Hierarchical patterns of symbiotic orchid germination linked to adult proximity and environmental gradients

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Summary

1 The recruitment stage can be critical in determining plant population dynamics, as juveniles encounter a range of abiotic and biotic stressors in the environments where they land after dispersal, and often experience high mortality rates. Although both abiotic and biotic constraints on recruitment are often assumed to operate at multiple scales, these relationships are rarely quantified in ways that enable prediction of recruitment dynamics across environmental gradients.

2 In plants such as orchids, an important bottleneck on recruitment may arise from an obligate dependence on mycorrhizal fungi in order to reach photosynthetic stage. Very little is known, however, about the factors controlling the distribution of the fungi or how fungal distributions may constrain orchid recruitment and distributions.

3 In this study, seed introduction experiments were used to test for relationships between symbiotic germination success of an orchid, *Goodyera pubescens*, and both proximity to adult orchids and soil abiotic conditions. Experiments were linked to ongoing demographic monitoring across a large environmental gradient to place patterns of germination better in the context of longer-term demographic patterns.

4 A hierarchical Bayesian statistical framework was used to evaluate explicitly how recruitment varies across scales and how this variation is explained by key abiotic variables. This framework was further utilized to link hypothesis testing to model building, and thereby improve our ability to make predictions across a range of environmental conditions.

5 At a microsite scale (< 4 m^2) strong evidence was found for decreasing germination success further from adult plants. At distances greater than 1 m from adults, increased germination success was associated with higher soil moisture, higher organic content and lower pH, although the strength of these relationships varied regionally. Patterns of symbiotic germination success were also highly correlated with observed recruitment patterns at the population level.

6 Explicitly linking plant recruitment to underlying abiotic gradients and key biotic interactions using a hierarchical, predictive modelling framework is essential for understanding basic plant population processes and building capacity to make predictions of how species and communities may respond to environmental changes.

Key-words: germination, hierarchical Bayesian, mycorrhizae, Orchidaceae, prediction

Introduction

The recruitment of new individuals into a population can be an important process for determining population dynamics and community structure (Clark *et al*. 1998; HilleRisLambers *et al*. 2002). Restrictions on fecundity and dispersal can limit the number of propagules available for subsequent development, and juveniles often

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experience high mortality rates. Thus, an understanding of patterns of recruitment is important for inferring many aspects of population dynamics, including population spread rates and responses to changing climate and land use (Silvertown *et al*. 1993; Clark *et al*. 1999; Munzbergova & Herben 2005; Uriarte *et al*. 2005).

Although recruitment and population dynamics are understood to be influenced by both abiotic and biotic factors, more studies are needed that explicitly link these processes to biotic interactions across measured abiotic

gradients (Beckage & Clark 2003). Explicit evaluation of environmental controls on recruitment is important for basic ecological theory, such as assessing niche vs. neutrality models of community assembly (Clark *et al.* 1998; Gravel *et al.* 2006), as well as the more applied interest in predicting species- and community-level responses to changing environmental conditions. The majority of studies linking recruitment to biotic factors fall within the context of testing for negative distance or density-dependence, often in the context of the Janzen–Connell hypothesis (Janzen 1970; Connell 1971; Nathan & Casagrandi 2004), but much less is known about how mutualisms may influence patterns of recruitment (e.g. Hood *et al.* 2004; Kiers *et al.* 2000). Mutualistic or commensal relationships may be expected to confer a positive distance and/or density dependence on recruitment processes if the species involved are co-distributed. Although new technologies are helping to uncover the identity and functions of soil organisms (Fitter 2005), this basic understanding of plant–symbiont biology must be extended to explore the implications for ecological processes, including recruitment patterns.

The extensive literature on the importance of scale for ecological processes (Turner *et al.* 1989; Kotliar & Wiens 1990; Levin 1992) also suggests the need for a better understanding of the scales at which recruitment and population dynamics vary, and how abiotic and biotic factors help explain variability at these scales. Incorporating scale-explicit fieldwork and statistical analysis may thus be critical for evaluating theory and developing predictive models of responses to changing climate and land use. Increasing availability of computationally intensive statistics is now enabling estimation of such multi-level models that incorporate often complex, hierarchical datasets.

In this study I used a combination of targeted field experiments, concurrent abiotic and demographic monitoring, and a hierarchical Bayesian statistical framework to explore patterns of symbiotic germination of the terrestrial orchid *Goodyera pubescens* across multiple spatial scales. As for all of the Orchidaceae, *G. pubescens* is obligately dependent on specific groups of fungi in order to reach photosynthetic stage (Rasmussen & Whigham 1998b). The fungi associated with *G. pubescens* consist of only one or two species within the predominantly saprotrophic genus *Tulasnella*, representing a more specific relationship than originally thought likely for autotrophic plants (McCormick *et al.* 2004), but consistent with the specificity found in a wider group of non-photosynthetic plants (Taylor & Bruns 1997, 1999). The obligate nature of these relationships suggests the potential for a strong influence on orchid recruitment, but 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 & Whigham 1998a), and a study of an Australian 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).

I test the following three specific hypotheses: (i) even within the small scale of 1 m from adult plants, symbiotic germination will be more likely in close proximity to adults because of a co-distribution of adult plants and symbionts; (ii) patterns of successful germination are related to specific edaphic factors that contribute to the site suitability for saprotrophic fungi, namely soil organic content, pH, carbon : nitrogen ratios and moisture availability. The hierarchical statistical framework is used to identify the scales at which germination is most variable and to evaluate explicitly the scales at which abiotic variables help explain this variability. Finally, (iii) I use concurrent demographic monitoring to test the hypothesis that differences in the probability of symbiotic germination result in subsequent population-level differences in seedling recruitment across the 120-km study gradient.

A further, general goal of this study was to begin to bridge the gap between what is known about the recruitment biology of forest herbs and the implications for population dynamics across environmental gradients. In a recent review of woodland herb ecology, Whigham (2004) highlights the fact that although much is known about their basic biology, there have been few detailed studies that allow the prediction of population dynamics over larger spatial and temporal scales. This is likely to be true of a wide range of plant species. An important step for converting the growing understanding of species' basic biology into a predictive capacity of population dynamics is to design studies that link important demographic stages to underlying driving variables while explicitly quantifying the uncertainties of predictions (Franklin *et al.* 2000; Clark 2003). Thus, in the course of testing specific hypotheses, I use hierarchical Bayesian methods to show how hypothesis testing can be usefully placed within the context of building predictive models that translate experimental data into realistic predictions across observed and potentially unobserved conditions.

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. Both roots and leaves can be long-lived and are replaced on a continual basis (Rasmussen & Whigham 2002). Flowers develop in July and August, and, if pollinated, form capsules containing thousands of minute seeds (< 2 mm in length, < 0.5 mm in 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. Like all orchids, *G. pubescens* seeds lack significant
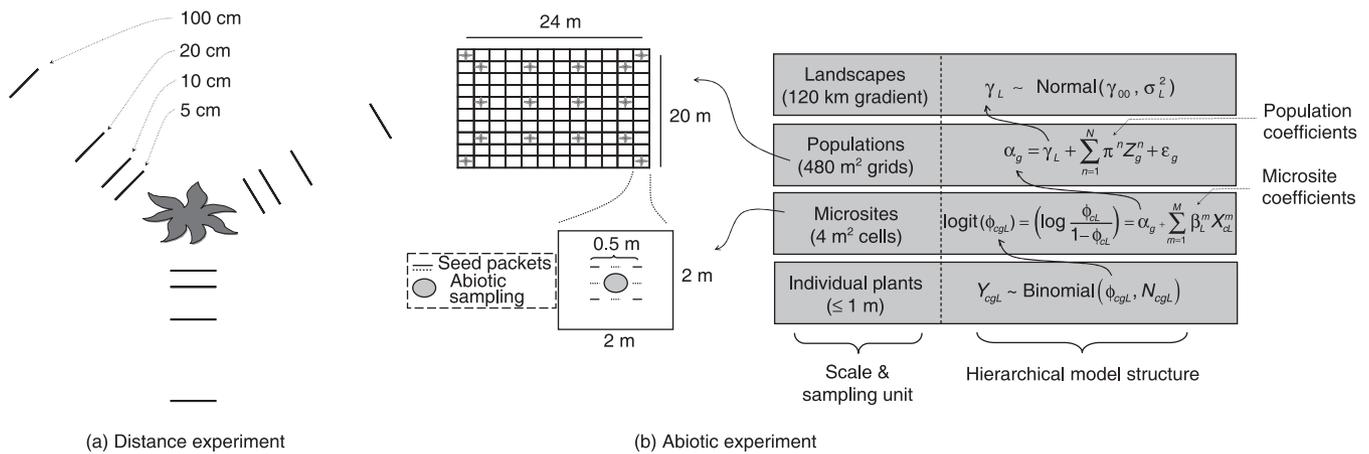
Fig. 1 Experimental design. (a) Experiment 1: seed packets, consisting of plankton netting folded within plastic slide mounts, were placed at four distance classes, in three directions, from adult plants; (b) Experiment 2: packets were placed in a hierarchical design around abiotic sampling points. At left is one study grid (of 16 in total) and one cell (of 256 in total), designed to sample the population and microsite scales, respectively. Eight seed packets were placed in each of the 16 cells per grid (marked by pluses), within 0.5 m of abiotic sampling. Solid seed packet symbols represent 2001–02 sampling and dotted symbols are for 2002–03. The model structure corresponding to this hierarchical experimental design is shown on the right.

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 2 years a seedling. If not colonized by fungi, seeds do not survive, so there is no persistent seed bank, although this is not the case for some other terrestrial orchids (Whigham *et al*. 2006). For the purposes of this report, I refer to symbiotic 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 (Whigham *et al*. 2006), and even adult plant roots remain heavily colonized by the fungi (Rasmussen & Whigham 2002).

The fungi associated with *G. pubescens* have been placed in the genus *Tulasnella* by McCormick *et al*. (2004) using DNA sequencing of the internal transcribed spacer region. Although it is 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, 2006). These saprotrophic fungi use decaying leaves and 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

As part of a larger study of the demography of forest herbs, experimental design and subsequent modelling were hierarchical to reflect different scales of ecological processes (Fig. 1). Study sites were selected across a geographical 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, 83°36′ W, 150–240 m elevation), the Nancytown area of northern Georgia, Habersham County (34°31′ N, 83°53′ W, 315–450 m elevation), and the Coweeta Hydrologic Laboratory in Macon County, North Carolina (35°03′ N, – 83°45′ W, 750–1500 m elevation). A total of 16 study grids (between 250 and 480 m² in size) were established in relatively mature (~80 years old) deciduous forest, and chosen to reflect the range of elevation, temperature and precipitation conditions experienced by the forbs under study. The size of the study grids was chosen to capture population-level processes of understorey plants, and were divided into 2×2-m cells to explore microsite dynamics of these plants. Thus, to emphasize the different ecological scales of interest, the remainder of the paper uses 'population scale' to refer to inference at the level of study grids and 'microsite scale' for inference at the level of 2×2-m cells (Fig. 1). These scales were chosen to reflect levels of variability in the abiotic environment in this system and the likely range of relevant plant processes and interactions (clonal growth, dispersal, etc.).

Seeds were collected from adult plants near the study sites within the Coweeta basin. Seed packets, using a modified design from Rasmussen & 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 Autumn, the 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 and dissecting scope. Seed packets were placed in the field in two experimental designs as described below.

EXPERIMENT 1: DISTANCE RELATIONSHIPS

To test for the effect of proximity to adult plants on protocorm formation, seed packets were placed at four distances from adult plants: 5, 10, 20 and 100 cm. Twenty-eight separate adult plants were selected from five of the study grids. Three transects of seed packets were placed around each adult plant (Fig. 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 m 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. In total, 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 have been collected, including soil moisture, organic content, carbon : nitrogen ratio (C : N) and pH (Fig. 1). A strong precipitation gradient from the Piedmont of the south-east up to the southern Appalachians (from average annual precipitations of 1231 mm yr^{-1} in Athens to 1809 mm yr^{-1} in Coweeta during the time period 1950–2003) is thought to be a major factor driving soil moisture gradients, vegetation patterns and a number of ecological processes in the region. Organic content of the soil is thought to be relevant to substrate availability for the saprotrophic fungi, C : N is generally a measure of substrate 'quality' for microbes and fungi and hence plant competition for available N, and soil pH regulates a number of plant–soil processes.

Seed packets (a total of 2048) were placed around the area of abiotic sampling in a square design (Fig. 1). Data from two consecutive years were pooled for analysis to increase sample size, which is justified given that the explanatory variables of interest exhibit greater spatial variability than temporal variability. For this experiment, care was taken to avoid placement of packets within 1 m of adult *G. pubescens*.

SOIL SAMPLING AND ANALYSIS

A hand-held Hydrosense Soil Water Content Measurement System (Campbell Scientific, Inc., Logan, UT, USA) was used to measure percentage soil moisture in the top 12 cm of soil at the same 16 cells in each grid, every 2 weeks during 2003 and 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 2005).

For characterization of the edaphic environment, three 12-cm soil cores were taken within a 0.5-m radius from the centre 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-mesh sieve, for a total of 256 samples. In a few circumstances, coarse rocky topsoil prohibited three complete 12-cm cores, and four more shallow cores within the 0.5-m radius were used. Total carbon, nitrogen and C : N were determined using dry Micro-Dumas combustion and analysis by gas chromatography (NA1500 C/H/N Analyser, Carlo Erba Strumentazione, Milan, Iatly). A 1 : 1 suspension of fresh soil in deionized water was measured for pH. Soil organic content was determined by percentage weight lost after combustion in a muffle oven. Soil texture was determined using the hydrometer method (Gee & Bauder 1986). As percentage sand and percentage clay were highly correlated, percentage 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, the R computing package (R, D.C.T. 2005) was used 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. For the purpose of all analyses, any seed packet containing ≥ 1 protocorm was considered a 'success'. There was variation in the number of protocorms per packet (between 0 and 13), but because the seeds were artificially placed within a single vertical slice of soil (unlike their natural dispersal pattern) packets were more appropriately scored in a binomial fashion.

With the result of a significant effect of distance on germination, I used Bayesian non-linear model fitting to infer further the shape of the relationship between germination and distance. I fit four competing models to the data and used the Deviance Information Criteria (DIC) to select the best model (see Supplementary Appendix S1). The DIC is an information criterion developed by Spiegelhalter *et al*. (2002) and like AIC (Akaike Information Criterion) is a function of model fit penalized for increasing model complexity (Burnham & Anderson 2002). The models consider each packet as one trial of a binomial process within each cell, such that $Y_d \sim$ Binomial(φ_d, n_d), 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 four competing models describing φ_d were chosen to reflect a range of possible forms that could describe the relationship between distance and germination success. These models were: (i) a negative exponential, which has been a common model for describing wind dispersal patterns; (ii) a logistic decay, which allows for a slower initial decline in probabilities that may represent a more discrete patch of fungi; (iii) a mixed exponential and power function, which also allows for both localized and longer distance components to the pattern (Bullock & Clarke 2000); and (iv) a uniform model in which probabilities do not change as

a function of distance. More detailed model descriptions and modelling methods are included in Appendix S1.

To assess the effect of soil abiotic variables on germination success, a hierarchical logistic regression approach was used to test the importance of hypothesized explanatory variables for determining suitability of a site for germination at both microsite and population scales. Although under-utilized in ecological studies, hierarchical linear models provide a natural framework for testing the significance of multiple variables simultaneously at multiple scales (Raudenbush & Bryk 2002). Inclusion of predictor variables at multiple levels of a model provides both an unbiased random effects model and unique inference on the scale-specific processes (J. Bafumi & A. Gelman, unpublished data). A Bayesian framework for the hierarchical models was chosen for more robust quantification of uncertainty given an unbalanced sampling design (Raudenbush & Bryk 2002). As discussed later, the Bayesian models also facilitate prediction at unobserved environmental states, while incorporating uncertainty from each level of the model. The hierarchical formulation allows for landscape-level differences owing to unmeasured site conditions, but a sharing of data across sites to reflect a common process across sites and allow greater inferential power (Gelman *et al.* 2004).

The hierarchical model begins with data at the individual packet level, where germination of seeds in each packet is considered a Binomial process within each cell, such that

$$Y_{cgL} \sim \text{Binomial}(\varphi_{cgL}, N_{cgL}),$$

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

$$\text{logit}(\phi_{cgL}) = \log\left(\frac{\phi_{cL}}{1 - \phi_{cL}}\right) = \alpha_g + \sum_{m=1}^{M} \beta_L^m X_{cL}^m.$$

The α_g are grid-level intercepts, β_L^m are M vectors of landscape-level regression coefficients, and X_{cL}^m are vectors of microsite covariate data (soil moisture, pH and organic content). The grid-level intercepts α_g provide the link between the cell and landscape levels as a random effect drawn from a global distribution with a grid-specific mean determined by a global intercept and grid-level covariates as follows:

$$\alpha_g = \gamma_L + \sum_{n=1}^{N} \pi^n Z_g^n + \varepsilon_g$$

Here, Z_g^n are N vectors of covariate data at the grid level (soil moisture, pH and organic content), and there are N regression coefficients, π^n, describing those relationships. The error terms at this level, ε_g, are normally

distributed $\varepsilon_g \sim \text{Normal}(0, \sigma_g^2)$, with the variation attributable to the grid level given a non-informative prior $\sigma_g^2 \sim \text{InverseGamma}(0.01, 0.01)$. The intercepts, γ_L, represent the regional means and are modelled with a non-informative global prior distribution $\gamma_L \sim \text{Normal}(\gamma_{00}, \sigma_L^2)$ and diffuse priors $\gamma_{00} \sim \text{Normal}(0, 1000)$ and $\sigma_L^2 \sim \text{InverseGamma}(0.01, 0.01)$. Regression coefficients are modelled hierarchically, with each of the M landscape-level parameters being drawn from a global distribution: $\beta_L^m \sim \text{Normal}(\beta_{\text{global}}^m, \sigma^2)$ and a global non-informative prior $\beta_{\text{global}}^m \sim \text{Normal}(0, 1000)$. These global parameters represent the overall effect (across all landscapes) of each covariate at the microsite scale. The N population-level parameters were given non-informative prior $\pi^n \sim \text{Normal}(0, 1000)$, allowing the data to drive their estimation. An unconditional model with no covariates at any level was initially fit in order to partition the variance in recruitment success among the different scales of the study.

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 the 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 local distances (≤ 1 m) from adult plants, and then across a range of abiotic conditions experienced by the species in the wider region. Ideally, synthetic models might be estimated that include both distance and abiotic constraints, but in this case the distance-dependence is too local (< 1 m) to be informed by abiotic measurements, as they were collected in this study for assessing larger scale patterns.

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 (Gilks *et al.* 1996; Spiegelhalter *et al.* 2000). Convergence of three independent chains was assessed via the Gelman–Rubin statistic, and sufficient burn-in periods and thinning rates were used to ensure satisfactory sampling of the posterior distributions (see Appendix S1).

Results

DISTANCE

Germination probability sharply declined with distance within 1 m from adult plants. Maximum-likelihood estimates of the probabilities of germination at four distances within this 1 m from adults showed a declining probability
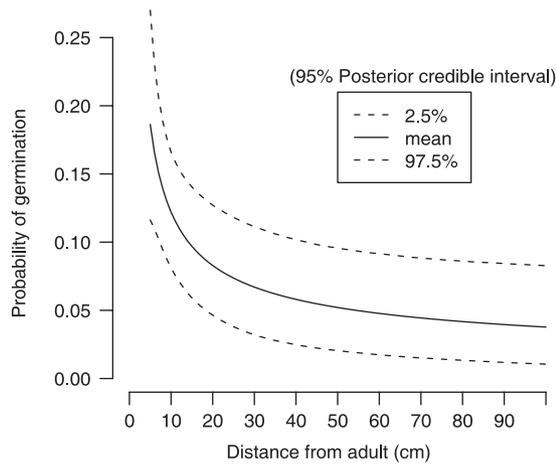
Fig. 2 Predicted germination probabilities as a function of distance. Probabilities of germination here reflect the probability that one or more seeds (out of approximately 150) within a seed packet were found germinated. Using estimated parameters of the most well-supported model (the mixed model), predictions are made within the MCMC statistical routine across the entire first metre from adult plants. Dotted lines indicate the 95% credible interval for the model predictions.

with distance (probabilities of 0.202, 0.119, 0.071 and 0.036 at distances of 5, 10, 20 and 100 cm, respectively). The distance effect was significant when a binomial generalized linear model was fit to the dataset ($P = 0.0187$).

Of the four Bayesian models fit to the distance data, the best fitting model as judged by the DIC was the mixed exponential and power function (DICs of 20.67, 24.11, 34.04 and 45.45 for the Mixed, Logistic, Uniform and Negative Exponential models, respectively). By balancing model fit and complexity, the model selection process provides a useful way to judge the relative fit of multiple plausible forms of the relationship, and the selection of the mixed model over the uniform model is also a more informative way to confirm the rejection of the null hypothesis of no effect of distance on germination. The small difference in DIC between the mixed and logistic models suggests that there may not be enough information really to distinguish between them. They both suggest an initial flatness relative to the exponential, and decline to a background probability at some distance.

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 cm (Fig. 2). Although probabilities of germination are low at all distances, the model predicts a sharp decline in germination 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 Fig. 2. If one were interested in using predictions for subsequent models of population dynamics, the entire posterior distributions could be used.

ABIOTIC FACTORS

The key abiotic variables, as well as the probabilities of successful symbiotic germination, were hierarchically

structured, with substantial variability within populations but also clear population and regional differences (Fig. 3), thus highlighting the motivation for hierarchical models. The unconditional model showed most of the variation in recruitment to be at the landscape level (75%), followed by the microsite level (18%) and then the population level (7%). Tested within the hierarchical model framework, explanatory variables were evaluated at each scale. Again, the 95% credible intervals are reported as a convenient summary of the posterior distribution of each parameter. As the posteriors give direct probabilities for 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. At the microsite scale, symbiotic germination was positively correlated with soil organic content and average soil moisture, and negatively correlated with pH (Fig. 4). The 95% intervals for the relationship with percentage organic content and soil moisture did not overlap zero for the Coweeta landscape, whereas intervals for the other landscapes were highly positively skewed but overlapping zero. Likewise, the relationship with pH was clearly negative in the Whitehall landscape, strongly skewed negative in Nancytown and broadly overlapping zero in Coweeta. At the population level, the 95% intervals for these explanatory variables were overlapping zero (Fig. 4). Both C : N and soil texture were broadly overlapping zero and not included in the final models.

Given the strong 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. 5). 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.

There was also a significant positive relationship between seed germination rates and observed demography within populations (Fig. 6). The Pearson's product-moment correlation was 0.87 ($P \ll 0.001$). 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's combination of field experiments, abiotic monitoring and hierarchical statistical analysis allowed a unique estimation of the spatial scales at which abiotic and biotic factors constrain recruitment success of a terrestrial orchid. The data showed a strong pattern
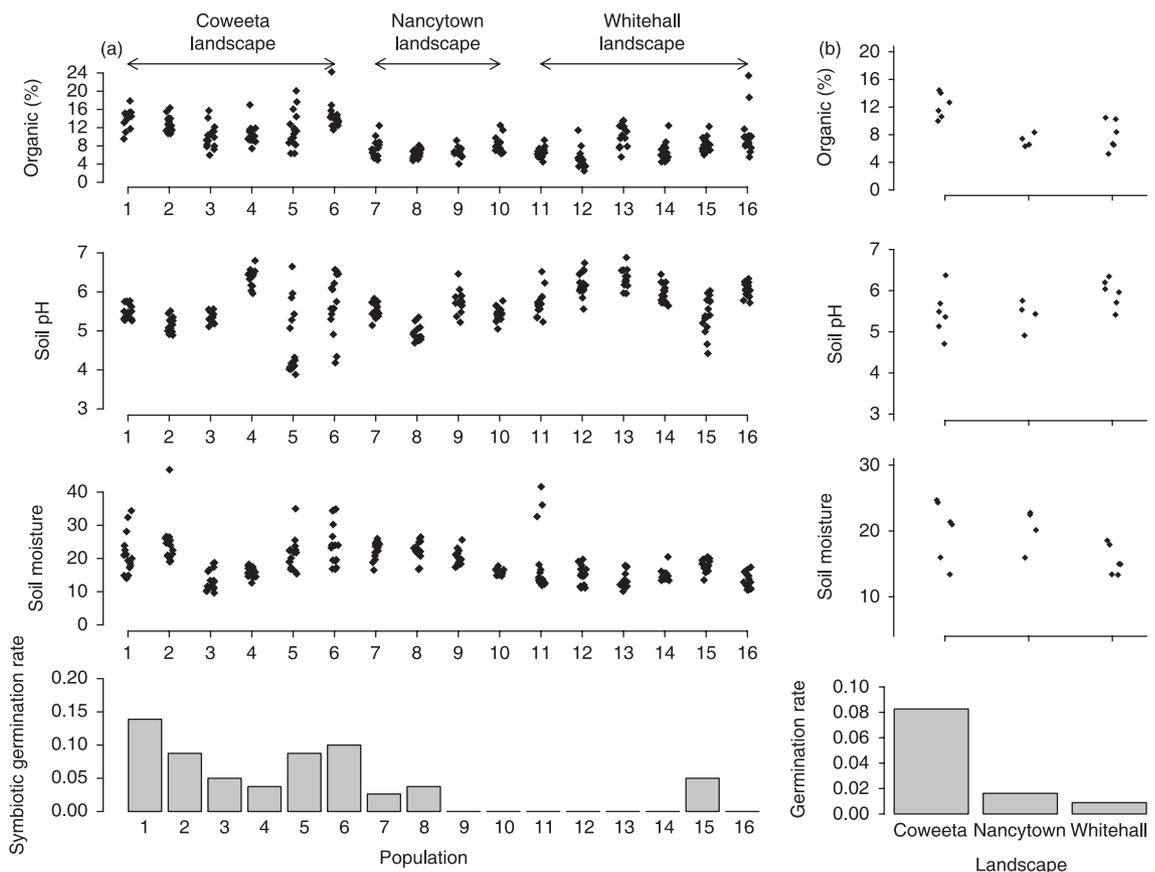
Fig. 3 Structured abiotic environment and germination rates. Distribution of abiotic variables and symbiotic germination success within and among study sites: (a) percentage organic content, soil moisture, pH and germination success in the 16 study grids show variability within sites but distinct site differences as well; (b) grid averages for each of the three regions (Coweeta, Nancytown, Whitehall) also show variability within each landscape but regional differences at this scale as well. Abiotic data are 'jittered' (randomly offset in the *x*-axis direction) for ease of viewing.

of declining probability of symbiotic germination within 1 m from adult plants, and increased germination in sites with higher soil moisture and organic content and lower pH. These abiotic effects were more significant at the microsite level than at the population level, but there were additional large regional differences in recruitment success. The unconditional model showed that the majority of variation in recruitment success was in fact at the landscape level, followed by the microsite level. The relative paucity of variation at the population scale may account for the lack of significance of abiotic explanatory variables at this scale. 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.

Several aspects of the analysis used in this study will be useful for other studies of plant recruitment, population dynamics and broader ecological studies. The hierarchical structure of the models allows explicit evaluation of which variables, and which scales, are most important for understanding and predicting recruitment success. In this case the same key abiotic variables were considered at the different scales, but if additional variables were measured at these different scales they

could be used at the appropriate scale (see Raudenbush & Bryk 2002 for detailed account of building hierarchical models). Building scale-explicit links between recruitment processes and abiotic variables will help avoid misinterpreting the importance of effects at the wrong scales, while better reflecting the spatial structure thought to predominate in these ecological processes. The Bayesian framework can help in parameter estimation of multi-level models (Raudenbush & Bryk 2002) and, importantly, help provide realistic estimates of the uncertainty associated with making predictions about ecological processes (Wikle 2003a,b; Clark 2005). It was shown here how even rather simple hierarchical models may be effectively used explicitly to evaluate abiotic effects at different scales and build a predictive understanding across these scales.

Finally, it was shown how the model-selection approaches becoming more widely used by ecologists may be extended to provide a smooth transition from hypothesis testing to prediction. Our understanding of recruitment dynamics will be enhanced by consideration of multiple models and building predictive models that incorporate uncertainty, instead of simply testing for significant effects of distance on recruitment probabilities. Selection of the mixed model is conditional on
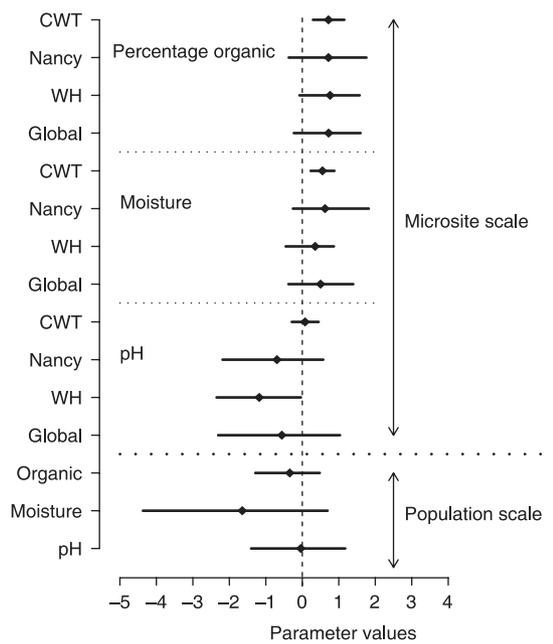
Fig. 4 Hierarchical abiotic effects. Lines represent 95% posterior credible intervals for estimated effects of percentage organic content, soil moisture and pH in the three landscapes (Coweeta, Nancytown, Whitehall); diamonds indicate means. At top are estimates of abiotic effects at the microsite (2 × 2 m) scale, corresponding to the, coefficients in Fig. 1, while the bottom three estimates represent population-level effects, corresponding to the π values in Fig. 1. Those variables whose intervals do not overlap the dashed zero line may be considered significantly different from zero.

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.

IMPLICATIONS FOR PLANT POPULATION DYNAMICS

The results and methods of this study contribute to the general understanding of plant recruitment with respect to distance to adults and key abiotic variables. Much of the research conducted on patterns of plant recruitment as a function of distance from adults has focused on tree species in the context of testing the Janzen–Connell hypothesis (Connell 1971; 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 pressure near parent plants) can contribute to the maintenance of species diversity. Studies of several tree species support the hypothesis of negative density dependence in recruitment (e.g. Augspurger & Kelly 1984; Packer & Clay 2000, 2003; Tomita *et al.* 2002), while the strength of this form of recruitment limitation as a mechanism of species co-existence is still under exploration. The current study targeted distance as an independent variable instead of

density *per se*, but the density of adults also declined as a function of distance due to the sampling design. Density of adults, in addition to distance to the nearest adult, may be of particular significance for species that may form mycorrhizal connections with neighbouring trees.

Previous studies with herbaceous plants are scarcer, and those few with orchids in particular have reported mixed results with regard to the importance of proximity to adults for recruitment. Although some studies have suggested a positive association between germination and adult locations for myco-heterotrophic orchids (McKendrick *et al.* 2000, 2002; Leake *et al.* 2004), and 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-m spatial scale used in this study (Masuhara & Katsuya 1994; McKendrick *et al.* 2000).

The declining probability of germination with distance from adults found in this study suggests positive density dependence for recruitment of these orchids. Although 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 cm of adults. The implications of these patterns for population dynamics will depend on subsequent protocorm and seedling survival rates. The demographic monitoring of the plants helped place the seed packet experiments in the context of how these early germination rates translate to population-level recruitment rates. The high correlation between seed germination rates and seedling recruitment rates 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 (my unpublished data). Even when accounting for decreased detection probabilities further from adult plants, it remains highly likely that seedling recruitment is most common within existing patches of adults. The lack of observed 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. Moreover, a couple of sites with adult plants and some protocorm formation in seed packets had zero observed natural recruitment, suggesting the existence of factors that are limiting at seed production and/or seedling stages.

MYCORRHIZAL ECOLOGY

Although the importance of mycorrhizal fungi to the ecology of adult plants is increasingly documented, less is known about how these fungi may influence initial patterns of plant recruitment. The obligate dependence on fungi for symbiotic germination in the Orchidaceae may make orchids particularly subject to recruitment constraints. Although all orchids are dependent on
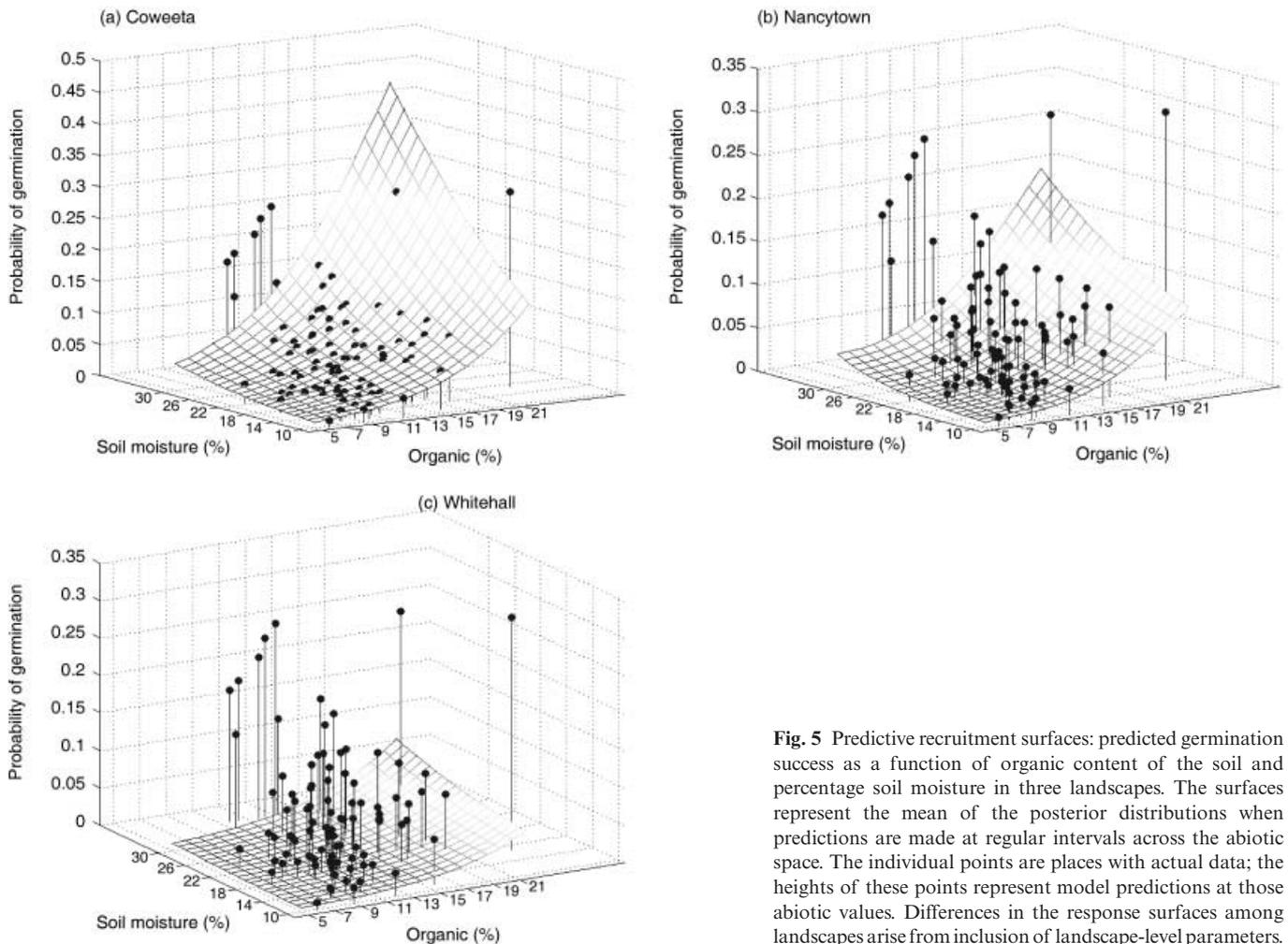
(a) Coweeta

(b) Nancytown

(c) Whitehall

Fig. 5 Predictive recruitment surfaces: predicted germination success as a function of organic content of the soil and percentage soil moisture in three landscapes. 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 heights of these points represent model predictions at those abiotic values. Differences in the response surfaces among landscapes arise from inclusion of landscape-level parameters.
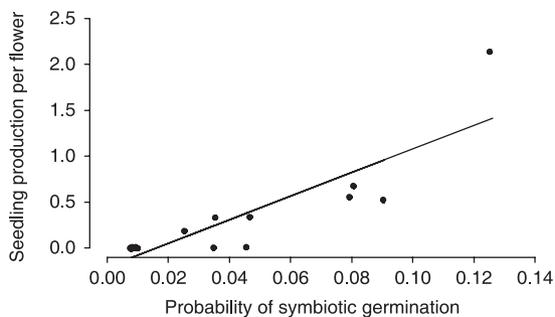

Fig. 6 Observed seedlings. The observed probability of seedling production is significantly related to germination probability in seed packets at the population level. The *y*-axis represents the total number of seedlings observed in each of the 16 populations between 2001 and 2004, divided by the number of observed flowers. The *x*-axis is the observed population-level seed packet success rate (finding a protocorm in a packet). Linear regression, $P < 0.001$ ($R^2 = 0.74$, $F_{1,14} = 42.88$).

found to maintain quite specific relationships that may include connections to neighbouring ectomycorrhizal trees (Taylor & Bruns 1997, 1999). Although basic evolutionary theory may suggest 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). Thus, factors that influence the abundance of these saprotrophic fungi, such as substrate availability and suitable abiotic conditions, may be important for their recruitment.

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 substrates necessary. The fungi within the genus *Tulasnella* are able to utilize cellulose, but not lignin, and in laboratory studies perform well on a range of raw wood and leaf substrates, as well as other basic media (Cairney 2005; McCormick *et al*. 2006). Although inferring fine-scale habitat suitability for fungi in natural systems is a challenge, a number of laboratory studies

fungi in order to reach photosynthetic stage, there appears to be some variation across species in both the specificity and the identity of fungal symbionts. Non-photosynthetic orchids ('myco-heterotrophs') have been

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 Ettema & Wardle 2002). Forest soils can be very heterogeneous both abiotically (Ettema *et al.* 2000; Fraterrigo *et al.* 2005) and biotically (Ettema *et al.* 1998; Diez 2005), so it is likely 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 ecology.

An alternative, but not mutually exclusive, explanation to this 'habitat suitability' hypothesis is that declining abundance away from adult plants may reflect decreased fungal access to those sites. The frequency and abundance of spore production of these fungi in nature are unknown, as well as their potential to spread clonally into new microhabitats. Because adult plants remain heavily colonized by fungi, and patches of plants often contain the same fungal genet (Bidartondo *et al.* 2000), 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 (Dahlberg 1997). More targeted experiments are 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. Dickie *et al.* 2002). In fact some studies have found significant clumping of ectomycorrhizal fungi around host plants (Bidartondo *et al.* 2000). As many of the ectomycorrhizal species are obligately associated with plants, it might be expected that saprotrophic species, such as in *Tulasnella*, 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.

Conclusions

Understanding how abiotic and biotic factors combine across multiple scales to constrain patterns of plant recruitment remains an important goal for plant ecologists. Translating this understanding into predictive capacity across spatial and temporal environmental gradients will require experimental and statistical methods that are scale-explicit and provide direct links to measurable abiotic variables. This study has demonstrated how hierarchical Bayesian models may be useful for linking patterns of germination to underlying environmental variables at different spatial scales. Such approaches that can incorporate observational and experimental data and prediction over unobserved states, while providing explicit estimates of uncertainty, will facilitate anticipation of species responses to environmental change.

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Supplementary material

The following supplementary material is available for this article:

Appendix S1 Distance model details.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2745.2006.01194.x.

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