationships, symbioses may be dynamic interactions both ecologically and evolutionarily, unfolding across a heterogeneous template of abiotic and biotic

variables that may alter the nature of the interactions (Thompson, 1999*b*). Mycorrhizal relationships between plant roots and soil fungi are one of the most ubiquitous terrestrial symbioses, known at least to influence plant nutrition and possibly a range of fitness-related aspects of performance (van der Heijden and Sanders, 2002). The effects of specific associations on fungal fitness are even less well known, although host-specific growth rates have been suggested for at least some groups of fungi (Bever, 2002).

In this study we tested hypotheses about the partitioning of spatial genetic variation, at multiple scales, of a terrestrial orchid, *Goodyera pubescens*, and its mycorrhizal fungi from the basidiomycete genus *Tulasnella*. The perennial, outcrossing and clonal life histories of many forest herbs, including this orchid, lead to a range of possibilities for the distribution of genetic diversity within and among populations. Moreover, understory plants of eastern deciduous forests experience a very heterogeneous forest floor environment, which could also influence demographic rates, selection, and distribution of genetic variability (Whigham, 2004). Among herbs, orchids are unique for reasons that have intrigued ecologists for many years, including their complex pollinator relationships and specific mycorrhizal relationships that are needed to grow to photosynthetic stage. The tiny orchid seeds lack a developed endosperm and depend on nutrition from associated fungi at least until reaching photosynthetic stage (Rasmussen, 1995) and to some extent thereafter (Gebauer and Meyer, 2003). These obligate and rather specific relationships may have implications for patterns of recruitment and genetic diversity of both plants and fungi, however very little is known about the distribution of the fungi in nature or the strength of coevolutionary pressure between the two groups.

There is now a large body of studies that describe the partitioning of genetic variation within and among populations of plants (reviewed by Hamrick and Godt, 1996; Nybom, 2004). The potential for long-distance dispersal of the tiny seeds leads to the expectation of high rates of gene flow among populations of orchids, which has been confirmed in a few studies (Brzosko et al., 2002; Trapnell et al., 2004; Smith et al., 2002, but also see Sun and

Wong, 2001). Studies of basidiomycete soil fungi have likewise found a large portion of the genetic variability within populations (Kauserud and Schumacher, 2003; Kretzer et al., 2005; Hogberg et al., 1999), although some spatial structuring has been found at large spatial scales (James et al., 1999). Much has also been learned in recent years about the structure of plant genetic diversity at very local scales (Vekemans and Hardy, 2004), and within population studies of mycorrhizal fungi have demonstrated individual clone sizes ranging from several meters to tens of meters (Redecker et al., 2001; Gherbi et al., 1999; Zhou et al., 1999; Kretzer et al., 2004). Because the plants and the fungi are capable of both sexual and clonal reproduction, and clones are visually indistinguishable from sexual recruits in the field, the relative importance of these different forms of reproduction in establishing population structure is unclear. However, there have been few studies of within-population spatial genetic structure of terrestrial orchids and no such studies of their mycorrhizal fungi. Moreover, no study to our knowledge has simultaneously investigated the spatial genetic variability of any plant and its associated mycorrhizal fungi.

The orchid mycorrhiza is a tight physiological symbiosis, and obligate at least for the plants, but the degree of association between particular genotypes of plants and fungi is unknown. Some work has suggested strain-specific functional differences within the *Tulasnella* studied in this research (McCormick et al., 2005), which may increase the likelihood of strain-level selection. Work with symbioses across taxonomic lines suggests that the function, specificity and genetic variability of closely coupled relationships such as between these plants and fungi, are likely to vary across environmental gradients as well (Thompson, 1999*a*; Bronstein, 1994). Thus, it is reasonable to expect that the correspondence observed between host and symbiont genetic diversity may depend on environmental conditions. However, while many studies have quantified the partitioning of genetic variance, far fewer have been able to explore the underlying causes of that structure, which may be both abiotic and biotic. Previous work with understory herbs has suggested that patterns of genetic variability may

be linked to the abiotic conditions at the forest floor, including light (Kudoh et al., 1999) and soil moisture (Pulliam et al. manuscript, Gonzales, unpublished data).

In this study we use inter-simple sequence repeat (ISSR) markers to test a series of hypotheses about the spatial genetic structure of 5 populations of the terrestrial orchid *Goodyera pubescens*, and its mycorrhizal fungi from the genus *Tulasnella*, across a large environmental gradient. Specifically, we test the following hypotheses: (1) The high potential rates of gene flow of both the plants and fungi, together with their clonal growth form, lead us to hypothesize that most of the genetic variability is found within populations, but significant spatial structuring (autocorrelation) of genetic variability within populations would result from the clonality. (2) Based on the potential for co-migration under clonal growth of plants, we use local neighborhood analysis to test the hypothesis that the observed spatial genetic autocorrelation (in 2-dimensional space) of the plants and fungi are congruent within populations. (3) Although there is no evidence of strong strain-specific selection between the plants and fungi, we use the paired plant-fungal sampling to test the hypothesis that there is covariance between the genetic relatedness of the plant and associated fungi, and that this covariance changes across environmental gradients. Finally, (4) although we use the population-level abiotic and demographic data to test the hypothesis that patterns of genetic diversity, and particularly clonal diversity, should be related in predictable ways to underlying abiotic and demographic processes. Specifically, previous research and basic biology led us to predict that variation in soil moisture and light availability would influence the clonality of plants, while soil moisture and organic content would in part drive the clonality of the fungi.

Materials and Methods

Study Species

Goodyera pubescens is a terrestrial, evergreen, clonal orchid distributed throughout eastern North America, from Florida, west to Arkansas, north and east into Canada. The species'

main above and belowground growing period is from summer to fall (Rasmussen and Whigham, 2002), and spreads clonally via branching rhizomes. Roots coming from the rhizome are long-lived, as in many terrestrial orchids (Rasmussen, 1995). Flowers develop in July and August, which if pollinated form capsules containing thousands of tiny seeds (< 2 mm length, < 0.5 mm across). *G. pubescens* has been found to be self-compatible but not self-fertilizing (Kallunki, 1981), and although likely pollinated by bees, the pollinators at our study sites are unknown. Gravity and wind dispersal of the seeds begins with dehiscence of the capsules in September, but can be spread over several months, with some seeds occasionally still remaining in the pods by early Spring (J.D., pers. obs.). Like most orchids, *G. pubescens'* seeds lack significant nutrient reserves and are therefore dependent on the transfer of carbohydrate energy, water and mineral nutrition from mycorrhizal fungi, at least until reaching photosynthetic stage.

G. pubescens associates with mycorrhizal fungi from the genus *Tulasnella*, a group of saprotrophic basidiomycetes from the order *Tulasnellales*. McCormick et al. (2004) determined through ITS sequencing that the *Tulasnella* associating with *G. pubescens* likely represent only one or two species, most closely aligning with *T. bifrons*. Isolates from *Goodyera* roots have been shown to utilize cellulose but not lignin (e.g. from dead leaves and wood) as substrates. Moveover, using isolations from multiple roots from single adults McCormick et al. (2005) have shown that *G. pubescens* associates with only one fungal strain at a time. Little is known about the nature of the relationship beyond the seedling stage, although adults remain heavily colonized by the fungi (Rasmussen, 1995; Rasmussen and Whigham, 1998*b*). While achlorophyllous, 'myco-heterotrophic' plants have been known to depend on fungal associates for nutrition and energy (Leake, 1994), recent stable isotope evidence suggests that even autotrophic terrestrial orchids continue to gain both carbon and nitrogen from their associated fungi (Gebauer and Meyer, 2003).

Study Design and Sampling Protocol

Individually mapped plants within five populations were monitored yearly for growth and reproduction from 1999 to 2004 on roughly 500 m^2 study grids. These populations are located across a geographic gradient of approximately 120 km. from the Piedmont of Georgia to the southern Appalachian Mountains, encompassing much of the range of abiotic conditions experienced by forest herbs in the region. Because the rhizome of *G. pubescens* is above-ground, distinctions could be made between separate and connected rosettes. Each ramet was marked and it was noted when a single ramet clearly split into multiple ramets via clonal growth. Seed pods on flowering stalks were counted and careful searches were conducted each year in the early fall to find new plants, including seedlings. The southernmost population was located in Whitehall Forest in Athens-Clarke County, GA (33°92' N latitude, 150-240 m elevation), one in the Nancytown area of north Georgia (34°31' N latitude, 315-450 m elevation), and three within the Coweeta Hydrologic Laboratory (35°03' N latitude, 750 - 1,500 m elevation) in Macon County, N.C. The three Coweeta populations were significantly closer together than the other two, but still considered here as separate populations due to quite different abiotic and demographic characteristics.

Tissue from forty individuals within each of the populations was sampled during the Fall and Winter of 2004, for a total initial sample size of 200 plants. As *G. pubescens* tend to occur in clusters of ramets, care was taken to sample some plants within the same clusters and from clusters distributed throughout the population, to assure the sampling of pairs of plants at a range of geographic distances, from 10 cm up to 24 meters. Roughly a quarter of one full healthy leaf was sampled from each selected plant, and one root was taken for later extraction of mycorrhizal fungi. Roots were stored in a moist towel until extraction within 24 hours of sampling. Roots were cleaned, scraped, and single fungal pelotons were serially washed in sterile water before being plated on a modified basal salts medium (Caldwell et al., 1991) or potato dextrose medium. Once hyphal growth was observed, mycelium from

a single peloton was transferred to new medium under sterile conditions and ultimately to liquid medium, from which tissue was extracted for subsequent DNA extraction.

Genetic Analyses

DNA was extracted using a combination of modified CTAB buffer extraction, and Qiagen DNEasy kits. Each ISSR reaction contained 1μL of DNA, 10.25μL H_2O, 1.25μL of primer, and 12.5μL of Gene Choice RedMix PLUS. Preceded by 2 minutes at 96° C, thirty-five cycles of 1 min. at 94° C, 1 minute at 44° C, and 2 min. at 72° C were used. After screening 56 ISSR primers, we found three primers that yielded a total of 17 polymorphic loci for the plants, and a single primer that yielded 35 polymorphic bands for the fungi.

Abiotic Monitoring

Each study grid was divided into 2 x 2 meter cells for sampling the abiotic environment. The handheld Soil Water Content Measurement System from Hydrosense was used to measure volumetric soil moisture in the top 12 cm of soil on 16 'intensive' cells within each of the study grids every two weeks between the years 2003-2004. These 16 cells were distributed evenly across the grid. Average values for the summer growing season were used in analyses. Values from an adjacent study grid 100 m from the Whitehall population were used for lack of equivalent monitoring in the population sampled for this study.

Understory light availability, as measured by Photosynthetic photon flux densities (% PPFD), was measured at the level of forest ground cover (0.5 m) using a handheld AccuPAR ceptometer. Eighty ceptometer readings were taken on each grid, with concurrent measurements taken in nearby open clearings, allowing for calculation of percent transmittance of incident canopy PAR to the forest floor. Measurements were taken between 10 AM and 2 PM on overcast days, thought to better represent overall light availability characteristics for a site (Gendron et al., 1998, 2001; Beaudet et al., 2004, but see Fladeland et al., 2003).

Statistical Methods

ISSR markers are inherited in a dominant fashion and thus each band is treated as either present or absent. Subsequent analysis is based on pair-wise genetic distances calculated using the Euclidean distance metric (Excoffier et al., 1992; Huff et al., 1993) as $d_{xy} = n - n_{xy}$: where n_{xy} is the number of loci at which two individuals share an allele, and n is the total number of polymorphic sites. The distance measure thus amounts to a tally of the number of differences in alleles between the individuals.

From the genetic distance matrix calculated for all plants, an AMOVA partitioning of the total genetic variation was used to obtain a measure of among and within population variation as

$$\phi_{PT} = \frac{V_{AP}}{(V_{AP} + V_{WP})} \tag{6.1}$$

where V_{AP} is the among population variation and V_{WP} is the within population variation. Intuitively then, higher ϕ_{PT} values indicate greater population differentiation, and serves as a multilocus analogue to G_{ST} for variance partitioning, suitable for binary data (Excoffier et al., 1992; Peakall et al., 1995). Nei's G_{ST} and the multilocus ϕ_{PT} have been found to be quite similar when compared using the same datasets (Nybom, 2004). Pairwise population-level relatedness was evaluated using Nei's genetic distance (Nei, 1972).

Pairwise genetic and geographic distance matrices for each population can be used to calculate a measure of autocorrelation, r, for each distance class, providing a measure of genetic similarity of individuals growing within a range of distances (see Smouse and Peakall, 1999 for details). Individual populations are evaluated separately, and then pooled together to increase statistical power for discerning patterns. Statistical significance of autocorrelation at each distance class was tested using permutation tests (1,000 permutations).

To identify potential spatial clusters, or 'hot-spots' of genetic relatedness, two-dimensional spatial autocorrelation analysis was also performed in which the correlation of genotypes was calculated for a moving three-neighbor window around each plant. Permutation tests in which correlations were calculated for randomly assembled neighborhoods were used to test

the significance of observed correlations. All autocorrelation and AMOVA calculations were performed using GenAlEx v6 (Peakall and Smouse, 2005).

Clonal diversity was assessed for each population in two ways: by a ratio of the number of genets to the number of ramets sampled, and using the Simpson's index, calculated as $\frac{n}{n-1}(1 - \sum p_i^2)$, where p_i is the frequency of genotype i. The Simpson's index was preferable to other diversity indices in this case because of its insensitivity to variable sample sizes. A pairwise bootstrapping approach was used to assess the significance of population differences in clonal diversity. Finally, permutation tests were used to explore the differences in genotypic composition of the different populations.

Partial Mantel tests were used to test for the correlation of plant and fungal genetic variability within populations while controlling for the similarity of genotypes simply due to spatial proximity. Both linear regressions and Spearman rank correlations were used to test for relationships between clonal diversity and demographic and abiotic variables. We used the R statistical computing package for regressions, Spearman correlations, and Mantel tests (R Development Core Team, 2005).

Results

Partitioning of Genetic Variation

The amount of polymorphism detected with ISSR primers proved sufficient for a reasonable degree of resolution in detecting clonality and describing patterns of genetic variation. Most of the genetic variability of both the plants and fungi was found within populations, with only 10 % of the genetic variation of the fungi, and 17 % for the plants, found among populations.

Spatial Genetic Structure

We also found significant spatial autocorrelation of genetic variation within populations for both the fungi and the plants, but the patterns vary among populations (Fig. 6.1). When considered together, the plants show significant autocorrelation only at the closest

distance class of 1 meter, but when analyzed separately there are moderate population differences, with all of the populations showing significant spatial structure within 1 meter, but population CWT12 exhibiting spatial structure up to 5 meters (Fig. 6.1(b)). The fungi exhibit spatial structure across a larger distance, with significant autocorrelation detected at both the 1 and 2 meter distance classes (Fig. 6.1(c)). As with the plants, there are also population differences in the degree of spatial autocorrelation, although the basic pattern is consistent(Fig. 6.1(d)). For both the plants and fungi, when identical clones are removed from analysis there was no significant spatial structure. The probabilities of observing the same multilocus genotype by chance was calculated for each genotype that was observed multiple times, and in all cases found to be less than 0.005, lending confidence that duplicate genotypes were indeed clones.

We also can see from the two-dimensional analysis that the spatial structure of the fungal genetic variation is heterogeneous within populations (Fig. 6.2). The plant two-dimensional analysis likewise revealed heterogeneous spatial structure of genetic variation within populations (Fig. 6.3).

Clonal Diversity

Clonal diversity differed among populations and also between plants and fungi. The plants ranged from a high *genet/ramet* ratio of .927 (Simpson's index of .996) in population CWT36G, to a low *genet/ramet* ratio of .707 (Simpson's index of .980) in the Nancytown population (Table 6.1). The fungi, with slightly less overall clonal diversity, ranged from a high *genet/ramet* ratio of .816 (Simpson's index of .995) in population CWT36G, to a low *genet/ramet* ratio of .581 (Simpson's index of .944) in the Whitehall population (Table 6.1). While only a couple of the populations showed significant differences from one another in clonal diversity using pair-wise permutation tests, more of the plant populations tend toward being significantly different from one another than the fungi (Table 6.2). All populations were significantly different from each other in genotypic composition ($p < .01$).

However the most clonally diverse plant populations did not necessarily associate with the most clonally diverse fungi (Spearman rank correlation: p=.23).

Covariance among plant and fungal genotypes

The partial Mantel tests suggested a correlation between plant and fungal genotypes, after controlling for spatial proximity among pairs of samples, for the Whitehall population and the Nancytown population, but not for the Coweeta populations (Table 6.3).

Relationships with Abiotic and Demographic Processes

We found substantial variation among populations in most key demographic parameters and abiotic variables. Comparisons between clonal diversity and abiotic and demographic variables were made at the population level, so the sample sizes were small (N=5). None of the relationships were statistically significant, but the patterns were suggestive of a relationship between both abiotic and demographic variables and clonal diversity in both the plants and the fungi. Spearman rank correlations (ρ) are given to indicate the direction and magnitude of the correlation. Both the plants and fungi showed a decreasing trend in clonal diversity with increasing light availability (Fig. 6.4(a); ρ = -0.56, plants; ρ = -0.8, fungi), and an increasing trend with moisture (Fig. 6.4(b); $\rho = 0.72$, plants; $\rho = 0.7$, fungi), suggesting that light contributes to clonal growth and higher moisture supports relatively greater sexual recruitment compared to clonal growth.

Using the direct plant demographic observations, we see a non-significant but decreasing overall relationship between clonal diversity and observed clonal production in the field (Fig. 6.4(d); ρ = -0.56, plants; ρ = -0.8, fungi), an increasing relationship with flower production at the population level (Fig. 6.4(e); $\rho = 0.56$), and a decreasing relationship with survival rates (Fig. 6.4(f); ρ = -0.46).

Finally, although again the sample size is small and not statistically significant, there was a suggestive pattern of increasing clonal diversity within the fungi with higher organic content of the soil (Fig. 6.4(c); $\rho = 0.5$).

Discussion

Overall genetic partitioning - fungi and plants

This study provides the first joint characterization of the spatial genetic structure of a plant and its mycorrhizal symbionts. Our results for the overall structuring of plant genetic diversity are in general concordance with other studies of orchids finding high within-population genetic variability relative to among populations (Trapnell et al., 2004; Smith et al., 2002), suggesting that diversity within populations is maintained by sufficient transfer of genes via either pollen or seed transport. The tens of thousands of tiny seeds produced per flowering individual likely enable at least periodic long-distance wind dispersal. A similar pattern was found for the fungi, suggesting sufficient long-distance dispersal of fungal spores to prevent major differentiation among populations. These *Tulasnella* fungi produce resupinate (flat) fruiting bodies, but spores may still be wind-blown for long distances. Direct estimates of long-distance dispersal of fungal spores are limited (Edman and Gustafsson, 2003; Hallenberg and Kuffer, 2001), and some evidence suggests shorter spore dispersal distances than conventional wisdom might suggest (James and Vilgalys, 2001). Other molecular studies of basidiomycete soil fungi have also found a large portion of the genetic variability within populations (Kauserud and Schumacher, 2003; Kretzer et al., 2005; Hogberg et al., 1999), however there are also examples of basidiomycetes exhibiting significant population structure at large spatial scales (e.g. James et al., 1999).

Spatial autocorrelation and 2D heterogeneity

Within populations we found significant spatial autocorrelation of genetic variability for both the plants and fungi. The fungi showed autocorrelation up to a spatial scale of 2 meters,

while the plants only showed significant structure within 1 meter. This kind of spatial genetic structure could arise from a number of processes that keep progeny close to parents, including limited propagule dispersal or pollen transfer distances, or clonal reproduction. When we removed duplicate genets from the analysis the autocorrelation disappeared, suggesting that most of the genetic autocorrelation appears to be driven by the clonal structure of both plants and fungi instead of by structuring via sexual recruits. These findings are consistent with spatial structure estimates of *Tulasnella* in Maryland (McCormick, unpub.data), and support other studies that have found small genet sizes less than a few meters for other mycorrhizal fungi (Redecker et al., 2001; Gherbi et al., 1999), although others have found larger genet sizes of tens of meters (Dahlberg, 1997).

The two-dimensional autocorrelation analysis revealed heterogeneous patterns of autocorrelation within populations, likely reflecting either an influence of micro-habitat variation within populations or colonization histories. Interestingly, however, the two-dimensional spatial patterns of the plants and fungi (Figures 6.2 and 6.3) appear not to be entirely the same for any given population, suggesting factors beyond simply co-migration of plant and fungal genets. A plausible explanation may be that small-scale variation in light drives plant clonality while soil conditions such as overall organic content, specific substrate availability, or moisture availability are more important for shaping patterns of fungal genetic structure.

Plant-fungal interactions

The paired plant-fungal sampling of the current study also allowed a unique assessment of the degree of correspondence between host and symbiont genotypes across an environmental gradient. We found that the two southernmost populations were the only two to show significant covariance between plant and fungal genetic variation after controlling for the effect of distance (Table 6.3). Because very little is known about the mechanisms underlying the formation of orchid symbioses or the pool sizes of available fungi in the soil, the observed pattern is difficult to interpret but suggestive of a couple possibilities. If the plants have a

role in selecting fungal strains from a larger pool of available soil fungi, these findings would suggest a more consistent selectivity in the southern populations. The southern populations receive significantly less precipitation than the more northern populations, which is reflected in consistently lower soil moisture levels (Diez, in prep). Other work in Maryland in which fungi were re-sampled from plants' roots in different years has suggested that symbiont switching may be related to stressful conditions (McCormick et. al. in press). Our findings of closer association between plant and fungal genotypes in drier habitats is consistent with this possibility. However, it may also be possible to achieve such a pattern without pair-wise selection of any kind if both plants and fungi are responding in a similar way to micro-habitat variations that structure genotypes independently of each other. Methods currently being developed to sample fungi from the soil (e.g. Fierer et al., 2005; O'Brien et al., 2005) will likely shed significant light on these relationships.

Abiotic and demographic relationships

The degree to which abiotic conditions were driving the patterns of genetic variability that we found in this study is unclear, however data at the population level suggested the possibility of a causal chain linking the abiotic conditions to demographic processes, which ultimately determine the structuring of genetic variability. Other intensive demographic work across a broader range of populations has found significant effects of soil moisture and light availability on this orchid's demographic processes (Diez and Pulliam, 2005).

There are a few plausible interpretations of the lack of statistically significant relationships between clonal diversity and measured abiotic and demographic variables. One possibility is that the abiotic environment across this geographic gradient may not be a major factor driving demographic rates and subsequent patterns of genetic diversity. Alternatively, and perhaps more believable given other work linking abioitic the same variables to demographic rates across a larger number of populations (Diez and Pulliam, 2005), we may not have sampled enough populations to see the relationship. A third possibility though, when

considering the apparent two-dimensional heterogeneity of genetic autocorrelation within populations, is that the abiotic drivers may be acting at a within-population scale. From what we know about heterogeneity of forest floors and the small scales at which plants can respond to abiotic environments (Hamrick and Holden, 1979; Hamrick and Lee, 1987), it is very possible that significant variation in clonal diversity can be explained by within-population abiotic heterogeneity.

The comparison of observed demography and genetic diversity also were not statistically significant, perhaps for similar reasons. One might expect a very direct relationship between observed demography and measured genetic diversity, but even four years of observed demography covers a shorter time frame than the processes operating to establish genetic variability in adult populations of these long-lived herbs. While direct monitoring can inform us of current demographic processes, population genetic sampling typically reflects processes operating over larger time frames, with notable exceptions such as parentage analysis (Sork et al., 1999).

Conclusions

Our research points toward a number of interesting areas to follow up on in orchid mycorrhizal research. Many of the implications for ecological and evolutionary dynamics between these groups will clearly need more experimental approaches to confirm, however characterizing patterns of genetic diversity is a useful first step. The lack of significant partitioning of genetic variability at the population level suggests that there is little local adaptation of these plants and fungi, but transplant experiments, though difficult to perform with mycorrhizal symbioses, would be needed to definitively address the degree of local adaptation. There is the preliminary suggestion that in more stressful environments there is greater correspondence between plant and fungal genotypes, which may help guide further experimental work. Moreover, the observed within-population heterogeneity suggests the scale at which demographic processes, responses to the abiotic environment, and possibly selection are operating

may be at the microsite level, which would not be too surprising in complex, heterogeneous environments such as eastern deciduous forest understories. Although the links to abiotic variability were not significant in this study, our understanding of how plants and their mycorrhizal fungi respond to global change will be facilitated by more studies attempting to link patterns of genetic variability to abiotic conditions at different spatial scales.

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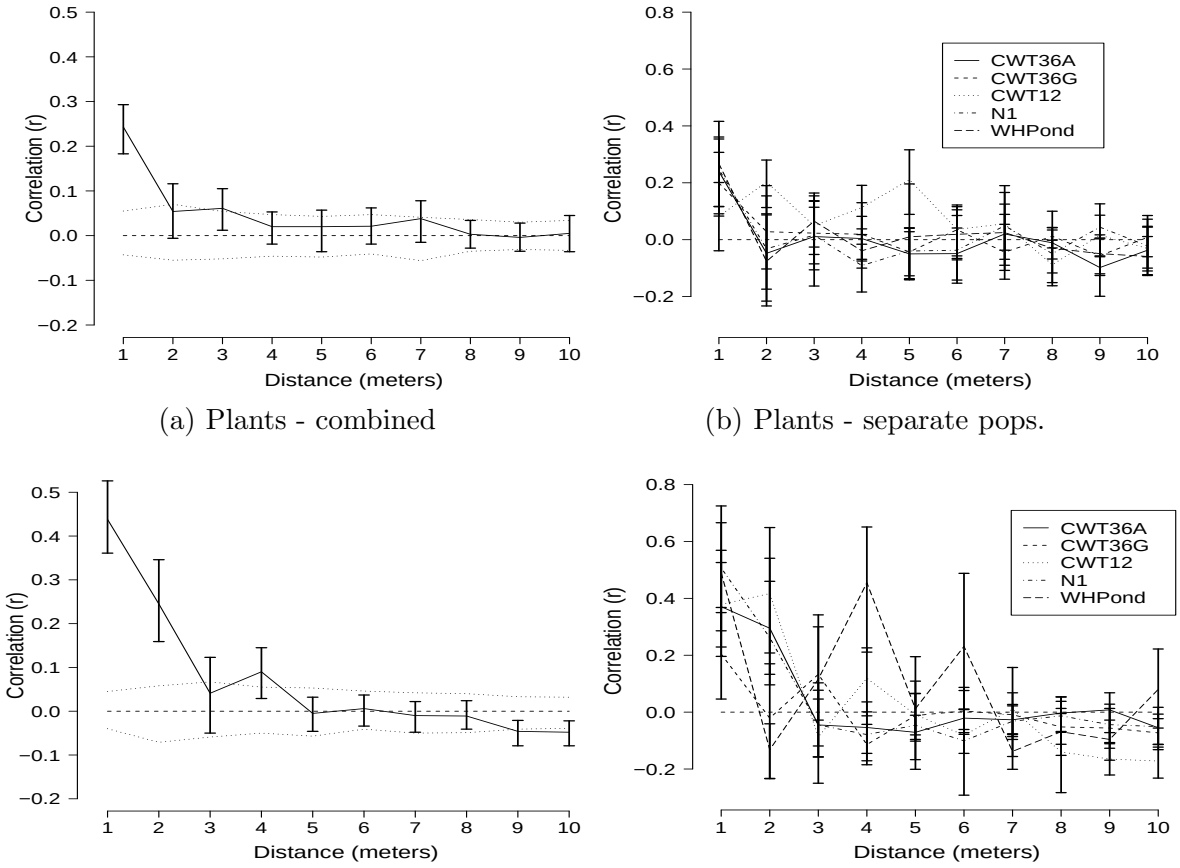
Table 6.1: **Clonal Diversity parameters:** N is the sample size in each population. *Geno* is the number of unique genotypes, and the Simpson's diversity index is defined as $\frac{n}{n-1}(1 - \sum p_i^2)$ where p_i is the frequency of genotype i.

Fungi				
Pop	N	*Geno*	$\frac{Geno}{N}$	Simpson's Index
CWT36A	38	31	0.816	0.987
CWT36G	39	35	0.897	0.995
CWT12	34	24	0.706	0.964
Nancytown	41	28	0.683	0.955
Whitehall	31	18	0.581	0.944
Plants				
Pop	N	*Geno*	$\frac{Geno}{N}$	Simpson's Index
CWT36A	41	32	0.780	0.987
CWT36G	41	38	.927	0.996
CWT12	40	32	0.800	0.982
Nancytown	41	29	0.707	0.980
Whitehall	40	31	0.775	0.987



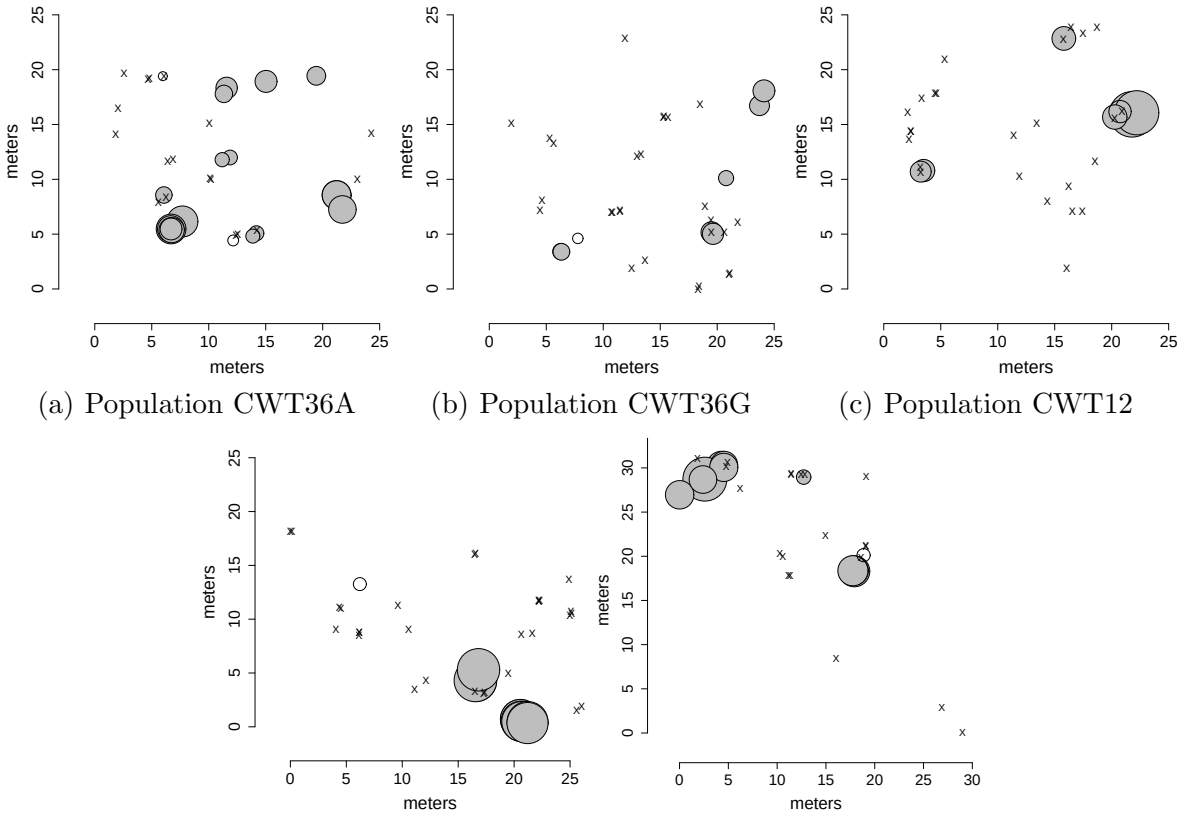
(a) Plants - combined

(b) Plants - separate pops.

(c) Fungi - combined

(d) Fungi - separate pops.

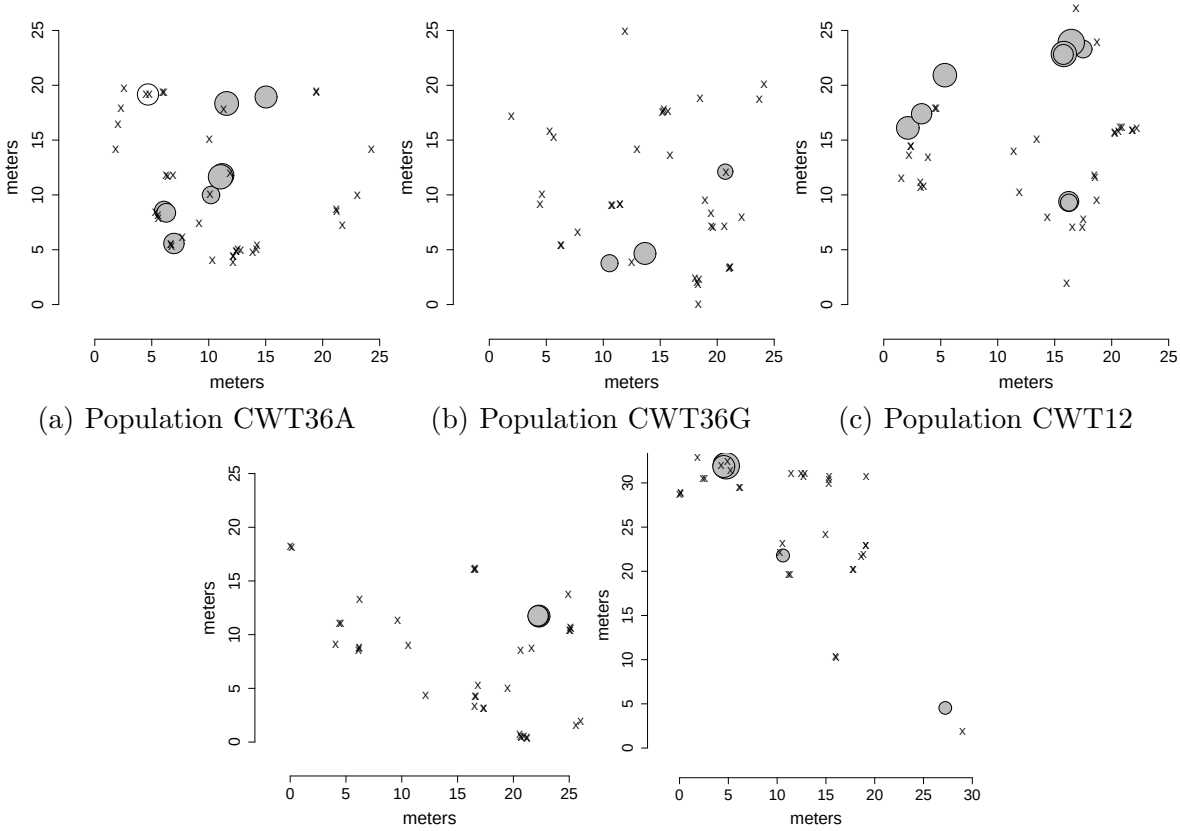
Figure 6.1: **Spatial genetic autocorrelation of plants and fungi:** Estimates are shown for the multivariate genetic autocorrelation coefficient, r, which is an estimate of genetic similarity based on multi-locus genotypes (see Smouse and Peakall, 1999). Permutation tests were used to construct 95% confidence intervals at each distance by drawing with replacement from the set of possible comparisons in each distance class (error bars), and comparing to expected values with random draws of individuals without regard to distance class (dotted lines). This procedure was performed for (a) all plants pooled together, (b) separate geographic plant populations, (c) all fungi pooled together, and (d) separate geographic fungal populations.



(a) Population CWT36A (b) Population CWT36G (c) Population CWT12

(d) Population North (e) Population WH

Figure 6.2: **2-D Autocorrelation of Fungal Genotypes:** Shaded circles represent locations where there was significant positive genotypic autocorrelation among the fungi with the closest 3 neighbors, as determined by permutation tests, using a .05 probability cutoff. The size of the circle represents the magnitude of correlation. A few scattered hollow circles represent negative correlations with the neighboring fungi. X's are those sampled plants with no significant correlation with neighbors.



(a) Population CWT36A (b) Population CWT36G (c) Population CWT12

(d) Population North (e) Population WH

Figure 6.3: **2-D Autocorrelation of plant genotypes:** Shaded circles represent plant locations where there was significant positive genotypic autocorrelation with the closest 3 neighbors, as determined by permutation tests, using a .05 probability cut-off. The size of the circle represents the magnitude of correlation. A few scattered hollow circles represent negative correlations with the neighboring plants. X's are those sampled plants with no significant correlation with neighbors.

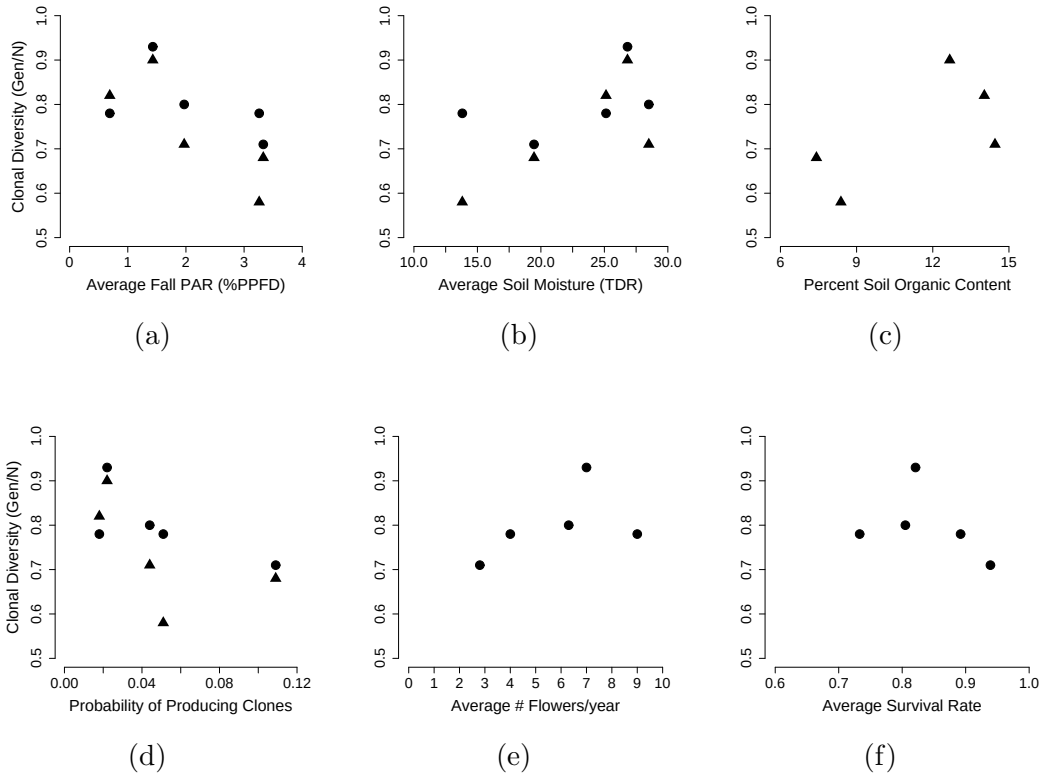


Figure 6.4: **Genetic Diversity Relationships with Abiotic Conditions and Demography:** Circles represent plant populations and triangles are fungi. The response variable in all graphs is a measure of clonal diversity ($genets/ramets$), as influenced by: (a) Light availability, as measured by average Fall PAR readings over the 2001-2004 period; (b) Average soil moisture readings, TDR, during the summer months over the 2001-2004 period; (c) Organic content of soil; (d) Observed clonality in the field; (e) Flowering; (f) Survival probabilities. See Spearman rank correlations in text; all p-values >> .1

Table 6.2: **Population Comparisons of Clonal Diversity:** Pairwise comparisons of clonal diversity. Statistical test based on 1000 bootstrap samples for each pairwise comparison, such that $p(A <= B)$ is interpreted as the probability that the populations have the same clonal diversity. In bold are those comparisons for which there is reason to believe there are differences between populations.

		$p(A <= B)$	
pop A	pop B	Fungi	Plants
CWT36G	CWT36A	0.74	0.82
CWT12	CWT36A	0.17	.375
CWT12	CWT36G	**0.09**	.15
Nancytown	CWT36A	**0.09**	.29
Nancytown	CWT36G	**0.07**	**.07**
Nancytown	CWT12	0.30	.48
Whitehall	CWT36A	**0.08**	.56
Whitehall	CWT36G	**0.05**	.19
Whitehall	CWT12	0.26	0.71
Whitehall	Nancytown	0.44	.74

Table 6.3: **Mantel Tests:** Reported are the Mantel correlation r statistics for the correlation between plant and fungal genetic distance matrices, and the probability p of observing this level of correlation by chance alone, based on 1000 permutations. Under "Partial" are partial Mantel tests, comparing the same two genetic distance matrices after controlling for geographic distance. Those populations meeting the p < .05 criteria are in bold

	Mantel test		Partial	
Population	r	p	r	p
CWT36A	.024	.344	.030	.332
CWT36G	.06	.193	.056	.204
CWT12	**.104**	**.014**	.048	.115
Nancytown	**.177**	**.001**	**.175**	**.001**
Whitehall	**.237**	**.001**	**.197**	**.001**

Chapter 7

Conclusions

This study has demonstrated significant relationships between abiotic environment and plant demographic rates and patterns of distributions. Moreover, significant relationships were found between the mycorrhizal germination and soil organic content and soil moisture. Overall, this work provides an example of the growing utility of hierarchical models for investigating complex, multi-level ecological processes. The ability to incorporate multiple sources of information and obtain realistic estimates of uncertainty will prove invaluable for ecological research in years to come.

Literature Cited

Abbott, M. B. and J. C. Refsgaard, editors. 1996. Distributed Hydrological Modelling. Kluwer Academic, Dordrecht Boston.

Allen, T. F. H. and T. B. Starr. 1982. Hierarchy. Perspectives for ecological complexity. University of Chicago Press, Chicago, Illinois.

Altwegg, R., S. Dummermuth, B. R. Anholt, and T. Flatt. 2005. Winter weather affects asp viper Vipera aspis population dynamics through susceptible juveniles. Oikos **110**:55–66.

Andrewartha, H. G. and L. C. Birch. 1954. The distribution and abundance of animals. University of Chicago Press, Chicago.

Araujo, M. B. and R. G. Pearson. 2005. Equilibrium of species' distributions with climate. Ecography **28**:693–695.

Armstrong, D. P. 2005. Integrating the metapopulation and habitat paradigms for understanding broad-scale declines of species. Conservation Biology **19**:1402–1410.

Augspurger, C. K. and C. K. Kelly. 1984. Pathogen Mortality of Tropical Tree Seedlings - Experimental Studies of the Effects of Dispersal Distance, Seedling Density, and Light Conditions. Oecologia **61**:211–217.

Band, L. E., C. L. Tague, P. Groffman, and K. Belt. 2001. Forest ecosystem processes at the watershed scale: hydrological and ecological controls of nitrogen export. Hydrological Processes **15**:2013–2028.

Banerjee, S., B. P. Carlin, and A. E. Gelfand. 2004. Hierarchical Modeling and Analysis for Spatial Data. Chapman and Hall CRC, Boca Raton.

Batty, A. L., K. W. Dixon, M. Brundrett, and K. Sivasithamparam. 2001. Constraints to symbiotic germination of terrestrial orchid seed in a mediterranean bushland. New Phytologist **152**:511–520.

Beaudet, M., C. Messier, and A. Leduc. 2004. Understorey light profiles in temperate deciduous forests: recovery process following selection cutting. Journal of Ecology **92**:328–338.

Benayas, J. M. R., M. Garcia, S. Colomer, and N. Escudero. 2004. Landscape- and field-scale control of spatial variation of soil properties in Mediterranean montane meadows. Biogeochemistry **69**:207225.

Besag, J. 1974. Spatial interaction and the statistical analysis of lattice systems (with discussions). Journal of the Royal Statistical Society, Series B **36**:192–236.

Beven, K. 2001. How far can we go in distributed hydrological modelling? Hydrology and Earth System Sciences pages 1–12.

Beven, K. J. and M. J. Kirkby. 1979. A physically based variable contributing area model of basin hydrology. Hydrologic Science Bulletin **24**:43–69.

Bever, J. D. 2002. Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant and Soil **244**:281–290.

Boddy, L. 2000. Interspecific combative interactions between wood-decaying basidiomycetes. Fems Microbiology Ecology **31**:185–194.

Boyer, D. G., R. J. Wright, W. M. Winant, and H. D. Perry. 1990. Soil-Water Relations on a Hilltop Cornfield in Central Appalachia. Soil Science **149**:383–392.

Brewer, C. A. and L. J. Gross. 2003. Training ecologists to think with uncertainty in mind. Ecology **84**:1412–1414.

Bronstein, J. L. 1994. Conditional Outcomes in Mutualistic Interactions. Trends in Ecology and Evolution **9**:214–217.

Brzosko, E., A. Wroblewska, and M. Ratkiewicz. 2002. Spatial genetic structure and clonal diversity of island populations of lady's slipper (Cypripedium calceolus) from the Biebrza National Park (northeast Poland). Molecular Ecology **11**:2499–2509.

Cairney, J. W. G. 2005. Basidiomycete mycelia in forest soils: dimensions, dynamics and roles in nutrient distribution. Mycological Research **109**:7–20.

Caldwell, B. A., M. A. Castellano, and R. P. Griffiths. 1991. Fatty-Acid Esterase Production by Ectomycorrhizal Fungi. Mycologia **83**:233–236.

Canham, C. D., J. S. Denslow, W. J. Platt, J. R. Runkle, T. A. Spies, and P. S. White. 1990. Light Regimes beneath Closed Canopies and Tree-Fall Gaps in Temperate and Tropical Forests. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **20**:620–631.

Caswell, H. 2001. Matrix population models : construction, analysis, and interpretation. Sinauer Associates.

Chen, J. Q., S. C. Saunders, T. R. Crow, R. J. Naiman, K. D. Brosofske, G. D. Mroz, B. L. Brookshire, and J. F. Franklin. 1999. Microclimate in forest ecosystem and landscape ecology - Variations in local climate can be used to monitor and compare the effects of different management regimes. Bioscience **49**:288–297.

Churkina, G., S. W. Running, and A. L. Schloss. 1999. Comparing global models of terrestrial net primary productivity (NPP): the importance of water availability. Global Change Biology **5**:46–55.

Clark, J. S. 2003. Uncertainty and variability in demography and population growth: A hierarchical approach. Ecology **84**:1370–1381.

Clark, J. S. 2005. Why environmental scientists are becoming Bayesians. Ecology Letters **8**:2–14.

Clark, J. S., B. Beckage, P. Camill, B. Cleveland, J. HilleRisLambers, J. Lighter, J. McLachlan, J. Mohan, and P. Wyckoff. 1999. Interpreting recruitment limitation in forests. American Journal of Botany **86**:1–16.

Clark, J. S. and C. N. Bjornstad. 2004. Population time series: Process variability, observation errors, missing values, lags, and hidden states. Ecology **85**:3140–3150.

Clark, J. S., S. R. Carpenter, M. Barber, S. Collins, A. Dobson, J. A. Foley, D. M. Lodge, M. Pascual, R. Pielke, W. Pizer, C. Pringle, W. V. Reid, K. A. Rose, O. Sala, W. H. Schlesinger, D. H. Wall, and D. Wear. 2001. Ecological forecasts: An emerging imperative. Science **293**:657–660.

Clark, J. S., G. A. Ferraz, N. Oguge, H. Hays, and J. DiCostanzo. 2005. Hierarchical Bayes for structured, variable populations: From recapture data to life-history prediction. Ecology **86**:2232–2244.

Clark, J. S. and S. L. LaDeau, 2005. Synthesizing ecological experiments and observational data with Hierarchical Bayes. *in* J. S. Clark and A. Gelfand, editors. Adventures in Hierarchical Modeling of the Environment. In press.

Clark, J. S., M. Lewis, J. S. McLachlan, and J. HilleRisLambers. 2003*a*. Estimating population spread: What can we forecast and how well? Ecology **84**:1979–1988.

Clark, J. S., J. Mohan, M. Dietze, and I. Ibanez. 2003*b*. Coexistence: How to identify trophic trade-offs. Ecology **84**:17–31.

Coleman, D. C., E. P. Odum, and D. A. Crossley. 1992. Soil Biology, Soil Ecology, and Global Change. Biology and Fertility of Soils **14**:104–111.

Collins, B. S., K. P. Dunne, and S. T. A. Pickett, 1985. Responses of forest herbs to canopy gaps. *in* S. T. A. Pickett and P. S. White, editors. The Ecology of Natural Disturbance and Patch Dynamics. Academic, London.

Coulson, T., E. A. Catchpole, S. D. Albon, B. J. T. Morgan, J. M. Pemberton, T. H. Clutton-Brock, M. J. Crawley, and B. T. Grenfell. 2001. Age, sex, density, winter weather, and population crashes in Soay sheep. Science **292**:1528–1531.

Coulson, T., P. Rohani, and M. Pascual. 2004. Skeletons, noise and population growth: the end of an old debate? Trends in Ecology and Evolution **19**:359–364.

Dahlberg, A. 1997. Population ecology of Suillus variegatus in old Swedish Scots pine forests. Mycological Research **101**:47–54.

Davis, A. J., L. S. Jenkinson, J. H. Lawton, B. Shorrocks, and S. Wood. 1998. Making mistakes when predicting shifts in species range in response to global warming. Nature **391**:783–786.

DeWoody, Y. D., Z. L. Feng, and R. K. Swihart. 2005. Merging spatial and temporal structure within a metapopulation model. American Naturalist **166**:42–55.

Diez, J. M., 2005. Niche variation of a terrestrial orchid symbiosis. Ph.D. thesis, UGA.

Diez, J. M. and H. R. Pulliam. 2005. Demographic Responses of a Terrestrial Orchid to Abiotic Gradients. manuscript to be submitted to Ecology .

Dunne, J. A., S. R. Saleska, M. L. Fischer, and J. Harte. 2004. Integrating experimental and gradient methods in ecological climate change research. Ecology **85**:904–916.

Eckstein, R. L. 2005. Differential effects of interspecific interactions and water availability on survival, growth and fecundity of three congeneric grassland herbs. New Phytologist **166**:525–536.

Edman, M. and M. Gustafsson. 2003. Wood-disk traps provide a robust method for studying spore dispersal of wood-decaying basidiomycetes. Mycologia **95**:553–556.

Ek, H., M. Sjgren, K. Arnebrant, and B. Sderstrm. 1994. Extramatrical mycelial growth, biomass allocation and nitrogen uptake in ectomycorrhizal systems in response to collembolan grazing. Applied Soil Ecology **1**:155–169.

Ellison, A. M. 1996. An introduction to Bayesian inference for ecological research and environmental decision-making. Ecological Applications **6**:1036–1046.

Ellison, A. M. 2004. Bayesian inference in ecology. Ecology Letters **7**:509–520.

Engelbrecht, B. M. J. and T. A. Kursar. 2003. Comparative drought-resistance of seedlings of 28 species of co-occurring tropical woody plants. Oecologia **136**:383–393.

Ettema, C. H., D. C. Coleman, G. Vellidis, R. Lowrance, and S. L. Rathbun. 1998. Spatiotemporal distributions of bacterivorous nematodes and soil resources in a restored riparian wetland. Ecology **79**:2721–2734.

Ettema, C. H., S. L. Rathbun, and D. C. Coleman. 2000. On spatiotemporal patchiness and the coexistence of five species of Chronogaster (Nematoda : Chronogasteridae) in a riparian wetland. Oecologia **125**:444–452.

Ettema, C. H. and D. A. Wardle. 2002. Spatial soil ecology. Trends in Ecology and Evolution **17**:177–183.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes - Application to Human Mitochondrial-DNA Restriction Data. Genetics **131**:479–491.

Fieberg, J. and S. P. Ellner. 2001. Stochastic matrix models for conservation and management: a comparative review of methods. Ecology Letters **4**:244–266.

Fierer, N., J. A. Jackson, R. Vilgalys, and R. B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology **71**:4117–4120.

Fitter, A. H. 2005. Darkness visible: reflections on underground ecology. Journal of Ecology **93**:231–243.

Fladeland, M. M., M. S. Ashton, and X. H. Lee. 2003. Landscape variations in understory PAR for a mixed deciduous forest in New England, USA. Agricultural and Forest Meteorology **118**:137–141.

Fleishman, E., R. Mac Nally, J. P. Fay, and D. D. Murphy. 2001. Modeling and predicting species occurrence using broad-scale environmental variables: an example with butterflies of the Great Basin. Conservation Biology **15**:1674–1685.

Franklin, A. B., D. R. Anderson, R. J. Gutierrez, and K. P. Burnham. 2000. Climate, habitat quality, and fitness in Northern Spotted Owl populations in northwestern California. Ecological Monographs **70**:539–590.

Fraterrigo, J. M., M. G. Turner, S. M. Pearson, and P. Dixon. 2005. Effects of past land use on spatial heterogeneity of soil nutrients in southern appalachian forests. Ecological Monographs **75**:215–230.

Freville, H., B. Colas, M. Riba, H. Caswell, A. Mignot, E. Imbert, and I. Olivieri. 2004. Spatial and temporal demographic variability in the endemic plant species Centaurea corymbosa (Asteraceae). Ecology **85**:694–703.

Gabriel, W., B. Luttbeg, A. Sih, and R. Tollrian. 2005. Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. American Naturalist **166**:339–353.

Gange, A. C. and V. K. Brown, 2003. Actions and Interactions of Soil Invertebrates and Arbuscular Mycorrhizal fungi in affecting the structure of plant communities. *in* M. G. A. van der Heijden and I. R. Sanders, editors. Mycorrhizal Ecology. Springer.

Gebauer, G. and M. Meyer. 2003. N-15 and C-13 natural abundance of autotrophic and mycoheterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. New Phytologist **160**:209–223.

Gee, G. W. and J. W. Bauder, 1986. Particle size analysis. Pages 383–411 *in* A. Klute, editor. Methods of soil analysis. Part 1. Madison, WI., 2nd ed edition.

Gelfand, A. E., A. M. Schmidt, S. Wu, J. A. Silander, A. Latimer, and A. G. Rebelo. 2005. Modelling species diversity through species level hierarchical modelling. Journal of the Royal Statistical Society Series C-Applied Statistics **54**:1–20.

Gelfand, A. E., J. A. Silander, W. Shanshan, A. M. Latimer, P. O. Lewis, A. G. Rebelo, and M. Holder. 2003. Explaining Species Distribution Patterns through Hierarchical Modeling. Bayesian Analysis **1**:1–47.

Gelman, A., J. B. Carlin, and H. S. S. B. Rubin. 2004. Bayesian Data Analysis, 2nd ed. Chapman and Hall CRC, NY.

Gendron, F., C. Messier, and P. G. Comeau. 1998. Comparison of various methods for estimating the mean growing season percent photosynthetic photon flux density in forests. Agricultural and Forest Meteorology **92**:55–70.

Gendron, F., C. Messier, and P. G. Comeau. 2001. Temporal variations in the understorey photosynthetic photon flux density of a deciduous stand: the effects of canopy development, solar elevation, and sky conditions. Agricultural and Forest Meteorology **106**:23–40.

Gherbi, H., C. Delaruelle, M. A. Selosse, and F. Martin. 1999. High genetic diversity in a population of the ectomycorrhizal basidiomycete Laccaria amethystina in a 150-year-old beech forest. Molecular Ecology **8**:2003–2013.

Gilks, W. R., S. Richardson, and D. J. Spiegelhalter, editors. 1996. Markov chain Monte Carlo in Practice. Chapman and Hall, London, UK.

Gordon, W. S. and J. S. Famiglietti. 2004. Response of the water balance to climate change in the United States over the 20th and 21st centuries: Results from the VEMAP Phase 2 model intercomparisons. Global Biogeochemical Cycles **18**.

Grayson, R. B. and G. Bloschl, editors. 2000. Spatial Patterns in Hydrological Processes: Observations and Modelling. Cambridge Univ. Press, Cambridge, UK.

Guisan, A. and W. Thuiller. 2005. Predicting species distribution: offering more than simple habitat models. Ecology Letters **8**:993–1009.

Hackl, E., G. Bachmann, and S. Zechmeister-Boltenstern. 2004. Microbial nitrogen turnover in soils under different types of natural forest. Forest Ecology and Management **188**:101–112.

Hallenberg, N. and N. Kuffer. 2001. Long-distance spore dispersal in wood-inhabiting Basidiomycetes. Nordic Journal of Botany **21**:431–436.

Hallett, T. B., T. Coulson, J. G. Pilkington, T. H. Clutton-Brock, J. M. Pemberton, and B. T. Grenfell. 2004. Why large-scale climate indices seem to predict ecological processes better than local weather. Nature **430**:71–75.

Hampe, A. 2004. Bioclimate envelope models: what they detect and what they hide. Global Ecology and Biogeography **13**:469471.

Hamrick, J. L. and M. J. W. Godt. 1996. Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **351**:1291–1298.

Hamrick, J. L. and L. R. Holden. 1979. Influence of Microhabitat Heterogeneity on Gene-Frequency Distribution and Gametic Phase Disequilibrium in Avena-Barbata. Evolution **33**:521–533.

Hamrick, J. L. and J. M. Lee. 1987. Effect of Soil Surface-Topography and Litter Cover on the Germination, Survival, and Growth of Musk Thistle (Carduus-Nutans). American Journal of Botany **74**:451–457.

Harte, J., E. Conlisk, A. Ostling, J. L. Green, and A. B. Smith. 2005. A theory of spatial structure in ecological communities at multiple spatial scales. Ecological Monographs **75**:179–197.

Harte, J. and R. Shaw. 1995. Shifting Dominance within a Montane Vegetation Community - Results of a Climate-Warming Experiment. Science **267**:876–880.

Helvey, J. D., J. J. Hewlett, and J. E. Douglass. 1972. Predicting Soil-Moisture in Southern Appalachians. Soil Science Society of America Journal **36**:954–959.

HilleRisLambers, J., J. S. Clark, and B. Beckage. 2002. Density-dependent mortality and the latitudinal gradient in species diversity. Nature **417**:732–735.

Hogberg, N., O. Holdenrieder, and J. Stenlid. 1999. Population structure of the wood decay fungus Fomitopsis pinicola. Heredity **83**:354–360.

Hooten, M. B., D. R. Larsen, and C. K. Wikle. 2003. Predicting the spatial distribution of ground flora on large domains using a hierarchical Bayesian model. Lands. Ecol. **18**:487–502.

Horvitz, C. C. and D. W. Schemske. 1995. Spatiotemporal Variation in Demographic Transitions of a Tropical Understory Herb - Projection Matrix Analysis. Ecological Monographs **65**:155–192.

Huff, D. R., R. Peakall, and P. E. Smouse. 1993. Rapd Variation within and among Natural-Populations of Outcrossing Buffalograss [Buchloe-Dactyloides (Nutt) Engelm]. Theoretical and Applied Genetics **86**:927–934.

Hutchings, M. J., E. A. John, and D. K. Wijesinghe. 2003. Toward understanding the consequences of soil heterogeneity for plant populations and communities. Ecology **84**:2322–2334.

James, T. Y., D. Porter, J. L. Hamrick, and R. Vilgalys. 1999. Evidence for limited intercontinental gene flow in the cosmopolitan mushroom, Schizophyllum commune. Evolution **53**:1665–1677.

James, T. Y. and R. Vilgalys. 2001. Abundance and diversity of Schizophyllum commune spore clouds in the Caribbean detected by selective sampling. Molecular Ecology **10**:471–479.

Janzen, D. H. 1970. Herbivores and Number of Tree Species in Tropical Forests. American Naturalist **104**:501–and.

Jolls, C. L., 2003. Populations of and Threats to Rare Plants of the Herb Layer. *in* F. S. Gilliam and M. R. Roberts, editors. The Herbaceous Layer in Forests of Eastern North America. Oxford University Press, Oxford.

Kadmon, R. and K. Tielborger. 1999. Testing for source-sink population dynamics: an experimental approach exemplified with desert annuals. OIKOS **86**:417–429.

Kalisz, S. and M. A. McPeek. 1992. Demography of an Age-Structured Annual - Resampled Projection Matrices, Elasticity Analyses, and Seed Bank Effects. Ecology **73**:1082–1093.

Kallunki, J. A. 1981. Reproductive-Biology of Mixed-Species Populations of Goodyera (Orchidaceae) in Northern Michigan. Brittonia **33**:137–155.

Kampichler, C., J. Rolschewski, D. P. Donnelly, and L. Boddy. 2004. Collembolan grazing affects the growth strategy of the cord-forming fungus Hypholoma fasciculare. Soil Biology and Biochemistry **36**:591–599.

Kauserud, H. and T. Schumacher. 2003. Genetic structure of Fennoscandian populations of the threatened wood-decay fungus Fomitopsis rosea (Basidiomycota). Mycological Research **107**:155–163.

Kearney, M. and W. P. Porter. 2004. Mapping the Fundamental Niche: Physiology, Climate, and the Distribution of a Nocturnal Lizard. Ecology **85**:3119–3131.

Keitt, T. H. and D. L. Urban. 2005. Scale-specific inference using wavelets. Ecology **86**:2497–2504.

Kery, M., K. B. Gregg, and M. Schaub. 2005. Demographic estimation methods for plants with unobservable life-states. Oikos **108**:307–320.

Knapp, A. K., P. A. Fay, J. M. Blair, S. L. Collins, M. D. Smith, J. D. Carlisle, C. W. Harper, B. T. Danner, M. S. Lett, and J. K. McCarron. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. Science **298**:2202–2205.

Knapp, L. B. and C. D. Canham. 2000. Invasion of an old-growth forest in New York by Ailanthus altissima: sapling growth and recruitment in canopy gaps. Journal of the Torrey Botanical Society **127**:307–315.

Knoepp, J. D. and W. T. Swank. 2002. Using soil temperature and moisture to predict forest soil nitrogen mineralization. Biology and Fertility of Soils **36**:177 – 182.

Koop, A. L. and C. C. Horvitz. 2005. Projection matrix analysis of the demography of an invasive, nonnative shrub (Ardisia elliptica). Ecology **86**:2661–2672.

Kretzer, A. M., S. Dunham, R. Molina, and J. W. Spatafora. 2004. Microsatellite markers reveal the below ground distribution of genets in two species of Rhizopogon forming tuberculate ectomycorrhizas on Douglas fir. New Phytologist **161**:313–320.

Kretzer, A. M., S. Dunham, R. Molina, and J. W. Spatafora. 2005. Patterns of vegetative growth and gene flow in Rhizopogon vinicolor and R-vesiculosus (Boletales, Basidiomycota). Molecular Ecology **14**:2259–2268.

Kudoh, H., H. Shibaike, H. Takasu, D. F. Whigham, and S. Kawano. 1999. Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, Uvularia perfoliata. Journal of Ecology **87**:244–257.

Kulheim, C., J. Agren, and S. Jansson. 2002. Rapid regulation of light harvesting and plant fitness in the field. Science **297**:91–93.

Lambers, H., F. S. Chapin, and T. Pons. 1998. Plant physiological ecology. Springer-Verlag, New York.

Latimer, A. M., S. Wu, A. E. Gelfand, and J. A. Silander. 2005. Building statistical models to analyze species distributions. Ecological Applications (in press) .

Leake, J. R. 1994. The Biology of Myco-Heterotrophic (Saprophytic) Plants. New Phytologist **127**:171–216.

Leake, J. R., S. L. McKendrick, M. Bidartondo, and D. J. Read. 2004. Symbiotic germination and development of the myco-heterotroph Monotropa hypopitys in nature and its requirement for locally distributed Tricholoma spp. New Phytologist **163**:405–423.

Leirs, H., N. C. Stenseth, J. D. Nichols, J. E. Hines, R. Verhagen, and W. Verheyen. 1997. Stochastic seasonality and nonlinear density-dependent factors regulate population size in an African rodent. Nature **389**:176–180.

Lewontin, R. C. and D. Cohen. 1969. On Population Growth in a Randomly Varying Environment. Proceedings of the National Academy of Sciences of the United States of America **62**:1056–and.

Lichstein, J. W., T. R. Simons, S. A. Shriner, and K. E. Franzreb. 2002. Spatial Autocorrelation And Autoregressive Models In Ecology. Ecological Monographs **72**:445463.

Lockwood, J. L. 1977. Fungistasis in Soils. Biological Reviews of the Cambridge Philosophical Society **52**:1–43.

Lookingbill, T. and D. Urban. 2004. An empirical approach towards improved spatial estimates of soil moisture for vegetation analysis. Landscape Ecology **19**:417–433.

Luoto, M., J. Poyry, R. K. Heikkinen, and K. Saarinen. 2005. Uncertainty of bioclimate envelope models based on the geographical distribution of species. Global Ecology and Biogeography **14**:575–584.

Mahmood, R. 1996. Scale issues in soil moisture modelling: Problems and prospects. Progress in Physical Geography **20**:273–291.

Masuhara, G. and K. Katsuya. 1994. In-Situ and in-Vitro Specificity between Rhizoctonia Spp and Spiranthes-Sinensis (Persoon) Ames Var Amoena (M Bieberstein) Hara (Orchidaceae). New Phytologist **127**:711–718.

Maurer, B. A. and M. L. Taper. 2002. Connecting geographical distributions with population processes. Ecology Letters **5**:223–231.

McCormick, M. K., D. Whigham, D. Sloan, K. O. O'Malley, and B. Hodkinson. 2005. Orchid-fungal fidelity: a marriage meant to last? accepted, Ecology .

McCormick, M. K., D. F. Whigham, and J. O'Neill. 2004. Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytologist **163**:425–438.

McKendrick, S. L., J. R. Leake, and D. J. Read. 2000*a*. Symbiotic germination and development of myco-heterotrophic plants in nature: transfer of carbon from ectomycorrhizal Salix repens and Betula pendula to the orchid Corallorhiza trifida through shared hyphal connections. New Phytologist **145**:539–548.

McKendrick, S. L., J. R. Leake, D. L. Taylor, and D. J. Read. 2000*b*. Symbiotic germination and development of myco-heterotrophic plants in nature: ontogeny of Corallorhiza trifida and characterization of its mycorrhizal fungi. New Phytologist **145**:523–537.

McKendrick, S. L., J. R. Leake, D. L. Taylor, and D. J. Read. 2002. Symbiotic germination and development of the myco-heterotrophic orchid Neottia nidus-avis in nature and its requirement for locally distributed Sebacina spp. New Phytologist **154**:233–247.

McKenzie, D., D. W. Peterson, D. L. Peterson, and P. E. Thornton. 2003. Climatic and biophysical controls on conifer species distributions in mountain forests of Washington State, USA. Journal of Biogeography **30**:1093–1108.

McMahon, S. M. and J. M. Diez. 2005. Scales of association: using hierarchical linear models to measure ecological systems. Submitted to Ecology .

Morris, W. F. and D. F. Doak. 2005. How general are the determinants of the stochastic population growth rate across nearby sites? Ecological Monographs **75**:119–137.

Munzbergova, Z. and T. Herben. 2005. Seed, dispersal, microsite, habitat and recruitment limitation: identification of terms and concepts in studies of limitations. Oecologia **145**:1–8.

Muraoka, H., Y. Tang, H. Koizumi, and I. Washitani. 1997. Combined effects of light and water availability on photosynthesis and growth of Arisaema heterophyllum in the forest understory and an open site. Oecologia **112**:26–34.

Nei, M. 1972. Genetic Distance between Populations. American Naturalist **106**:283–and.

Nuismer, S. L., J. N. Thompson, and R. Gomulkiewicz. 1999. Gene flow and geographically structured coevolution. Proceedings of the Royal Society of London Series B-Biological Sciences **266**:605–609.

Nybom, H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molecular Ecology **13**:1143–1155.

Oberhuber, W. and H. Bauer. 1991. Photoinhibition of photosynthesis under natural conditions in ivy (Hedera helix L.) growing in an understory of deciduous tress. Planta **185**:545–553.

O'Brien, H. E., J. L. Parrent, J. A. Jackson, J. M. Moncalvo, and R. Vilgalys. 2005. Fungal community analysis by large-scale sequencing of environmental samples. Applied and Environmental Microbiology **71**:5544–5550.

Okuyama, T. and B. M. Bolker. 2005. Combining genetic and ecological data to estimate sea turtle origins. Ecological Applications **15**:315–325.

Packer, A. and K. Clay. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature **404**:278–281.

Packer, A. and K. Clay. 2003. Soil pathogens and Prunus serotina seedling and sapling growth near conspecific trees. Ecology **84**:108–119.

Parmesan, C., S. Gaines, L. Gonzalez, D. M. Kaufman, J. Kingsolver, A. T. Peterson, and R. Sagarin. 2005. Empirical perspectives on species borders: From traditional biogeography to global change. Oikos **108**:58–75.

Pavao-Zuckerman, M. A. and D. C. Coleman. 2005. Decomposition of chestnut oak (Quercus prinus) leaves and nitrogen mineralization in an urban environment. Biology and Fertility of Soils **41**:343–349.

Peakall, R. and P. E. Smouse, 2005. GenAlEx 6: Genetic Analysis in Excel. Population genetic software for teaching and research.

Peakall, R., P. E. Smouse, and D. R. Huff. 1995. Evolutionary Implications of Allozyme and Rapd Variation in Diploid Populations of Dioecious Buffalograss Buchloe Dactyloides. Molecular Ecology **4**:135–147.

Pearson, R. G. and T. P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global Ecology and Biogeography **12**:361–371.

Penuelas, J., C. Gordon, L. Llorens, T. Nielsen, A. Tietema, C. Beier, P. Bruna, B. Emmett, M. Estiarte, and A. Gorissen. 2004. Nonintrusive field experiments show different plant responses to warming and drought among sites, seasons, and species in a north-south European gradient. Ecosystems **7**:598–612.

Pulliam, H. R. 1988. Sources, Sinks, and Population Regulation. American Naturalist **132**:652–661.

Pulliam, H. R. 2000. On the relationship between niche and distribution. Ecology Letters **3**:349–361.

R Development Core Team, 2005. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL `http://www.R-project.org`. ISBN 3-900051-07-0.

Rasmussen, H. N. 1995. Terrestrial orchids from seed to mycotrophic plant. Cambridge University Press.

Rasmussen, H. N. 2002. Recent developments in the study of orchid mycorrhiza. Plant and Soil **244**:149–163.

Rasmussen, H. N. and D. F. Whigham. 1993. Seed Ecology of Dust Seeds in-Situ - a New Study Technique and Its Application in Terrestrial Orchids. American Journal of Botany **80**:1374–1378.

Rasmussen, H. N. and D. F. Whigham. 1998*a*. Importance of woody Debris in seed germination of Tipularia discolor (Orchidaceae). American Journal of Botany **85**:829–834.

Rasmussen, H. N. and D. F. Whigham. 1998*b*. The underground phase: a special challenge in studies of terrestrial orchid populations. Botanical Journal of the Linnean Society **126**:49–64.

Rasmussen, H. N. and D. F. Whigham. 2002. Phenology of roots and mycorrhiza in orchid species differing in phototrophic strategy. New Phytologist **154**:797–807.

Raudenbush, S. W. and A. S. Bryk. 2002. Hierarchical linear models: applications and data analysis methods. Advanced quantitative techniques in the social sciences, Sage Publications.

Redecker, D., T. M. Szaro, R. J. Bowman, and T. D. Bruns. 2001. Small genets of Lactarius xanthogalactus, Russula cremoricolor and Amanita francheti in late-stage ectomycorrhizal successions. Molecular Ecology **10**:1025–1034.

Ridolfi, L., P. D'Odorico, A. Porporato, and I. Rodriguez-Iturbe. 2003. Stochastic soil moisture dynamics along a hillslope. Journal of Hydrology **272**:264–275.

Rosenzweig, M. l. 1968. Net Primary Productivity of Terrestrial Communities - Prediction from Climatological Data. American Naturalist **102**:67–and.

Roy, M., R. D. Holt, and M. Barfield. 2005. Temporal autocorrelation can enhance the persistence and abundance of metapopulations comprised of coupled sinks. American Naturalist **166**:246–261.

Silvertown, J., M. Franco, I. Pisanty, and A. Mendoza. 1993. Comparative Plant Demography - Relative Importance of Life-Cycle Components to the Finite Rate of Increase in Woody and Herbaceous Perennials. Journal of Ecology **81**:465–476.

Skov, F. and J. C. Svenning. 2004. Potential impact of climatic change on the distribution of forest herbs in Europe. Ecography **27**:366380.

Smith, J. L., K. L. Hunter, and R. B. Hunter. 2002. Genetic variation in the terrestrial orchid Tipularia discolor. Southeastern Naturalist **1**:17–26.

Smith, S. E. and D. J. Read. 1997. Mycorrhizal Symbiosis. 2nd ed. edition. Academic Press, London.

Smouse, P. E. and R. Peakall. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity **82**:561–573.

Sork, V. L., J. Nason, D. R. Campbell, and J. F. Fernandez. 1999. Landscape approaches to historical and contemporary gene flow in plants. Trends in Ecology and Evolution **14**:219–224.

Spiegelhalter, D. J., N. G. Best, B. R. Carlin, and A. van der Linde. 2002. Bayesian measures of model complexity and fit. Journal of the Royal Statistical Society Series B-Statistical Methodology **64**:583–616.

Spiegelhalter, D. T. A. and N. Best, 2000. WinBUGS User Manual. MRC Biostatistics Unit: Cambridge.

Stenseth, N. C., A. Mysterud, G. Ottersen, J. W. Hurrell, K. S. Chan, and M. Lima. 2002. Ecological effects of climate fluctuations. Science **297**:1292–1296.

Stephenson, N. L. 1998. Actual evapotranspiration and deficit: biologically meaningful correlates of vegetation distribution across spatial scales. Journal of Biogeography **25**:855–870.

Sun, M. and K. C. Wong. 2001. Genetic structure of three orchid species with contrasting breeding systems using RAPD and allozyme markers. American Journal of Botany **88**:2180–2188.

Taylor, D. L. and T. D. Bruns. 1997. Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. Proceedings of the National Academy of Sciences of the United States of America **94**:4510–4515.

Taylor, D. L. and T. D. Bruns. 1999. Population, habitat and genetic correlates of mycorrhizal specialization in the 'cheating' orchids Corallorhiza maculata and C-mertensiana. Molecular Ecology **8**:1719–1732.

Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. Collingham, B. F. N. Erasmus, M. F. de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. van Jaarsveld, G. F. Midgley, L. J. Miles, M. A. Ortega-Huerta, A. Townsend Peterson, O. Phillips, and S. E. Williams. 2004. Extinction risk from climate change. Nature **427**:145148.

Thompson, J. N. 1999*a*. The evolution of species interactions. Science **284**:2116–2118.

Thompson, J. N. 1999*b*. Specific hypotheses on the geographic mosaic of coevolution. American Naturalist **153**:S1–S14.

Thompson, J. N. 2005. The Geographic Mosaic of Coevolution. University of Chicago Press.

Thuiller, W., S. Lavorel, and M. B. Araujo. 2005. Niche properties and geographical extent as predictors of species sensitivity to climate change. Global Ecology and Biogeography **14**:347–357.

Thuiller, W., S. Lavorel, G. Midgley, S. Lavergne, and T. Rebelo. 2004. Relating plant traits and species distributions along bioclimatic gradients for 88 Leucadendron taxa. Ecology **85**:1688–1699.

Tilman, D. 2004. Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. Proceedings of the National Academy of Sciences of the United States of America **101**:10854–10861.

Tilman, D. and P. Karieva, editors. 1997. Spatial Ecology: The Role of Space in Population Dynamics and Interspecific Interactions. Monographs in Population Biology, Princeton University Press.

Tomita, M., Y. Hirabuki, and K. Seiwa. 2002. Post-dispersal changes in the spatial distribution of Fagus crenata seeds. Ecology **83**:1560–1565.

Trapnell, D. W., J. L. Hamrick, and J. D. Nason. 2004. Three-dimensional fine-scale genetic structure of the neotropical epiphytic orchid, Laelia rubescens. Molecular Ecology **13**:1111–1118.

Tuljapurkar, S. 1985. Population-Dynamics in Variable Environments .6. Cyclical Environments. Theoretical Population Biology **28**:1–17.

Turner, M. G., V. H. Dale, and R. H. Gardner. 1989. Predicting across scales: Theory development and testing. Landscape Ecology **3**:245–252.

Urban, D. L., C. Miller, P. N. Halpin, and N. L. Stephenson. 2000. Forest gradient response in Sierran landscapes: the physical template. Landscape Ecology **15**:603–620.

Uriarte, M., C. D. Canham, J. Thompson, J. K. Zimmerman, and N. Brokaw. 2005. Seedling recruitment in a hurricane-driven tropical forest: light limitation, density-dependence and the spatial distribution of parent trees. Journal of Ecology **93**:291–304.

Valverde, T. and J. Silvertown. 1997. A metapopulation model for Primula vulgaris, a temperate forest understorey herb. Journal of Ecology **85**:193–210.

van Asch, T. W. J., S. J. E. van Dijck, and M. R. Hendriks. 2001. The role of overland flow and subsurface flow on the spatial distribution of soil moisture in the topsoil. Hydrological Processes **15**:2325–2340.

van der Heijden, M. G. A. and I. R. Sanders. 2002. Mycorrhizal Ecology. Springer.

van Hinsberg, A. and P. van Tienderen. 1997. Variation in growth form in relation to spectral light quality (red far-red ratio) in Plantago lanceolata L in sun and shade populations. Oecologia **111**:452–459.

Vekemans, X. and O. J. Hardy. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. Molecular Ecology **13**:921–935.

Wade, P. R. 2000. Bayesian methods in conservation biology. Conservation Biology **14**:1308–1316.

Webb, W. L., W. K. Lauenroth, S. R. Szarek, and R. S. Kinerson. 1983. Primary Production and Abiotic Controls in Forests, Grasslands, and Desert Ecosystems in the United-States. Ecology **64**:134–151.

Wells, J. M. and L. Boddy. 2002. Interspecific carbon exchange and cost of interactions between basidiomycete mycelia in soil and wood. Functional Ecology **16**:153–161.

Weltzin, J. F. and G. R. McPherson. 2000. Implications of precipitation redistribution for shifts in temperate savanna ecotones. Ecology **81**:1902–1913.

Western, A. W., R. B. Grayson, and G. Bloschl. 2002. Scaling of soil moisture: A hydrologic perspective. Annual Review of Earth and Planetary Sciences **30**:149–180.

Whigham, D. E. 2004. Ecology of woodland herbs in temperate deciduous forests. Annual Review of Ecology Evolution and Systematics **35**:583–621.

Whigham, D. F. and J. ONeill, 1991. The dynamics of flowering and fruit production in two eastern North American terrestrial orchids, Tipularia discolor and Liparis lilifolia.

Page 89101 *in* T. C. E. Wells and J. H. Willems, editors. Population ecology of terrestrial orchids. SPB Academic Publishing, The Hague, Netherlands.

Whittaker, R. J., M. B. Araujo, J. Paul, R. J. Ladle, J. E. M. Watson, and K. J. Willis. 2005. Conservation Biogeography: assessment and prospect. Diversity and Distributions **11**:3–23.

Wikle, C. K. 2003*a*. Hierarchical Bayesian models for predicting the spread of ecological processes. Ecology **84**:1382–1394.

Wikle, C. K. 2003*b*. Hierarchical models in environmental science. International Statistical Review **71**:181–199.

Williams, B. K. 2001. Uncertainty, learning, and the optimal management of wildlife. Environmental and Ecological Statistics **8**:269–288.

Xiao, J. F. and A. Moody. 2004. Photosynthetic activity of US biomes: responses to the spatial variability and seasonality of precipitation and temperature. Global Change Biology **10**:437–451.

Xu, M., Y. Qi, J. Q. Chen, and B. Song. 2004. Scale-dependent relationships between landscape structure and microclimate. Plant Ecology **173**:39–57.

Yeakley, J. A., W. T. Swank, L. W. Swift, G. M. Hornberger, and H. H. Shugart. 1998. Soil moisture gradients and controls on a southern Appalachian hillslope from drought through recharge. Hydrology and Earth System Sciences **2**:41–49.

Zhou, Z. H., M. Miwa, and T. Hogetsu. 1999. Analysis of genetic structure of a Suillus grevillei population in a Larix kaempferi stand by polymorphism of inter-simple sequence repeat (ISSR). New Phytologist **144**:55–63.

Zinko, U., J. Seibert, M. Dynesius, and C. Nilsson. 2005. Plant species numbers predicted by a topography-based groundwater flow index. Ecosystems **8**:430–441.