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SPATIAL ENVIRONMENTAL VARIATION: WITHIN AND AMONG POPULATION EFFECTS IN *DANTHONIA SPICATA*

By

Melissa Kay McCormick

A DISSERTATION

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ABSTRACT

SPATIAL ENVIRONMENTAL VARIATION: WITHIN AND AMONG POPULATION EFFECTS IN *DANTHONIA SPICATA*

By

Melissa Kay McCormick

Understanding the role of spatial environmental heterogeneity on the ecological and evolutionary dynamics of plant populations is a major challenge for ecologists and evolutionary biologists. Spatially structured variation in resources is commonly measured, but its temporal persistence and relation to plant performance have rarely been assessed. Spatial structure in resources can affect ecological and evolutionary processes at all levels of organization, but the effect of resource variation on these processes will depend on the spatial and temporal scales at which it varies.

I used a series of field and greenhouse experiments to determine whether variation in soil resources (soil moisture, ammonia, and nitrate) was spatially structured and temporally consistent at scales relevant to the native, perennial grass *Danthonia spicata*. I then related these patterns of variation in soil resources to ecological and evolutionary differences within and among populations of *D. spicata*. Specifically, I tested whether *D. spicata* plants responded to measured differences in the spatial structure of soil moisture and nitrogen availability in different sites. I also related differences in dispersal distance among five populations of *D. spicata* to the amount of spatially structured variation that occurred in these sites. Interactions with other species may affect the responsiveness of a species to resource heterogeneity. I extended the population-level analyses of spatially

structured variation in soil resources to the community level by testing whether the interaction between *D. spicata* and the epiphytic fungus *Atkinsonella hypoxylon* was affected by differences in soil moisture and nitrogen.

I found that my five study sites differed in amount and consistency of spatially structured variation in soil moisture, ammonia, and nitrate. More importantly, I found that survival of *D. spicata* plants was affected by differences in spatially structured variation in soil resources among populations. This suggests that *D. spicata* plants "perceive" differences in spatially structured variation in soil resources similar to those I had measured.

In sites where soil resources are spatially structured, plants can take advantage of spatial structure and target their seeds to good environments by decreasing their dispersal distance. I found that *D. spicata* populations in sites with more spatially structured soil resource variation had shorter dispersal distances than those in less spatially structured sites. This finding was consistent with the hypothesis that spatially structured environmental variation selects for decreased dispersal distance.

In three populations of *D. spicata* infected by *A. hypoxylon*, I found that infected plants were distributed differently than uninfected plants with respect to soil moisture and ammonia supply. In greenhouse studies, infected plants performed less well in dry, low fertility conditions than uninifected individuals. These differences in the performance of infected and uninfected plants in response to soil resources structures the interaction between these two species and influences their distribution in the field.

ACKNOWLEDGMENTS

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Financial support for this research was provided by a National Science Foundation Dissertation Improvement Grant, the KBS Graduate Research Training Grant, the Department of Zoology, the College of Natural Science, MSU's Ecology, Evolutionary Biology, and Behavior Program, and the Kellogg Biological Station.

Thanks also go my parents, who instilled me with an appreciation of plants and the natural environment and who encouraged me to always do my best, with the assurance that they would love me no matter how things turned out. Finally, I would like to thank my husband, Mark, whose love, patience, tolerance of long working hours, and humor supported me throughout my graduate career.

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Chapter 1

INTRODUCTION

Environmental variation occurs at a variety of spatial and temporal scales in ecological systems. The challenge to ecologists is to determine the importance of environmental heterogeneity at different scales, and the linkages between scales, to develop a more complete understanding of how environmental variation can affect the distribution, abundance, and fitness of an organism. Spatial variation in soils is ubiquitous (e.g. Grime 1979, Tilman 1988, Schlesinger 1996, Gross et al. 1995), but the role of soil resource variation in determining plant-"perceived" resource heterogeneity is largely unknown (Robertson et al 1988, Ehrenfeld 1997). The effect of environmental variation on plants depends on the magnitude, scale, and temporal consistency of that variation (Miller et al. 1995, Stratton and Bennington 1998). It also depends on the proportion of variation that is spatially structured (the degree to which two points that are close together are more similar than two points chosen at random). These aspects of environmental variation interact with plant size, longevity, and dispersal characteristics to determine the potential evolutionary response of a population to its environment (Antonovics et al. 1987).

If environmental conditions are predictable (structured in time and space) at the scale of dispersal and individual longevity, then selection can result in locally adapted subgroups (Hoffman and Parsons 1991). Locally adaptive responses among populations to differences in mean environmental conditions have been well documented (e.g. Bennington and McGraw 1995, Schoen et al. 1986, Antonovics et al. 1987, Platenkamp

1990), but differences among populations in response to differing magnitudes of environmental variation have only rarely been examined (Sultan 1987, Stark 1994, but see Miller and Fowler 1994). The scale at which local adaptation to environmental conditions occurs depends on the strength and predictability of selection, the available genetic variation, and the interaction between gene dispersal distance and the scale of environmental structure.

Although dispersal distance influences the evolutionary impact of environmental variation at different scales, dispersal itself can also respond adaptively to differences in the scale of spatially structured environmental variation (Harper 1977, Venable and Brown 1988). The spatial scale of dispersal also affects the amount of genetic diversity that can be maintained within populations by determining the scale at which local adaptation can occur. Maintenance of genetic diversity by spatial variation has been theoretically demonstrated to be rare because dispersal distance and the scale of environmental variation must be precisely matched (e.g. Spieth 1979, Gillespie 1981). However, if populations can adjust dispersal distance to the scale at which environmental variation is structured, then conditions may be less restrictive (Hedrick 1986).

When environmental variation occurs at scales smaller than dispersal distance or is temporally inconsistent, populations cannot respond by local adaptation. Instead, populations must be phenotypically plastic in their response to environmental variation. Plastic responses to individual-scale environmental variation have been frequently demonstrated (e.g. Jackson and Caldwell 1993, Fitter 1994, Gross et al. 1993). However, the amount of phenotypic plasticity displayed by organisms in a population may be locally adapted to the amount of environmental variation that occurs at small scales.

Although local adaptation in phenotypic plasticity has occasionally been related to the temporal consistency of spatially structured environmental variation (Sultan and Bazzaz 1993), it has rarely been related to either the amount or scale of spatially structured environmental variation (but see Lotz et al. 1990, Miller and Fowler 1994).

The response of plants to environmental variation can also be modified by interactions with other species (Chesson 1985, Reader and Best 1989). For example, the growth of plants infected by mycorrhizae is often limited by light, while the growth of uninfected plants in the same community may be limited by soil nutrients (Harley 1969, Harley and Smith 1983). This suggests that plants with mycorrhizae "perceive" high nutrient, low light habitats as unsuitable, while plants without mycorrhizae "perceive" low nutrient, high light habitats as similarly unsuitable. This sort of environmentally-mediated tradeoff response of a species interaction may produce relatively discrete spatial patches of plants with and without mycorrhizae in a heterogeneous environment. Environmentally-dependent species interactions, such as this example, are most likely to be apparent between intimately associated species.

In this dissertation, I used a combination of field and greenhouse studies to study the response of *Danthonia spicata* to spatial environmental heterogeneity in soil resources. *Danthonia spicata* is a native, perennial, C_3 bunchgrass that grows in dry, low fertility old-fields and oak savannas throughout the northern and eastern United States and southern Canada. *Danthonia spicata* produces dimorphic seeds with different dispersal abilities (Figure 1.1). Allocation to the two seed types has a strong genetic basis and is extremely variable within and among *D. spicata* populations (Clay 1982), making it a good indicator of population differences in dispersal. I examined differences

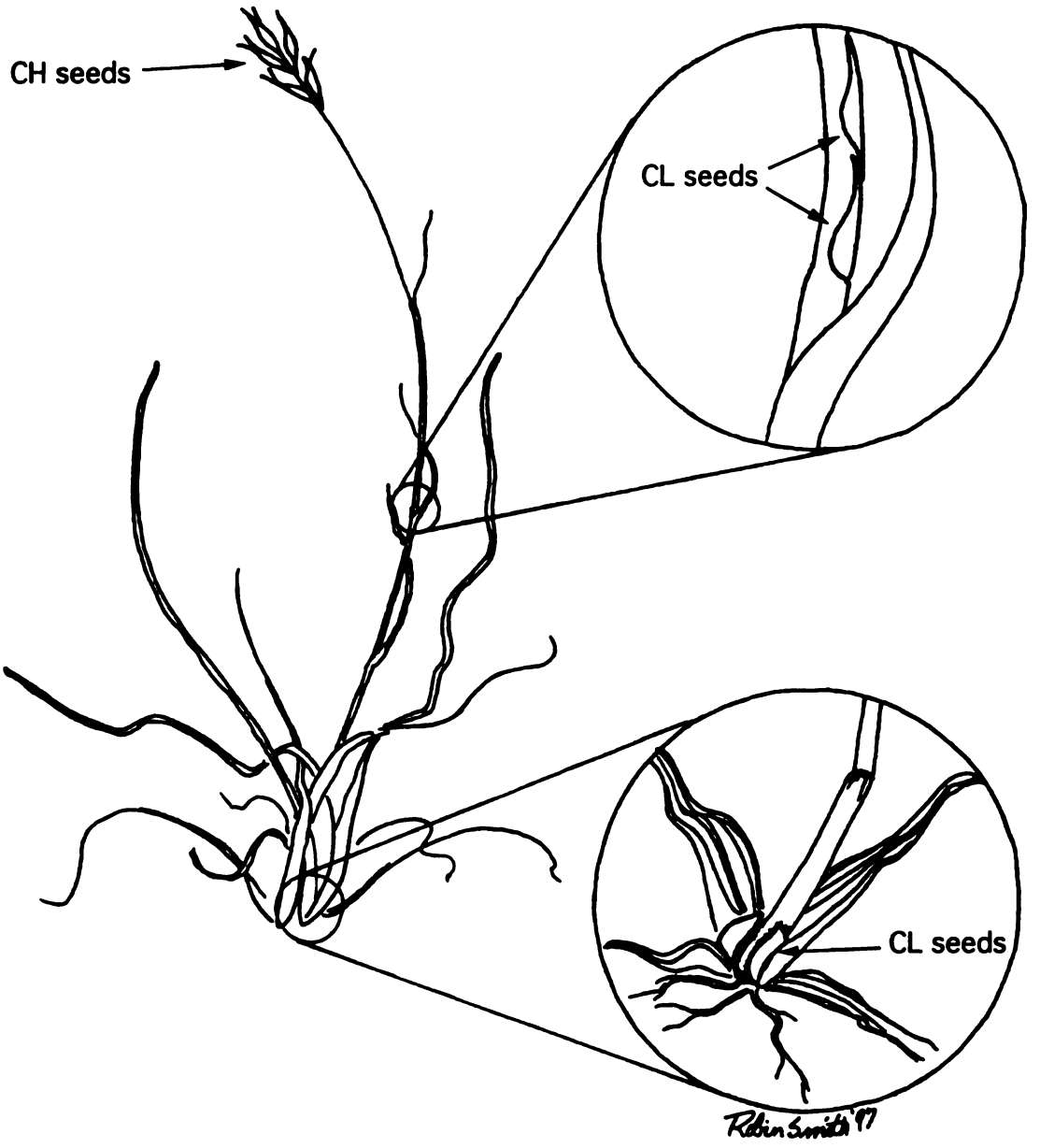

Figure 1.1: *Danthonia spicata*
Diagram of *Danthonia spicata* showing the location of cleistogamous (CL) and chasmogamous (CH) seeds.

among populations in dispersal distance, an explicitly spatial trait that could be related to differences in the spatial scale of resource heterogeneity among sites, and in three traits associated with nitrogen acquisition that were likely to be strongly influenced by temporal variation (i.e. be phenotypically plastic). I predicted that *D. spicata* plants from environments with more spatially structured variation in soil resources would have shorter dispersal distances and more phenotypic plasticity than plants from less variable environments. I then also examined the effect of spatial heterogeneity in soil resources on the interaction between *D. spicata* and the epiphytic fungus *Atkinsonella hypoxylon*. Because *A. hypoxylon* has been shown to affect the growth and competitive ability of *D. spicata* (Clay 1984, Kelley and Clay 1987), I predicted that infection by *A. hypoxylon* would affect plant "perception" of spatially variable soil resources.

DISSERTATION OVERVIEW

Chapter 2 is an analysis of the spatial structure and temporal consistency of variation in soil resources among five populations of *Danthonia spicata*. I measured heterogeneity in soil moisture and nitrogen availability; for nitrogen I measured inital pool as well as the supply rate of nitrate- and ammonia-nitrogen over 1-2 years. I then related these measures of soil resource heterogeneity to performance of *D. spicata* in a phytometer study. I used both direct measurements of soil moisture and nitrogen availability and plant performance in these sites to examine differences in the magnitude and scale of spatial environmental variation within populations. I predicted that soil resource heterogeneity would vary to differing degrees among sites and that plant

performance (survival, growth and tillering of *D. spicata*) would reflect these measured differences in soil conditions.

In chapter 3 I examined the relationship between dispersal distance and the scale of soil resource variation for five *D. spicata* populations. I used dispersal measurements and computer simulations to estimate the average population dispersal pattern and the amount of environmental variation (relative to parental conditions) that would be encountered within each population by dispersing offspring. I then related among-population differences in dispersal pattern to among-population differences in the scale of spatial heterogeneity in soil resources. I predicted that populations in sites with less spatially structured variation in soil resources would have primarily chasmogamous (far-dispersing) seeds and populations in sites with more spatially structured variation would have a large proportion of cleistogamous (near-dispersing) seeds.

The results of greenhouse experiments examining the relationship between phenotypic plasticity and spatial variation in soil nitrogen are presented in chapter 4. I examined differences in phenotypic plasticity of three traits related to nitrogen acquisition among five populations. I also compared the amount of phenotypic plasticity for nitrogen acquisition among differently-dispersing families within each population. I predicted that plants from populations with more spatial variability in nitrogen supply rate would have greater phenotypic plasticity than those from less variable poplations and that individuals from far-dispersing (low CL) families would be more plastic than those from near-dispersing (high CL) families.

Because interactions with other species can affect the responses of an organism to its environment, I also examined the impact of the interaction between *D. spicata* and the

epiphytic fungus *Atkinsonella hypoxylon* on plant perception of spatially heterogeneous soil resources. These results are summarized in Chapter 5. I used field experiments to examine the spatial distribution of *A. hypoxylon* in field populations of *D. spicata* in relation to soil moisture and nitrogen supply. I then used greenhouse experiments to explicitly address the effects of soil moisture and fertility on growth of plants infected and uninfected by *A. hypoxylon*. I predicted that plants infected by *A. hypoxylon* would be affected differently by soil moisture and fertility than uninfected plants.

I discuss the implications of these results for studies of evolution in spatially and temporally heterogeneous environments in a concluding chapter (6). I specifically relate my results to studies of measured environmental heterogeneity and to theoretical expectations for the joint evolution of dispersal (local adaptation) and phenotypic plasticity in response to spatially heterogeneous environments.

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Chapter 2

SPATIAL STRUCTURE IN SOIL RESOURCES: TEMPORAL CONSISTENCY AND "PERCEPTION" BY *DANTHONIA SPICATA*

(Manuscript co-authored with K.L. Gross)

INTRODUCTION

Spatial structure in soil resources can have an important role in generating patterns of genetic variation (Venable and Brown 1988, Bell and Lechowicz 1991, Stratton 1995) and structuring interactions in plant populations and communities. Spatial structure in soil resources has been measured in a number of systems (e.g. Grime 1979, Tilman 1988, Schlesinger 1990, Gross et al. 1995), but whether this measured spatial structure is relevant to plants is largely unknown (Robertson et al 1988, Ehrenfeld 1997). To be relevant to plants, resource heterogeneity must occur at appropriate spatial and temporal scales, determined by plant size, dispersal distance, and longevity. Temporal inconsistency may cause measured spatial heterogeneity to appear irrelevant to plants over the time scales at which ecological and evolutionary responses are produced.

In a spatially structured environment, plants and their dispersing offspring encounter environmental variation over a range of scales. Individual plants can average over temporal variation within their lifetimes and families can average over variation at longer time scales, but the ecological and evolutionary impact of environmental variation on plant populations may depend on how individuals and families experience that variation. Plant species that differ in growth phenology may be disproportionately affected by environmental variation that occurs during different times of the year or during different seasons. For example, a plant that grows, reproduces and goes dormant

in the spring may be little affected by environmental variation during the fall, while a plant that grows throughout the year may be affected by differently structured variation during different seasons. Patterns of environmental variation that were consistent among seasons or during a single season among years would affect these two plant species differently. Consequently, the temporal scale at which spatial structure would need to be consistent to produce ecological and evolutionary effects would differ among species with different phenologies. Temporal persistence of measured soil spatial structure has been assumed at many temporal scales, but rarely measured (but see Goovaerts and Chiang 1993). Several recent studies of evolution in heterogeneous environments have questioned the persistence of measured spatial environmental structure within populations (e.g. Sultan and Bazzaz 1993, Stratton and Bennington 1998).

Spatial structure in soil resources occurs over a wide range of scales from millimeters to hundreds of kilometers (e.g. Robertson and Gross 1994, Gross et al. 1995, Miller et al. 1995, Ehrenfeld et al. 1997). Individual size, offspring dispersal distance, and population size define scales at which spatial structure is likely to generate different evolutionary responses in plant populations (Miller et al. 1995). An individual plant may only experience a few cm^2 of the soil surface, but the spatial scale at which a plant experiences environmental structure can change as it grows and/or with life stage. A plant also "perceives" its environment through differential success of its offspring, which may be distributed over several m^2 or more (Bell and Lechowicz, 1994). The amount of spatial variation in soil resources that is encountered by individuals or dispersing offspring is determined by the spatial structure of environmental variation. The proportion of environmental variation that is spatially structured and the range over

which it is structured determine the extent to which environmental variation will increase with increasing distance between sample points (or between parent and offspring) and the distance over which it will increase. Spatial structure that occurs at scales smaller than dispersal distance must be tolerated or exploited through phenotypic plasticity, while spatial heterogeneity at larger scales may result in local adaptation. For measured spatial structure to affect the structure of plant populations and communities, it must be of a magnitude that affects plant performance, occur at spatial scales greater than dispersal distance, and persist over temporal scales longer than an individual lifetime.

Demonstrating environmental structure at spatial and temporal scales that are relevant to plants is an important step in relating measured variation to "perceived" variation. However, the definitive test of the relevance of field-measured soil heterogeneity to a particular plant species is to grow that species in intact field populations where variation in soil variables has been measured (Bell and Lechowicz 1991, Miller and Fowler 1997) and to relate plant performance to environmental measurements.

In this study, we combined data on single-time measures of soil moisture and extractable nitrogen, with longer-term measures of nitrogen supply rate and growth of phytometers to examine the spatial structure of soil moisture and nitrogen availability, the temporal persistence of these patterns, and their relevance to the growth and survival of *Danthonia spicata* in field populations. Nitrogen availability limits plant performance in many terrestrial plant communities (Chapin 1980) and variation in nitrogen availability has frequently been demonstrated on spatial scales ranging from centimeters to meters and much larger (Gross et al. 1995, Ehrenfeld et al. 1997). Although the impact of

uniformly increasing nitrogen availability on plant performance has often been demonstrated, the relevance of measured spatially structured variation in nitrogen to plants in the field is largely unknown (Miller and Fowler 1994). Competition for spatial heterogeneous soil resources may be particularly important for plants in low productivity environments (Tilman 1988). If so, then we might expect that plant populations in low productivity environments would be responsive (on both evolutionary and ecological time scales) to resource heterogeneity. We studied five field sites with populations of *D. spicata* and examined differences among the sites in the proportion, range and temporal consistency of spatial structure in soil moisture and nitrogen. We then related soil moisture and nitrogen measurements to growth and survival of *D. spicata* planted into the intact communities of each site where we had measured soil moisture and nitrogen.

MATERIALS AND METHODS

Study species

Danthonia spicata (Poaceae) is a native, perennial, C_3 bunch grass that commonly occurs in dry, nutrient-poor oak savannas, old-fields, and forest clearings throughout the eastern and northern United States and Canada (Darbyshire and Cayouette 1989). *Danthonia spicata* reproduces vegetatively by tillering, with the tillers forming a discrete clump. These semi-autonomous tillers can be separated, producing independent clonal replicates that we refer to throughout this paper as culms. *Danthonia spicata* produces two flower types. Potentially outcrossed, wind-pollinated chasmogamous (CH) flowers are produced at the tip of the reproductive stalk and disperse an average of 60 cm (Chapter 3), with possible additional secondary dispersal. Obligately self fertilized

cleistogamous (CL) flowers are produced in the axils of the leaf sheaths along and at the base of the reproductive stalk and disperse an average of 10 cm or less (Chapter 3).

There is substantial variation within and among *D. spicata* populations in percent allocation to shorter dispersing, CL, seeds (Clay 1982). Allocation to CL seeds affects mean dispersal distance, and thus the spatial scale over which offspring encounter the environment (Chapter 3). We measured spatial variation in soil resources and its consistency over time in five field sites across the state of Michigan that had populations of *D. spicata* (selected from a larger sample of eight sites; Chapter 3) that differed in average allocation to CL seeds. Plant populations in Sites A_2 and L_1 produced a large percent of CH seeds and so were far-dispersing populations. Populations in Sites D and L_2 had moderate proportions of CL seeds. Population R had a large proportion of CL seeds and was a short-dispersing population (Figure 2.1; Chapter 3).

The five sites in which we examined the relationship between environmental heterogeneity and *D. spicata* performance all had communities that were dominated by perennial, herbaceous vegetation, especially *Andropogon virginicus*, *Schizachirium scoparium*, and *Carex* spp.. None of the sites had been plowed or tilled for at least 45 years. Site A_2 was located in a remnant oak savanna in the Allegan State Game Area near Allegan, Michigan. Sites L_1 and L_2 were located in old-fields that had been abandoned from agriculture at the W.K. Kellogg Biological Station. Site R, also an old-field site, was located in the Rose Lake Wildlife Research Area near East Lansing, Michigan. Site D was located in an alvar grassland in the Maxton Plains Preserve on Drummond Island, Michigan.

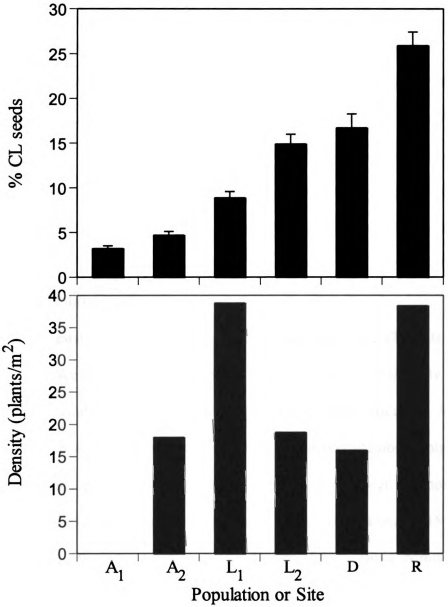

Figure 2.1: Population characteristics
Percent of cleistogamous (CL) seeds ± 1 s.e. **(a.)** and plant density **(b.)** in selected study populations of *Danthonia spicata*. Population A_1 was only used as a source of phytometers for the two population planting experiment.

Quantifying spatial variation:

We used a stratified, nested sampling design to measure soil moisture, current nitrate and ammonia availability (standing pool sizes) and relative supply of nitrate- and ammonia-nitrogen in a 4 x 6 m permanent plot in four of the five study sites (A_2, L_1, L_2, R). Using a stratified, nested sampling design allowed us to detect spatially structured environmental variation over a wide range of scales (Corbett 1998). Additional points were nested at 5 and 10 cm to provide additional power to detect spatial variation within *D. spicata*'s dispersal distance.

To establish this sampling design, we randomly chose 43 points to sample within each plot. We then randomly chose 13 of these 43 points to potentially receive one or more of 20 randomly distributed additional sample points 5 cm away. When an initial point was chosen more than once, we located the second additional point 5 cm from the first additional point (10 cm from the initial point). This design produced 20 pairs of points 5 cm apart and eight pairs 10 cm apart. To generate a total of 15 pairs of points separated by 10 cm, we randomly selected seven of the 13 initial points to receive an additional point 10 cm away. Again, when a point had already received additional points, this additional point was placed 10 cm from the most recently added point, so that all additional points were in a line (see Figure 2.2). This produced a total of 70 sample points within each permanent plot across a range of scales.

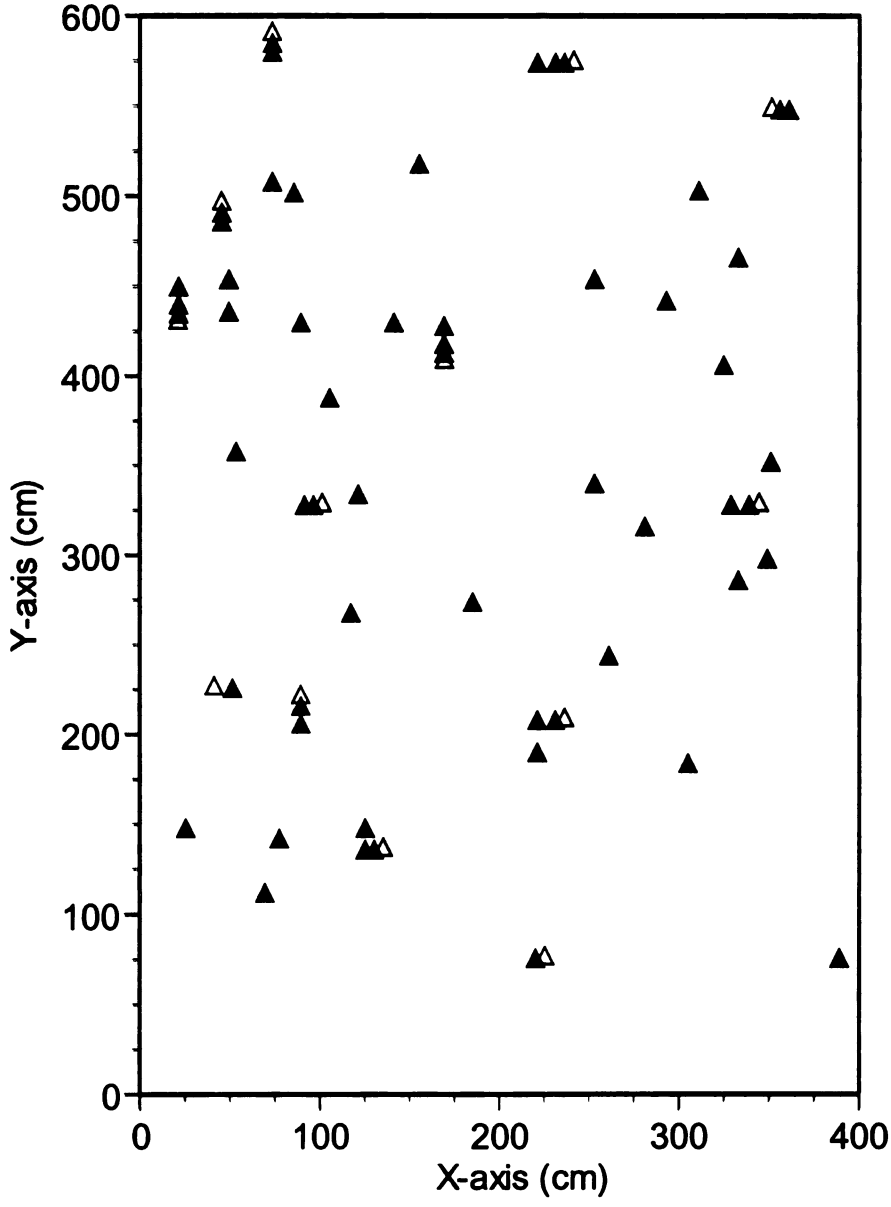

Figure 2.2: Soil sampling design
Diagram of sample point locations in the permanent plot in each population. Of 43 base points, 13 (Δ) were chosen to receive one or more additional points at 5 or 10 cm away. Total N = 70 points.

Soil sampling and processing

To estimate environmental conditions at a single point in time, we sampled soil moisture and current availability of nitrate- and ammonia-nitrogen in four of these five sites (A_2, L_1, L_2, R). In Sites L_1 and L_2, we removed a soil core 2.5 cm in diameter and 10 cm deep from each sample point in June 1996. We immediately placed the soil cores in plastic bags in an ice-filled cooler, where they remained until further processing (~ 12 hours). Each soil core was then passed through a 2 mm sieve to remove large rocks and root masses.

We weighed a 10 g subsample from each sieved soil sample and dried it for 72 hours at 65°C. Dried samples were then reweighed to determine the percent of soil moisture in g H_2O g dry soil^{-1}. We extracted available nitrogen from an additional 10.00 g subsample of soil with 50.0 ml of 1M KCl. Extracted samples were shaken for 1 min. and allowed to incubate at room temperature for 24 hours before filtering through a 1 μm Gelman glass fiber filter. Filtered extracts were stored at ~ 3°C for 5-8 days before analysis for nitrogen concentration. We analyzed extracts for nitrate- and ammonia-nitrogen using segmented flow colorimetry (Alpkem). Nitrogen concentration of each sample was then corrected for initial soil moisture and expressed as g N g dry soil^{-1}.

Extractable nitrogen in soil cores gave us an estimate of nitrogen availability at a single point in time. We used ion exchange resin bags placed at the same sampling points to estimate the relative supply rate of nitrate and ammonia (a longer-term, or more integrated measure of nitrogen availability). After removal of each soil core, we inserted a mesh bag (PeCap 120 mm polyester mesh, Tetko, Inc., Briarcliff Manor, NY) filled with 1-2 tablespoons of mixed bed ion exchange resin (Dowex MR-3) so that each resin

bag was positioned 5 cm beneath the soil surface. These resin bags remained undisturbed in the soil for 12 months (until June 1997), when they were removed and replaced with new resin bags. We rinsed the removed resin bags with double distilled water to remove soil particles and dried them at 40°C. We weighed out a 2.00 g subsample of dry resin from each bag and extracted nitrogen bound to the resins with 30 ml 1M KCl. Resin bag extracts were filtered and analyzed for nitrate and ammonia using the same protocol as for soil extracts.

We sampled soil moisture and extractable nitrate- and ammonia-nitrogen in Sites A_2 and R in June 1997 and placed resin bags in each sample location, as we had done for Sites L_1 and L_2. In October 1997, we removed the resin bags that were placed in the field in June 1997 (from all four sites) and replaced them with new resin bags. The shorter sample period used for resin bags in 1997 divided the year into two seasons (summer 1997 and spring 1998) corresponding to *D. spicata*'s two growing periods and allowed an examination of temporal nitrogen dynamics among seasons. In May 1998 we removed the resin bags that we placed in the field in October 1997 (spring 1998). At the same time, we resampled soil moisture in all four sites by removing a second soil core immediately adjacent to each initial sampling location.

In the fifth site (D), the soils were too shallow to allow coring or resin bag installation (mean depth 4 cm), so we used soil depth as an indication of soil moisture. We expected soil depth would have a strong effect on microhabitat suitability in this site because it largely determined the availability of water, with deeper locations retaining pools of water long after shallow locations had become extremely dry (McCormick, personal observation). We used the same sampling design that was used for soil cores in

the other four sites to sample soil depth in this site in June 1997. We did not resample soil depth, because it was largely the result of topography of the underlying bedrock, which did not change appreciably among years (McCormick, personal observation).

Data analysis (spatial structure)

We used data from our 70 sample points to calculate mean soil moisture, nitrogen pools, and nitrogen supply in each of the four sites. To examine site differences in mean environmental conditions, we tested for differences in each factor among the four sites using an ANOVA.

We used semivariance analysis (GS+, v. 3.11.6, GammaDesign, Inc., Plainwell, MI) to calculate semivariograms, which describe the similarity between pairs of points as a function of how far apart they are, for each environmental variable (Robertson and Gross 1994). We examined each soil variable over the range 0 to 350 cm (active lag) using a 20 cm interval (step size). We then used a linear regression of semivariance on distance (Systat 8.0 for windows, 1997. SPSS, Chicago IL.) to determine whether semivariance increased with increasing distance between sample points at distances within and beyond *D. spicata*'s dispersal distance. These analyses provided an estimate of whether spatial structure was present over the range 0 to 350 cm, but could not detect spatial structure present only at very small scales (e.g. 10 to 15 cm).

We examined the temporal consistency of spatial structure in soil resources in each site by calculating a semivariogram for the sum of conditions at the two dates to be compared and testing for the presence of spatial structure in the covariogram calculated using the equation Variance(A + B) = Variance(A) + Variance(B) – 2*Covariance(A+B)

and Semivariance = Variance / 2. The covariogram, composed of the covariance calculated for each distance class, describes spatial structure that is consistent across the sample periods compared. We used the significance of the linear regression of covariance on separation distance to test for significant temporal consistency of spatial structure in each soil variable. If there is consistent spatial structure, we expect that the covariance will increase with distance. Because the covariance will not increase with distance if there is no spatial structure in the two samples being compared, the covariogram can only reflect presence of consistent structure, not a consistent lack of structure.

We examined the temporal consistency of spatial structure in soil nitrate and ammonia over two time periods. First we tested for significant spatial structure among seasons within a year. This compared the June to October 1997 (summer 1997) resin bag measurements to the October 1997 to May 1998 (spring 1998) measurements. We could not calculate summer 1997 spatial structure for ammonia or nitrate supply in Site A_2 because foraging animals unearthed 50 of the 70 resin bags we installed in June 1997. We also examined the relationship between spatial structure of standing pool nitrogen availability (from soil cores) and longer-term measures of nitrogen supply (from resin bags). This comparison may indicate consistency of nitrogen availability in a season among years. Because soil cores were sampled in the spring (1996 or 1997), we compared the spatial structure of standing pool nitrogen to the spatial structure of spring 1998 resin bag measurements. For populations L_1 and L_2 we also compared 1996 full year measurements to 1997 measurements (summer 1997 + spring 1998). For soil

moisture, we examined the spatial structure in the covariogram comparing the two sample times (1996 or 1997 with 1998 measurements) as described above.

Plant perception of environmental variation-variable scale planting

Plant performance is the best indication of whether measured spatial environmental variation is "perceived" by plants in a population. To determine whether *D. spicata* plants could perceive differences in the measured scale of spatial variation in two sites (L_1, L_2), we conducted a phytometer experiment, using *D. spicata* culms as our measurement tools. We used individual *D. spicata* plants collected from three populations as phytometers: A_1 (5 individuals), L_2 (5) and L_1 (6) in April 1995. Population A_1 was an oak savanna site that was located approximately 5 km away from Site A_2. Site A_1 was very similar to Site A_2 in soil moisture, nitrate and ammonia availability (McCormick, unpublished data). The *D. spicata* population in sites A_1 and A_2 had a similar average proportion of CL seeds (Figure 2.1).

We divided each of the 16 parent plants into 26 to 30 culms, each of which was individually potted into 10 cm square pots filled with silica sand and grown at 24 to 32° under ambient light in the greenhouse. Plants were grown without fertilizer for 13 months, and then planted in the field (May 1996). Between 22 and 26 culms from each parent were randomly assigned to one of two sites (L_1 or L_2). We planted the phytometers (culms) into randomly selected points in one half of each permanent plot in which we measured the spatial variation of soil moisture and nitrogen, with the stipulation that each source population had to be represented in each plot half. Planting culms into only two sites allowed us to have greater power to detect differences in plant

performance across a range of scales within each site. Using plants from three source populations allowed us to determine whether plants from different populations perceived measured spatial variation differently. The culms were grown in the field for 25 months (May 1996 - June 1998). We then harvested all above-ground biomass from each surviving culm, noting the number of tillers each had produced and whether they had produced seeds. Harvested biomass was dried at 45°C for 48 hours and then weighed (± 0.01 g) to determine above-ground dry weight.

We established a separate common garden in a tilled 2 x 2 m (tilled 20 cm deep) plot adjacent to the L_1 site in May 1996 to distinguish differences in culm growth resulting from genetic differences among source populations from differences in culm growth resulting from differences in "perceived" spatial variation in the intact field sites (L_1 and L_2). We planted four culms from each parent into randomly selected locations on a 10 x 10 cm grid established in the central 1 x 1 m of the tilled area. We planted additional, randomly selected, culms around the perimeter of the common garden to reduce edge effects on the study plants. We measured the initial size of planted culms by counting the number of living leaves at the time of planting.

The common garden was regularly weeded and culms monitored until June 1997 (13 months), when we harvested above-ground biomass from all surviving culms. We determined final plant size by measuring above-ground dry weight and by counting the number of tillers each culm produced. We used an ANOVA to compare the growth (dry weight and number of tillers) of culms from different source populations across both intact sites (L_1 and L_2) and in the tilled common garden. Both dry weight and number of tillers were log-transformed to improve normality. We used a second ANOVA to

compare growth of plants from all source populations in the common garden and the two recipient sites (L_1, L_2).

To determine whether variation in culm final weight was spatially structured, we conducted a semivariance analysis of plant weight as a function of planting location in each of the two intact recipient sites (L_1, L_2). However, this analysis only allowed us to examine spatial structure in growth variation among surviving culms and did not take into account spatial structure in culm mortality. We used joincount analysis (Joincounts, Epperson 1992), a measure of spatial structure in discrete variables, to determine whether, and at what scales, culm survival was spatially structured in these two sites. In joincounts, a significant, negative, standard normal deviate statistic (SND < -1.96) indicates that survival was significantly spatially structured.

Comparing plant performance with environmental variables

To compare plant growth and survival in these two sites with our measures of environmental variables, we used kriging analysis (block kriging, GS+, v. 3.11.6, GammaDesign, Inc., Plainwell, MI) to estimate values of sol moisture and ammonia for each culm planting location. Kriging analysis uses the eight sample points nearest the point for which an estimate is desired and weights them according to their distance from the estimate location and the spatial structure of that variable in that site (Robertson and Gross 1994). In a site with substantial spatial structure, sample points near the estimate location would be weighted much more than points farther away, while in a site with little spatial structure there would be little difference in weighting, regardless of distance from the estimate location. We examined the significance of regressions of growth of the

planted culms on soil moisture, and nitrate and ammonia supply estimates. To examine environmental effects on culm survival we used t-tests to compare estimates of soil variables for locations where planted culms survived and where they died.

Plant perception of environmental variation-dispersal scale planting

We conducted a second phytometer experiment to expand the range of sites in which we measured spatial structure in plant growth and survival. We collected four randomly chosen adult *D. spicata* from each of four populations (A_2, D, L_1, R) in February 1997. We separated these plants into individual culms, potted each culm in a separate pot, and fertilized potted culms twice per week with Peter's Peat lite (20-20-20) fertilizer to encourage vegetative growth. In late March 1997, all culms that had produced additional tillers (reproduced vegetatively) were further divided and repotted. In mid-April we ceased fertilizing all plants in preparation for planting.

We designed this phytometer experiment to determine whether differences in the spatial structure of soil resources among sites would affect plants. We specifically focussed on spatial scales representative of CH and CL seed dispersal distances (10 cm for CL seeds and 60 cm for CH seeds). Two of the sites used in this phytometer experiment had *D. spicata* populations with a high or moderate proportion of short-dispersing CL seeds (D, R) and two had populations with primarily far-dispersing CH seeds (L_1, A_2).

We established four randomly located subplots (43 cm x 43 cm) within the permanent plot in each study area. We placed a 7 x 7 cm quadrat at each corner of each subplot and planted a culm of *D. spicata* at each corner of each quadrat (Figure 2.3). We

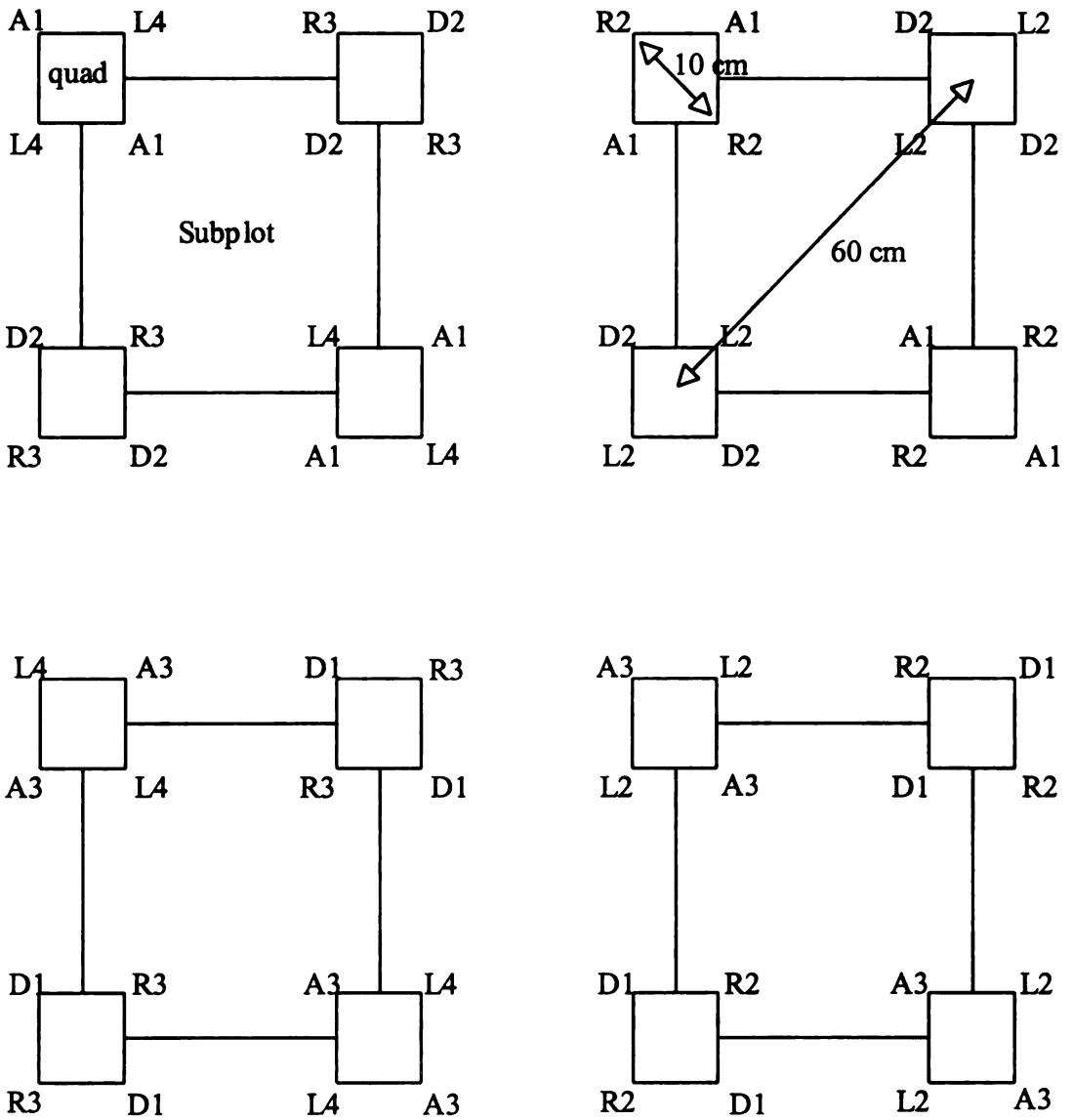

Figure 2.3: Four site planting design
Diagram of design used to plant culms in each population in the four population phytometer experiment. Shown are four subplots located within a plot. Diagonal distances across quadrats (10 cm) and across subplots (60 cm). Distances were chosen to reflect the distances dispersed by CL and CH seeds, respectively. Letters in the quadrat corners represent the source population of each culm. A refers to culms from Population A_2, D and R refer to culms from Populations D and R, respectively, and L refers to culms from Population L_1. The number after the population letter refers to the parent number in each population (1-4).

planted each subplot with culms from four parent plants, one from each source population. Within each subplot, culms from a single parent were planted in two opposing corners of a quad in each of two opposing quads in the subplot (see Figure 2.3). Culms from each parent were represented in two randomly chosen subplots (of the four possible) in one site for a total of 8 culms from each of eight parental plants. Eight culms from each parent were planted into two sites; one site with a high proportion of short-dispersing seeds and one with predominantly far-dispersing seeds). This gave us a total of 256 plants across four sites.

We planted culms in Sites A_2, L_1, and R over a period of two days in early May 1997. We watered the culms three times during the first week and once the following week to ease transplant shock. Culms were planted into Site D one week later, immediately after a heavy rain and transplants into this site were not watered after planting. We measured culm survival and growth (number of tillers produced) in early June 1998, when all culms were harvested. Plants were dried at 40°C, and weighed to determine final above-ground biomass. The number of tillers at the time of planting was used as a measure of initial size.

We tested for differences in number of tillers and biomass (both log transformed to improve normality) among source populations and among sites using ANOVAs. All culms planted into Site A_2 died within one month of planting, so it was not included in any analyses. We used logistic regression (Systat 8.0 for windows, 1997. SPSS, Chicago IL.) to test for differences in culm survival among the four source populations and the three remaining sites. Because culm survival was low, the two culms planted into a quadrat rarely both survived, so we could not use semivariance analysis to examine

spatial structure in culm growth at that scale. However, we used joincount analysis (Joincounts, Epperson 1992) to compare similarity in culm survival within versus among quads in a subplot. A significant, negative, standard normal deviate statistic (SND < -1.96) at the within-quadrat scale indicates that survival was significantly more similar within than among quadrats and, therefore, that it was spatially structured.

RESULTS

Site differences in soil resources

There were significant differences among sites in soil moisture (1998), and in nitrate and ammonia supply rates in both summer (1997; Figure 2.4e, g) and spring (1998; $p < 0.001$ for each; Figure 2.4f, h). Sites L_1 and L_2 had higher soil moisture and nitrate supply than Sites A_2 and R in the spring (1998; Figure 2.4b, f). Site A_2 had higher soil nitrate and ammonia supply than L_1, L_2, and R in the summer (1997; Figure 2.4e, g). The four sites had similar ammonia supply in the spring (1998; Figure 2.4h). We could not directly compare initial nitrogen availability (from soil extracts) or soil moisture across sites because these measures were made in different years in the four sites. Comparing within a measurement year, Site R was consistently more moist and had lower nitrate and ammonia than A_2 (Figure 2.4).

Spatial structure of soil resources

Using semivariance analysis, we found that the spatial structure of soil moisture and nitrogen within these sites was often spatially structured (Figure 2.5, 2.6, 2.7). As expected, soil resources were more spatially structured in some sites others. For example

Figure 2.4: Moisture and nitrogen levels across years at four study sites
Mean (± 1 s.e.; N = 58 to 68 samples each) soil moisture (**a, b**), the availability of nitrate (**c**) and ammonia (**d**) and relative supply of nitrate (**e, f**) and ammonia (**g, h**) nitrogen across four populations of *Danthonia spicata*. Soil moisture and initial pools of nitrate and ammonia were sampled in June 1996 in Populations L_1 and L_2 (■), and in June 1997 in Sites A_2 and R (■). Soil moisture was resampled in all four sites in 1998. Summer nitrogen supply measures were sampled from June to October 1997. Spring nitrogen supply measures were sampled from October 1997 to May 1998.

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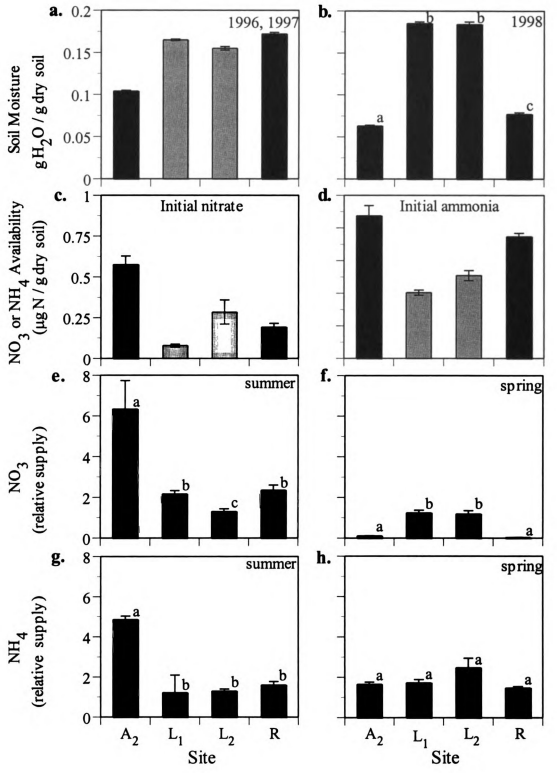


Figure 2.4: Moisture and nitrogen levels across years at four study sites

Figure 2.5: Semivariance and covariance of soil moisture over two years
Semivariograms and covariograms for soil moisture for two years in each study site. Year one measurements (◇, solid line) for semivariograms were collected in 1996 for Sites L_1 and L_2 and in 1997 for Sites A_2 and R. Year two measurements (Δ, dotted line) for semivariograms were collected in 1998 in all four sites. Covariograms show spatial structure that was consistent across year one and two measurements in each site. Asterisks (*) in the lower right corner of each semi- or covariogram indicate significant spatial structure at the $p \leq 0.05$ level. Non-significant spatial structure by a zero (o). For each semivariogram, the top symbol refers to the significance of the year one semivariogram and the bottom symbol to the year two semivariogram.

Figure 2.5: Semivariance and covariance of soil moisture over two years

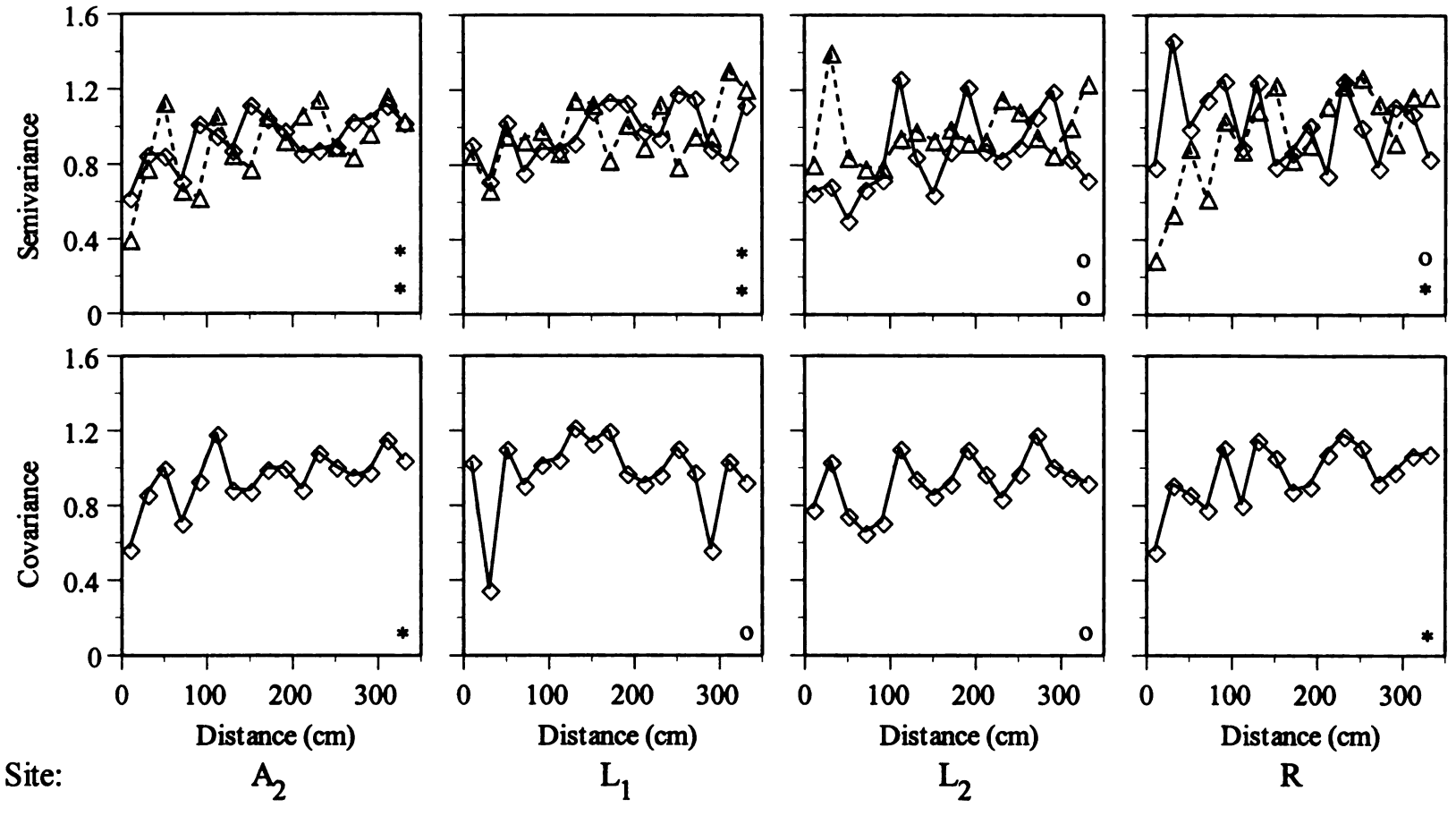


Figure 2.6: Semivariance in relative nitrogen supply
Summer 1997 (◇, solid line) and spring 1998 (Δ, dotted line) semivariograms for relative nitrate and ammonia supply during two seasons in three study sites (L_1, L_2, R). Asterisks (*) in the lower right corner of each semivariogram indicate significant spatial structure at the $p \leq 0.05$ level. Spatial structure that was almost significant ($0.05 < p < 0.10$) is designated by a minus (-) sign and non-significant spatial structure by a zero (o). For each semivariogram, the top symbol refers to the significance of the year one semivariogram and the bottom symbol to the year two semivariogram.

Figure 2.6: Semivariance in relative nitrogen supply

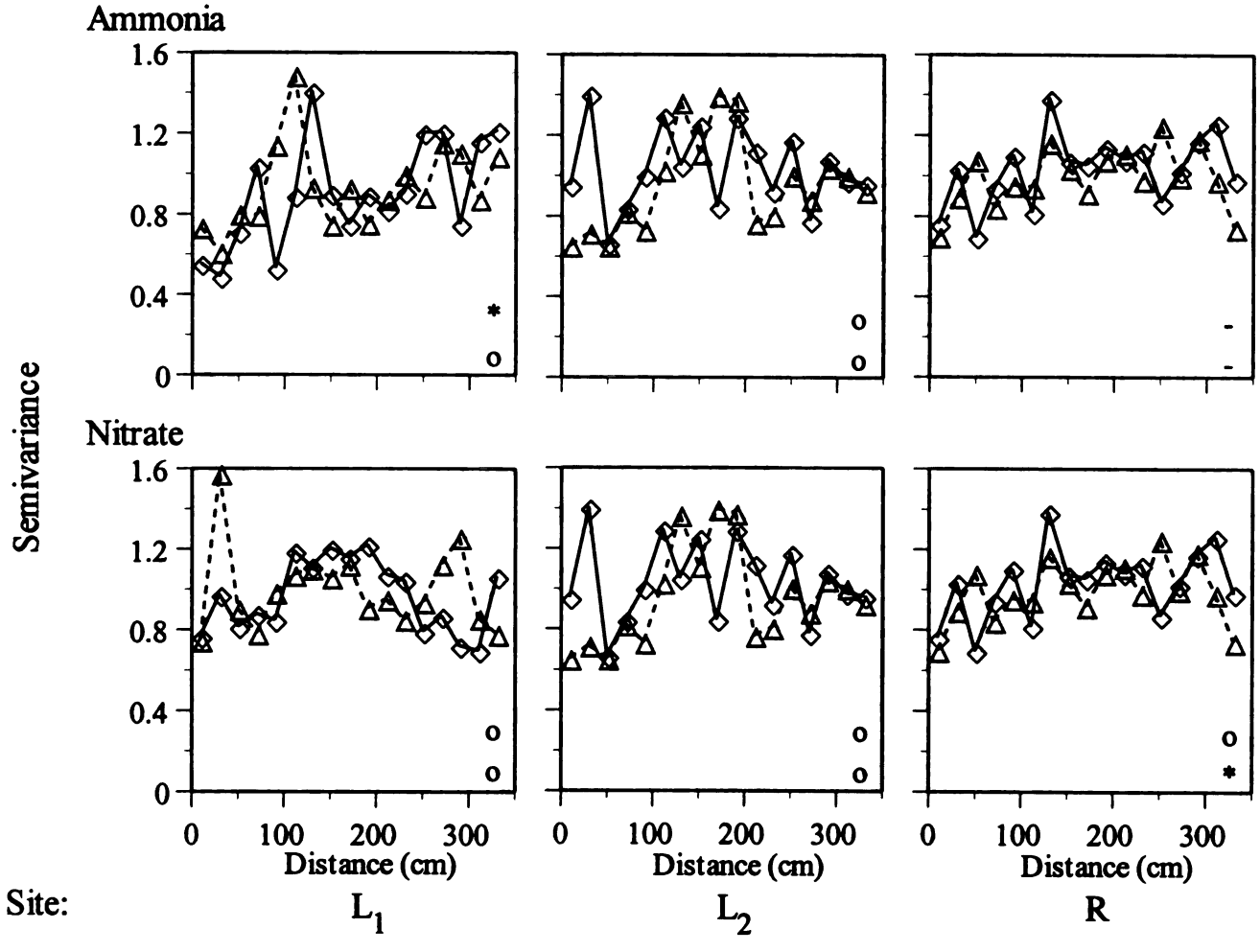
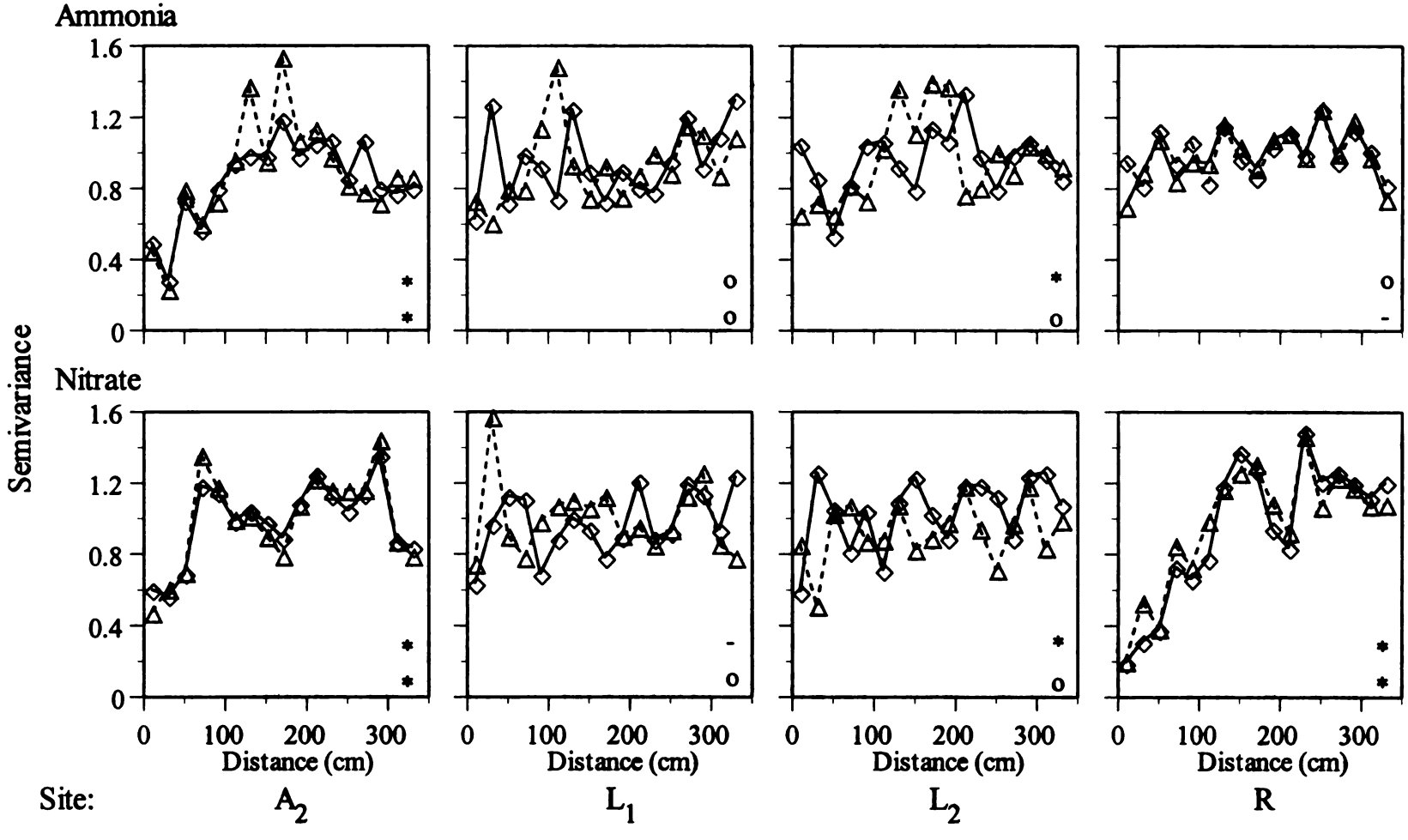


Figure 2.7: Comparison of spatial structure in initial availability and resin bag nitrogen measures
Spatial structure in ammonia and nitrate pools in 1996 for Sites L_1 and L_2 and in 1997 for Sites A_2 and R (◇, solid line) and spring 1998 (Δ, dotted line). Asterisks (*) in the lower right corner of each semivariogram indicate significant spatial structure at the $p \leq 0.05$ level. Spatial structure that was almost significant ($0.05 < p < 0.10$) is designated by a minus (-) sign and non-significant spatial structure by a zero (o). For each semivariogram, the top symbol refers to the significance of the year one semivariogram and the bottom symbol to the year two semivariogram.

Figure 2.7: Comparison of spatial structure in initial availability and resin bag nitrogen measures



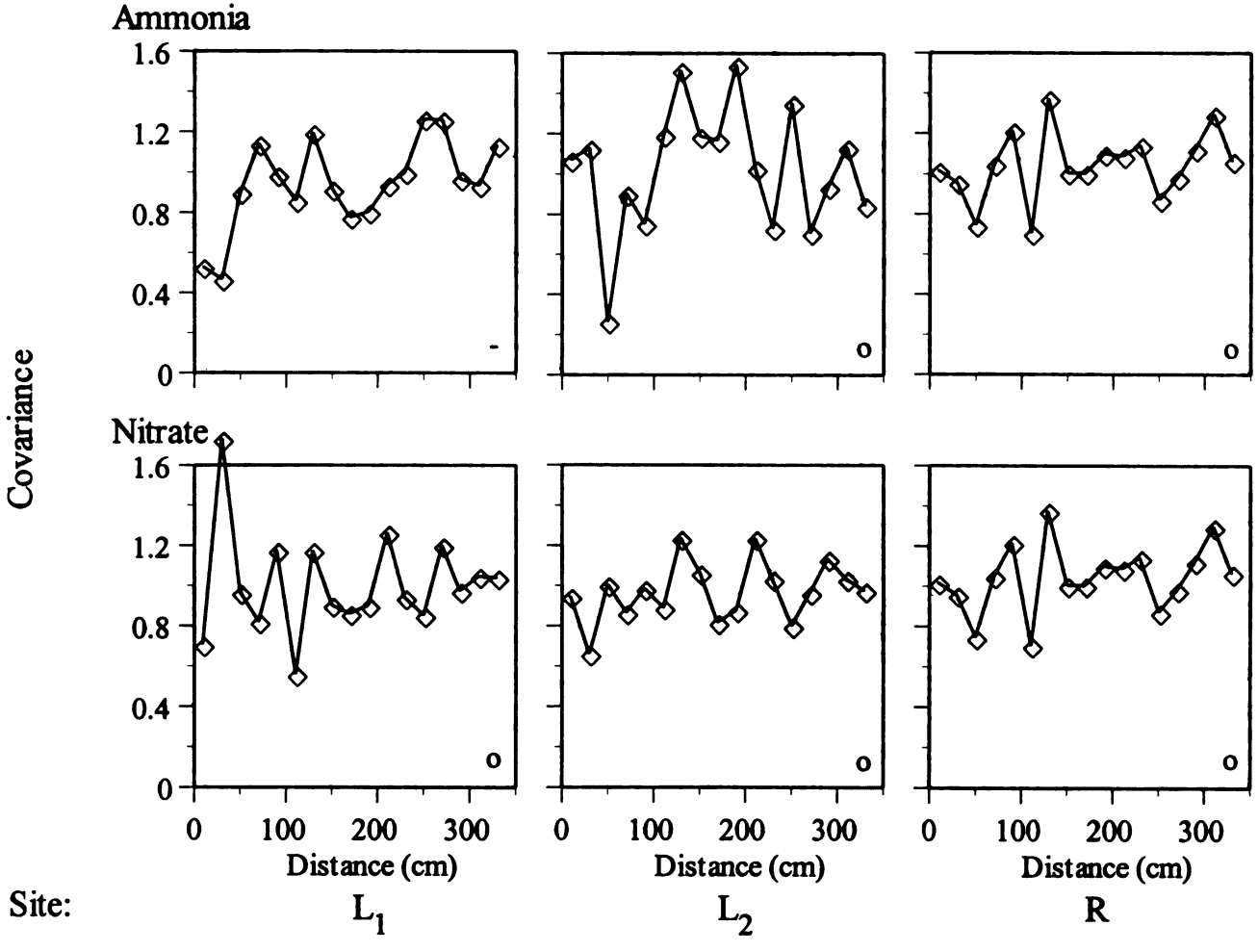
Site A_2 (the oak savanna) had significant temporally consistent spatial structure in soil moisture (Figure 2.5) and also in initial (soil core) nitrate and ammonia levels (Figure 2.7). All three old-field sites (L_1, L_2, and R) had some significant spatial structure in soil moisture or ammonia. However, Site L_1 had no temporally consistent spatial structure. In contrast, Site L_2 (spring ammonia and nitrate among years) and Site R(soil moisture and spring nitrate among years) had significant temporally consistent spatial structure. In Site D (the alvar grassland) soil depth (a surrogate for soil moisture) was not significantly structured ($p = 0.650$).

Temporal consistency of spatial structure in soil resources

Soil moisture showed consistent spatial structure among years in both Site A_2 and Site R, but not in Sites L_1 or L_2 (Figure 2.5). Neither ammonia nor nitrate was consistently spatially structured among seasons in any site (Figure 2.8). However, spatial structure in initial availability of nitrate and ammonia was frequently consistent with spatial structure in nitrogen supply detected either one (A_2, R) or two (L_1, L_2) years later from spring 1998 resin bag measures. Sites A_2 and L_2 had consistent spatial structure in both initial and relative supply for both nitrate and ammonia (Figure 2.9). In Site R, soil cores and resin bags detected similar (consistent) spatial structure for nitrate, but not for ammonia (Figure 2.9). The greater consistency between soil core and resin bag measures of nitrogen than among resin bag measurements in different seasons (summer 1997 and spring 1998) might suggest consistency among years but not among seasons. In Sites $L_1$and L_2, nitrogen supply was measured over two full years to allow patterns that persisted throughout the year to be compared among years. We summed 1997 summer

Figure 2.8: Covariance of ammonia and nitrate among seasons
Covariograms for ammonia and nitrate showing consistent spatial structure among seasons in three study sites (L_1, L_2, R). Asterisks (*) in the lower right corner of each covariogram indicate significant spatial structure at the $p \leq 0.05$ level. Spatial structure that was almost significant ($0.05 < p < 0.10$) is designated by a minus (-) sign and non-significant regressions by a zero

Figure 2.8: Covariance of ammonia and nitrate among seasons



(o).

Figure 2.9: Covariance of initial nitrogen pools and spring 1998 nitrogen supply
Covariograms for ammonia and nitrate showing consistent spatial structure between spring 1996 (Sites L_1 and L_2) or spring 1997 (Sites A_2 and R) nitrogen pool estimates and spring 1998 relative ammonia and nitrate supply in each study site. Asterisks (*) in the lower right corner of each covariogram indicate significant spatial structure at the $p \leq 0.05$ level. Spatial structure that was almost significant ($0.05 < p < 0.10$) is designated by a minus (-) sign and non-significant regressions by a zero (o).

Figure 2.9: Covariance of initial nitrogen pools and spring 1998 nitrogen supply

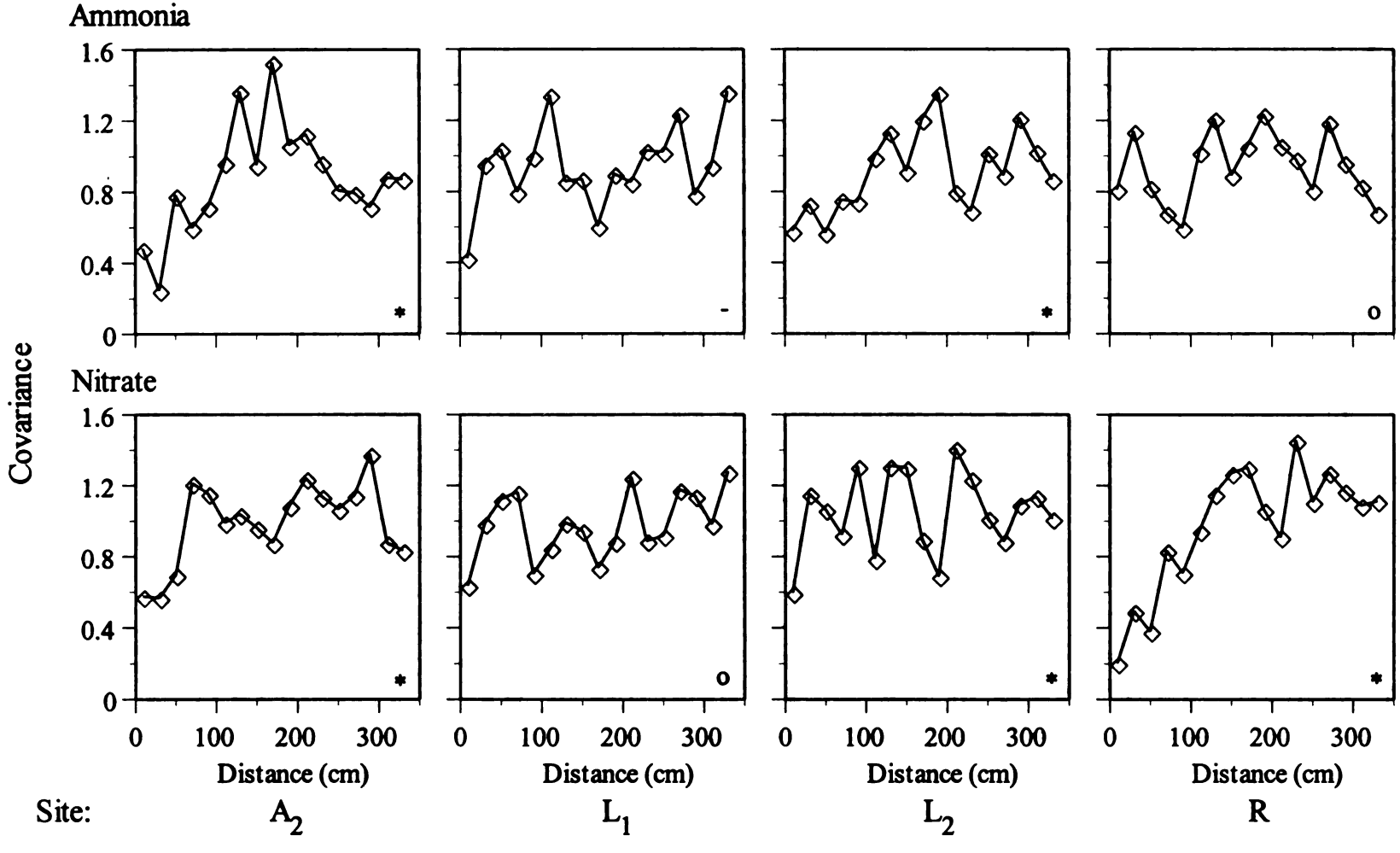
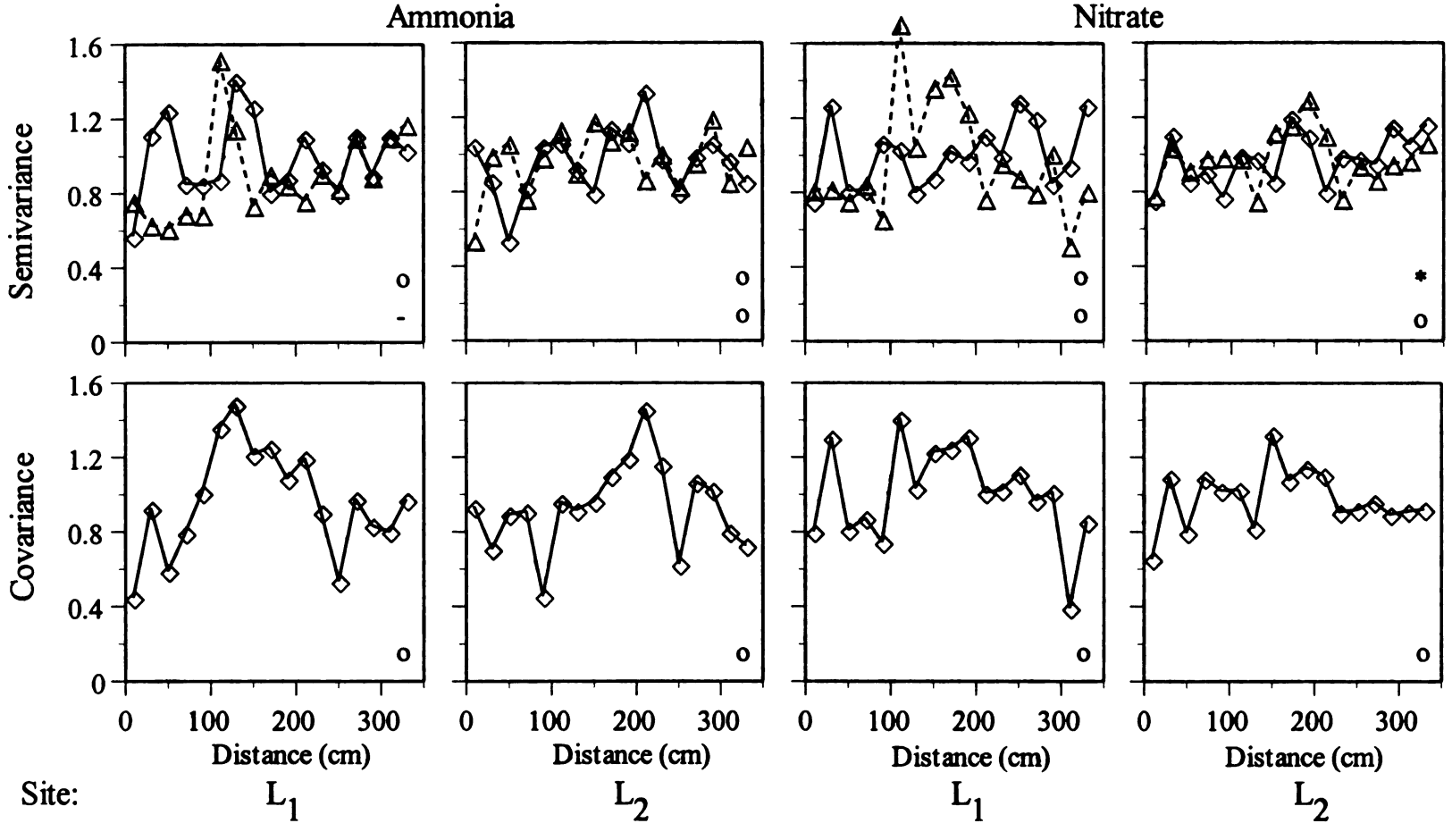


Figure 2.10: Spatial structure in nitrogen across years in two sites
Semivariograms for relative ammonia and nitrate supply in 1996 (◇, solid line) and 1997 (1997a + 1997b; Δ, dotted line) in Site L_1 and L_2. Covariograms show spatial structure that was consistent across 1996 and 1997 measurements in each site. Asterisks (*) in the lower right corner of each semi- or covariogram indicate significant spatial structure at the $p \leq 0.05$ level. Spatial structure that was almost significant ($0.05 < p < 0.10$) is designated by a minus (-) sign and non-significant spatial structure by a zero (o). For each semivariogram, the top symbol refers to the significance of the year one semivariogram and the bottom symbol to the year two semivariogram.

Figure 2.10: Spatial structure in nitrogen across years in two sites



and 1998 spring resin bag measures to get data comparable to 1996 full year measurements. Comparisons between 1996 and 1997 measures of relative nitrate and ammonia supply showed no temporally consistent spatial structure at the whole year time scale in Sites L_1 and L_2 (Figure 2.10). However, there was no significant spatial structure in Site L_1 or L_2 in either nitrate or ammonia supply during 1997 (summer + spring), so it was not possible to detect significant temporally consistent spatial structure in these two sites between 1996 and 1997.

These results show that four of the five study sites (excluding Site D) had spatial structure during some times. Site A_2 temporally consistent spatial structure among years in soil moisture, ammonia and nitrate. In contrast, Site L_1 had no consistent spatial structure among years. Site L_2 had somewhat less spatial structure that was consistent among years than A_2 (ammonia and nitrate were consistently structured, but soil moisture was not). Site D had no significant spatial structure in soil depth (a surrogate for soil moisture). Similar to Site L_2, Site R had less consistent spatial structure than A_2, but more than Site L_1 or D (soil moisture and nitrate were consistently structured, but ammonia was not). The real test of whether these differences among sites in amount of temporally consistent spatial structure are relevant to plants is to use phytometers to measure differences in plant "perceived" spatial structure.

Plant perception of environmental variation-variable scale planting

As might be expected, there were strong differences in growth between the common garden and field sites (L_1, L_2) for both biomass and number of tillers (each $p <$

0.001), but plants grew similarly in Sites L_1 and L_2 ($p = 0.142$). Source populations differed in the number of tillers produced in the common garden and field sites, with plants from Population A_1 producing more tillers than plants from Populations L_1 and L_2 ($p < 0.001$). But populations had similar biomass ($p = 0.271$). Differences in tiller production among the source populations were similar across the common garden and field sites ($p \geq 0.170$). Initial culm size was not a significant determinant of final size or survival ($P > 0.8$) and was not used in analysis of this experiment.

Three of the four parent plants collected from Population L_1 were infected with the epiphytic fungus, *Atkinsonella hypoxylon*. Plants infected with this fungus often have higher rates of tiller production (Clay 1984, Chapter 5). To determine whether parent and source population differences in tillering were a result of infected culms, which were only collected from Population L_1, we removed infected plants from the analysis and tested for differences among source populations and parents using only uninfected plants. However, removing infected plants from the analysis did not substantially affect population differences in tiller production or biomass.

Neither plant dry weight nor the number of tillers produced by culms (regression, $p > 0.210$ in all cases) were significantly related to estimates of soil moisture and relative supply rates of nitrate and ammonia nitrogen for individual planting locations in the two sites. However, in Site L_2, the estimates of soil moisture in 1996 and 1998 for locations where planted culms survived were significantly higher than estimates for locations where culms died (t-test, $P \leq 0.041$). Culms that survived in Site L_1 showed the same tendency, as in Site L_2, to be in higher moisture locations than culms that died, but this relationship was not significant in this site (t-test, $P \geq 0.320$). Average soil moisture was

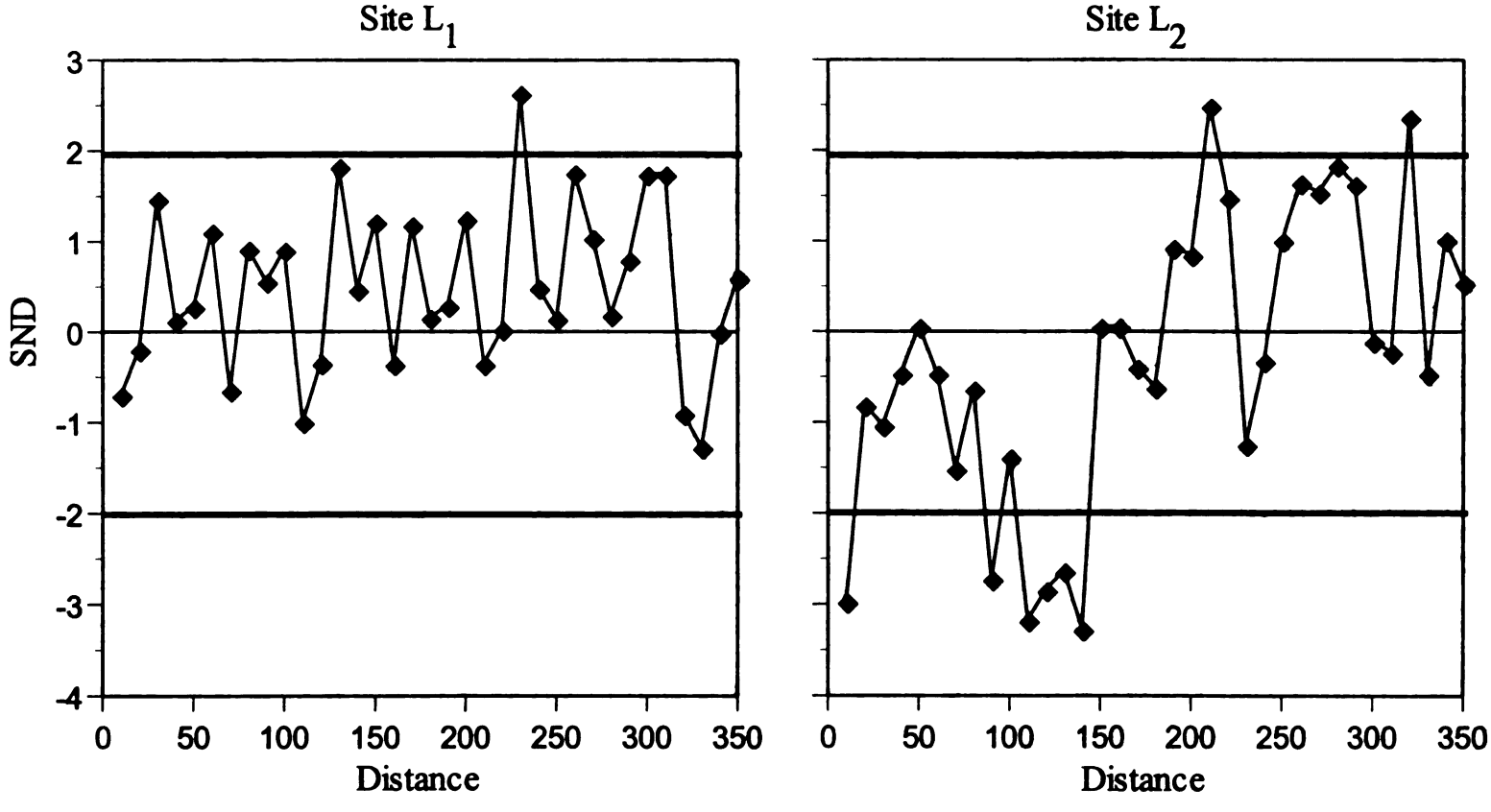
lower in Site L_2 than in L_1 (Figure 2.4), and so moisture may have been a more important determinant of culm survival. Estimated nitrate supply for 1996 and spring 1998 (t-tests, $P \leq 0.035$) and ammonia supply rates in all sampling periods (t-tests, $P \leq 0.035$) differed among locations where culms survived and locations where they died in Site L_2. Surprisingly, culms that died were in locations with higher estimated nitrate and ammonia supply than those that lived. Culms that survived in Site L_1 were similarly associated with lower nitrogen locations, but this difference was not significant.

Culm survival was spatially structured in Site L_2 at distance intervals less than ~150 cm but was not spatially structured in Site L_1 (Figure 2.11). Thus, in Site L_2, where similar spatial structure was detected by soil cores and resin bags for both nitrate and ammonia, culm survival was spatially structured, while in Site L_1, where we found very little spatial structure in any soil variable, culm survival was spatially independent. Neither plant dry weight nor the number of tillers produced by culms were significantly spatially structured in either site ($p \geq 0.090$ for both variables in both sites).

Plant perception of environmental variation-dispersal scale planting

We conducted a second phytometer experiment that was designed to examine differences among sites in spatially structured environmental variation "perceived" by plants at the spatial scales encountered by dispersing *D. spicata* seeds. In this experiment, initial plant size, measured as number of tillers, was a significant factor in determining final dry weight and number of tillers produced in this experiment (both log transformed to improve normality; $P = 0.012$ and 0.028, respectively), so it was included as a covariate in all analyses. No culms survived in Site A_2, so we could not include it in

Figure 2.11: Spatial structure of culm survival in two field sites
Standard normal deviate (SND) statistics calculated from a joincounts analysis are used to determine the significance of spatial structure in culm survival in the variable scale phytometer experiment. SND's of less than -1.96 indicate significant spatial structure. SND's of greater than +1.96 indicate significant overdispersion. SND's between 1.96 and -1.96 indicate a random distribution



any analyses. Final dry weight and number of tillers produced differed among the three other sites ($p = 0.006$ and $p = 0.010$, respectively). Plants from different source populations did not differ in final dry weight and, unlike in the variable scale planting, source populations also did not differ in the number of tillers they produced ($p = 0.433$).

However, culm survival differed among the three sites (Logistic regression, $P < 0.001$; Systat 8.0 for Windows, SPSS Inc. Chicago, Il.). Culms that survived were significantly larger initially than culms that died, so initial size (initial number of tillers) was included in this and subsequent logistic regressions. Source populations also differed in survival, accounting for initial size ($P = 0.002$), suggesting that culms from some source populations were more robust than others were (Table 1). However, there was not a significant interaction between source population and planting site ($p = 0.646$), so culms from different source populations did not differ in their "perception" of habitat quality.

Culm survival was more similar within than among quadrats in Site D (joincounts, SND = -2.47), indicating significant spatial structure in culm survival. Culm survival was not significantly structured in either Site L_1 (SND = 0.06) or R (SND = 0.93). However, power to detect spatial structure in discrete variables is highest when both categories, in this case, survival and death, are equally probable. We had equal power to detect structure in survival in Sites D and L_1, with 24 of 64 culms surviving in each. However, we had very little power to detect spatial autocorrelation in Site R, because only six of 64 culms survived in that site.

The results of the variable scale phytometer experiment suggest that *D. spicata* plants "perceive" spatially structured soil resources over a range of scales in Site L_2 but

not Site L_1. The dispersal scale phytometer experiment further supports the presence of differences in spatial structure among sites at the specific scales encountered by dispersing CL and CH seeds (see Chapter 3). The results of both phytometer experiments suggest that the effects of soil resource variation act through survival variation, rather than through differences in growth.

DISCUSSION

Structure of soil resources

Spatial structure of soil resources is well documented within and among populations (e.g. Robertson and Gross 1994, Gross et al. 1995, Miller et al. 1995, Ehrenfeld et al. 1997). However, few studies have explicitly assessed the temporal consistency of measured spatial structure of soil resources (but see Goovaerts and Chiang 1993) or examined its relevance to plant performance (but see Miller and Fowler 1997). In this study, we examined the spatial scale and temporal persistence of heterogeneity in soil moisture, nitrate, and ammonia across five herb-dominated communities and related this to spatial structure in the performance of *D. spicata* phytometers. We specifically focused on differences among sites in the magnitude of spatial structure in these resources and how this was "perceived" by *D. spicata*.

There were significant differences among sites in all of the soil variables we measured and also in the degree of spatial structure in soil resources and its temporal consistency. Each soil variable we measured was spatially structured in at least one site, during at least one sample time. Contrary to the expectation put forth by Grime (1994) that very unproductive habitats would be dominated by temporal rather than spatial

variation in resource conditions, the two driest sites that we measured (A_2, R), which had the lowest plant biomass production (McCormick, unpublished data), tended to have more spatial structure in soil resources than the two moister, more productive sites (L_1, L_2). However, Grime's expectation was based on shorter-term (within individuals in both space and time) nutrient dynamics than we examined. The disparity between his predictions and our results underscores the importance of assessing the spatial and temporal dynamics of soil nutrient variation across the multiple spatial and temporal scales relevant to plants. It seems possible that ephemeral plant effects on resource availability may dominate productive habitats while differences in soil type or long term plant effects dominate less productive habitats.

Environmental factors may differ in the temporal and spatial scales at which they are structured (Grime 1994). In this study, spatial variation in soil moisture was temporally consistent in the driest two sites we studied (A_2, R). Ammonia and nitrate supply were inconsistent among seasons in all three old-field Sites (L_1, L_2, and R). Goovaerts and Chiang (1993) found similar inconsistency in patterns of ammonia availability among seasons in a fallow agricultural field. This suggests that seasonal differences in spatial patterns of nitrogen availability may be common, at least in old-field sites.

Ammonia (Sites A_2 and L_2) and nitrate (Sites A_2, L_2 and R) both had consistent spatial structure among years in the spring in some sites. This suggests that consistent spatial structure during one season may be hidden in full year measurements by inconsistency among seasons. If so, then it is important to measure nitrogen supply during the time of year most important to plants of interest (e.g. during periods of intense

growth) to relate spatial environmental structure to plant performance. If soil resource structure varies among seasons, then plant species that differ phenologically may also "perceive" different amounts of resource structuring in the same community.

Plant species effects on the supply rate of both nitrate and ammonia can be substantial (e.g. Wedin and Tilman 1990, Wardle 1998) and could produce differences in spatial structure among seasons. Our study sites were dominated by long-lived perennial plants and if plant species affected the spatial structure of nitrate and ammonia, then they could produce spatially structured variation in soil resources that was consistent over many years. Strong species effects on soil nutrients have been detected in experimental studies with other grass species (Tilman and Wedin 1991) and also for nitrogen-fixing species (e.g. Vitousek et al. 1987, Wardle 1998). If species composition affects soil processes, and species occur in predictable locations over time, then spatial structure in soil nutrients may be more temporally consistent in communities that are dominated by long-lived species. Therefore mature grasslands with long-lived species, such as the habitats in which *D. spicata* grows (although *D. spicata* is relatively short-lived; Darbyshire and Cayouette 1989), may have more consistent spatial structure than habitats dominated by ephemeral species, like early successional fields.

Plant "perception" of environmental structure

Our phytometer experiments revealed similarities between measured spatial structure of soil resources and spatial structure "perceived" by *D. spicata* plants. The major effect of soil resource variation on *D. spicata* was to affect plant survival. We found little effect of spatial environmental structure on growth of plants that survived. In

the variable scale phytometer experiment, survival of culms planted into Site L_2, which had more spatial structure in soil nitrogen than L_1, was significantly spatially structured at scales less than ~150 cm, but culm survival was not spatially structured in Site L_1 (Figure 2.11).

Similarly, in our dispersal scale phytometer experiment, culm survival was significantly structured in Site D, but not in Site L_1, which had little spatial structure in any factor. Our analysis of spatial variation in Site D was limited to soil depth because soils in this site were too shallow to allow soil core removal or resin bag installation. For this variable, we found little evidence of spatial structure, but other factors may have affected plant survival in this site and culms planted in site D "perceived" the environment as spatially structured. Also, the randomly located sample points and phytometers in this study evaluated the way that environments would be sampled by randomly dispersing offspring. But when dispersal is non-random (as it is in *D. spicata*), other ways of measuring spatial environmental structure, and thus the spatial predictability of environmental conditions encountered by plants and their offspring may be more powerful (e.g. using dispersal curves to determine sampling designs; see Chapter 3). Spatially structured culm survival in Site D and unstructured survival in Site L_1 was consistent with predicted differences among the sites based on dispersal distance (see Chapter 3). However, extremely low survival of plants in Populations A_2 and R limited our ability to make general conclusions about the relationship between measured and "perceived" spatial environmental structure among populations from the dispersal scale phytometer experiment.

Our study, like many other studies (e.g. Sultan and Bazzaz 1993, Ehrenfeld 1997), documented that there is substantial temporal variation in soil nitrogen. Temporal variation in nitrogen in these sites was evident from the inconsistency of nitrate and ammonia supply (from resin bags) and from inconsistency among seasons (Figure 2.8). However, spatial structure of soil variables may be temporally inconsistent among seasons but consistent among years for a given season, as is suggested by the consistency between nitrogen pool and supply measurements (Figure 2.9). If a plant species is affected by soil resource variation during a specific time of year (determined by its phenology), then consistent spatial structure during that season may have a consistent effect on that species' population and community dynamics despite inconsistent resource structure among seasons. The differences in spatial structure of culm survival and their relation to soil moisture and nitrogen conditions (variable scale phytometer experiment) despite seasonal inconsistency of spatial structure in nitrogen supply supports this interpretation. Similarity between spatial structure in soil resources and spatial structure in phytometer survival suggests that temporally consistent spatial structure in soil resources among years is an important determinant of *D. spicata* performance, but experiments in additional sites and with other species are needed to expand the generality of these results.

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Chapter 3

THE ROLE OF SPATIAL ENVIRONMENTAL HETEROGENEITY IN THE EVOLUTION OF GENE FLOW DISTANCE IN *DANTHONIA SPICATA*

INTRODUCTION

The scale of gene flow within and among populations is critically important in governing the scale of local adaptation, the maintenance of genetic diversity, and the cohesiveness of populations. The evolution of gene flow distance in response to spatial heterogeneity has received theoretical, but little experimental attention (e.g. Spieth 1979, Gillespie 1981, Hedrick 1986, Venable and Brown 1988). Theory predicts that gene flow distance should evolve to match the spatial scale of variation in natural selection (Tonsor 1990, Balkau and Feldman 1973, Christiansen and Feldman 1975).

Gene flow in plants includes both pollen and seed dispersal. In many plants, gene flow occurs predominantly through pollen dispersal (Price and Waser 1979). In others, especially selfing plants, it is primarily through seed dispersal. It has been argued that some plants may have little ability to fine tune gene flow distances because of reliance on vectors over which they have little control (Waddington 1983). However, it is easy to imagine how genetic traits could influence the movement of pollen and seeds that are wind or ballistically dispersed (Okubo and Levin 1989).

Increased gene flow is favored by many factors including temporal variation in site quality among generations and competition with parents and siblings. These factors selecting for non-zero dispersal are likely always important, however, restricted gene flow may evolve when environmental heterogeneity is spatially structured (Tonsor 1990). The balance of the relative strengths of selection to increase and decrease dispersal determines the optimal dispersal distance. Although temporal variation among generations (e.g. Stratton and Bennington 1998) and the strength of competition between parents and offspring or among siblings undoubtedly differs among environments, the

major factor selecting for different dispersal distances among relatively similar populations is likely to be the strength of selection by spatially structured environmental variation to decrease dispersal distance.

Temporal variation at very small time scales (e.g. within a growing season) may be substantial at individual points within a population without affecting the mean value of a location relative to other locations in a site. Some traits, such as seed dispersal, have a strong spatial component (i.e. may directly affect the spatial distribution of plants or their offspring) and may evolve relatively independent of smaller scale (within generation) temporal variation. If traits affecting gene flow can adapt to spatial environmental variation, then gene flow evolution will be an important factor in tailoring population structure to the scale of environmental variation, thus affecting the process of local adaptation and the maintenance of genetic variation.

When environmental variation is temporally consistent and spatially structured, increasing seed dispersal results in offspring encountering environmental conditions that are increasingly less likely to resemble their maternal parent's environment. When spatial variation is consistent over time and most of the microhabitats in a site are unsuitable for growth, then a maternal environment will be better than a random location in the site. If a maternal location is better than an average location for seedling growth, then increasing similarity between maternal and offspring conditions may represent an increasing probability of offspring encountering good environments.

In populations where the difference between maternal and random locations is very strong, the evolution of dispersal may be dominated by spatial environmental variation and may be quite short (although still non-zero). However, in sites where there is either little spatial environmental variation or where spatial variation is not structured, the similarity between offspring and maternal conditions will not depend on offspring dispersal distance. In these populations, spatial environmental variation will not select for decreasing dispersal. Selection by other factors, such as competition with siblings and

parents, acting to increase dispersal may dominate the evolution of dispersal distance in environments with little spatially structured environmental variation.

One way to approach the question of whether spatial environmental variation can affect the evolution of gene flow distance among populations is to examine existing differences in seed dispersal distance among populations in sites that differ in amount of spatially structured environmental variation. I examined the correlation between spatial environmental variation and gene flow distance in *Danthonia spicata*. *Danthonia spicata* produces two differently-dispersing seed types and previous work has shown that there is substantial genetic variation within and among *D. spicata* populations for proportional allocation to these two seed types (Clay 1982). In plant species with heteromorphic seeds that disperse to different distances, the ability to modify dispersal distance may be particularly strong because dispersal distance can be strongly altered by allocating different proportions of seeds to the differently-dispersing structures (Lloyd 1984).

I used simulated dispersal distributions and the spatial structure of environmental variation to estimate the similarity of offspring to maternal conditions in five *D. spicata* populations. I then determined the effect that decreasing dispersal distance would have on the similarity between maternal and offspring conditions and compared that effect to the proportional allocation to short-dispersing seeds in these populations.

MATERIALS AND METHODS

Study species

Danthonia spicata (Poaceae) is a perennial, C_3 bunch grass that is native to North America and commonly occurs in dry, nutrient-poor oak savannas, old-fields, and openings throughout the eastern and northern United States and Canada (Darbyshire and Cayouette 1984). *Danthonia spicata* plants produce two flower types (chasmogamous and cleistogamous) that produce similar-sized seeds (Clay 1983). Potentially outcrossed, wind-pollinated chasmogamous (CH) flowers are produced at the tip of the reproductive

stalk. Obligately self-fertilized cleistogamous flowers are produced in the axils of leaf sheaths along and at the base of the reproductive stalk (Figure 3.1). Cleistogamous (CL) seeds, up to ten per leaf node, remain within the leaf sheath, attached to the reproductive stalk until germination, while CH seeds are dispersed individually by wind. These differences in dispersal mechanism mean that the two seed types are likely to have different dispersal potentials. Chasmogamous seeds of *D. spicata* are estimated to be less than 10% outcrossed and there is little quantitative genetic difference between the two seed types (Clay 1982). The relatively small genetic difference between the two seed types means that gene flow distance in *D. spicata* depends largely on seed dispersal distance. Genetic variation for the proportion of the two seed types among populations allowed me to investigate the relationship between spatial environmental heterogeneity and dispersal distance in this species.

Population and site characterization

I located eight sites where *D. spicata* occurred, all of which were dominated by perennial, herbaceous vegetation. None of these sites had been plowed or tilled for at least 45 years. Six of the survey sites were located in southwest Michigan; three were remnant oak savannas in the Allegan State Game Area near Allegan, Michigan (A_1, A_2, A_3); and three were old-fields near the W.K. Kellogg Biological Station (L_1, L_2, LA). A fourth old-field site was located in the Rose Lake Wildlife Research Area in central Michigan near East Lansing (R). The eighth survey site was an alvar grassland in the Maxton Plains Preserve on Drummond Island, in northern Michigan (D).

I established a 4 x 6 m permanent plot in each of the eight sites during the spring of 1996. I measured the percent of seeds that were cleistogamous (CL) in each population by collecting two reproductive stalks from each of 30 randomly selected *D. spicata* plants within each plot in June 1996. The location of each sampled plant was noted and the plant was marked with an aluminum tag so it could be relocated. In five of

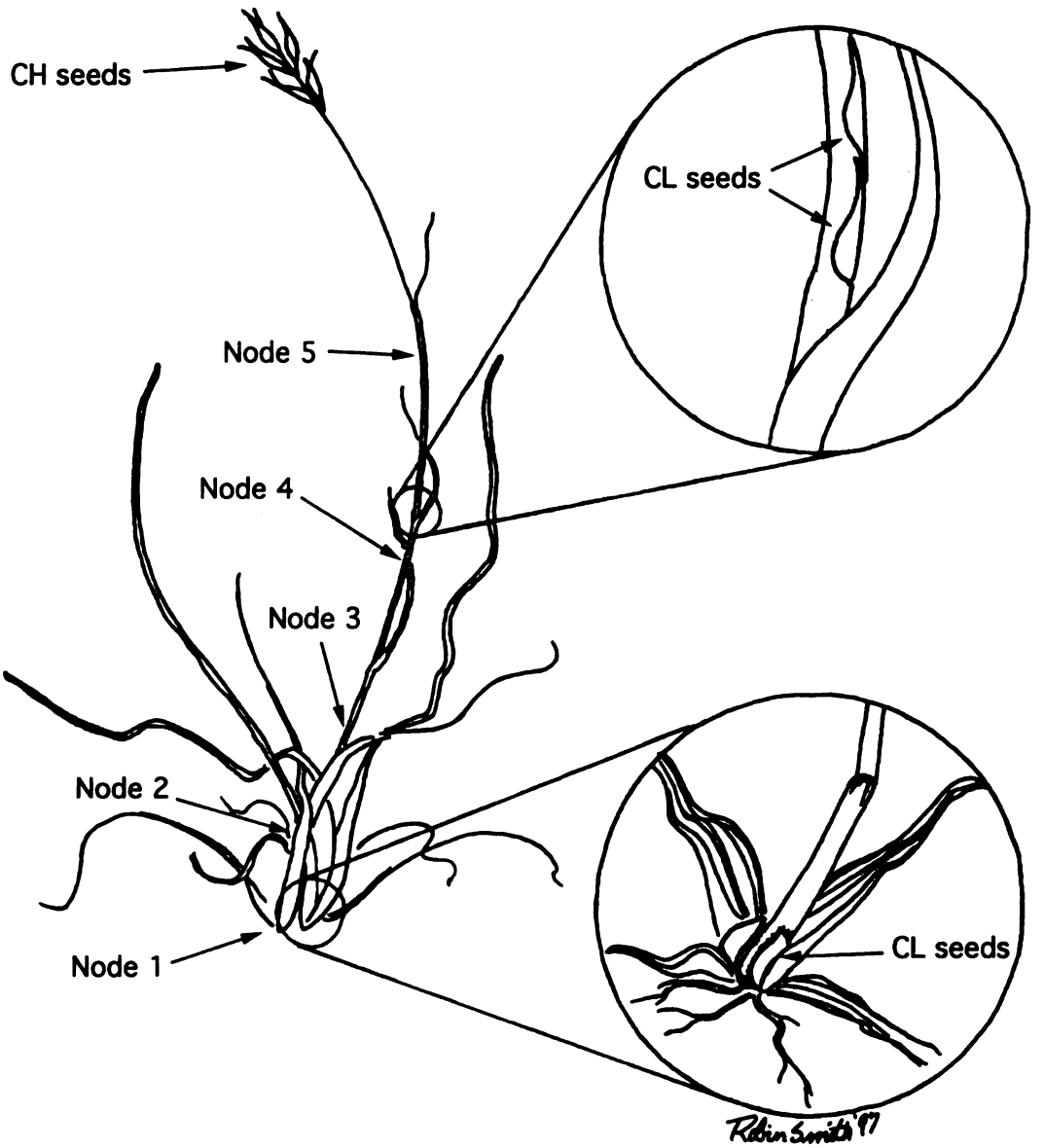


Figure 3.1: *Danthonia spicata* chasmogamous and cleistogamous seeds
Chasmogamous (CH) seeds in a terminal inflorescence and cleistogamous (CL) seeds within leaf sheaths at each node along the reproductive stalk. Nodes are numbered from the base of the plant towards the top, just below the CH inflorescence.

the populations (A_2, L_1, L_2, D, R), I resampled reproductive stalks from all tagged plants that reflowered in 1997 to verify that percent allocation to cleistogamy was consistent among years. Three of the eight populations (L_1, L_2, R) contained plants infected by the epiphytic fungus *Atkinsonella hypoxylon*. Because *A. hypoxylon* causes abortion of all CH seeds in infected plants (Clay 1984), I only sampled reproductive stalks from uninfected plants. In five of the eight sites (A_2, L_1, L_2, D, R), I measured plant density (plants m^{-2}) along two randomly located transects (25 cm x 12 m long).

On each sampled reproductive stalk, I counted the number of CH seeds and measured total reproductive stalk length to use in CH dispersal simulations. I also counted the number of CL flowers and initiated seeds at each node along the reproductive stalk, denoting the position of each node by a node number (Figure 3.1). Percent cleistogamy was calculated for each individual as the total number of CL flowers x (the total number of CH seeds and CL flowers)$^{-1}$. I used flowers and initiated CL seeds rather than mature seeds to estimate CL seed production, because *D. spicata*'s CH seeds mature and disperse before CL seeds mature. Very few individuals reflowered in 1997, so I was only able to assess the consistency of percent CL seeds in those few individuals in each population. I used an ANOVA assessing the effect of Population and 1996 percent cleistogamy on 1997 percent cleistogamy to determine the temporal consistency of allocation to CL seeds among populations.

Measuring dispersal distance

To estimate the primary dispersal distance of *D. spicata* seeds, I collected three plants with multiple (20 to 40) reproductive stalks and mature, but not yet dispersing, CH seeds from two of the study populations, two from Population L_1 and a third from L_2. Each plant was excavated in early June 1996 and placed in a 10 x 10 cm square pot. I then transferred each plant (and pot) to a dispersal arena in a field location at the W.K.

Kellogg Biological Station more than 500 m from the nearest naturally-occurring *D. spicata* plant.

Each dispersal arena consisted of a 3 m x 3 m sheet of plywood, the central 1.2 m x 1.2 m area of which was overlaid by an egg crate panel light diffuser. I sprayed the entire arena with Tanglefoot (Tanglefoot Company, Grand Rapids, MI) so that it would trap dispersed seeds (as per Thiede and Augspurger 1996). The potted experimental plant was placed in a 10 x 10 cm hole in the center of the diffuser and plywood. The three dispersal arenas were separated by at least 5 m. Using these dispersal arenas, I was only able to estimate primary dispersal as it might occur with no surrounding vegetation. However, *D. spicata* often grows in very open habitats, with little tall vegetation, so these estimates probably provide a good estimate of the primary dispersal distance of CH and CL seeds. Secondary dispersal of CL seeds in the field appears to be very limited, evidenced by the presence of persistent reproductive stalks near and attached to parent plants, from which CL seeds germinate (McCormick, personal observation).

I determined the distance traveled by dispersed seeds in early July 1996, after the reproductive stalks had senesced. I noted the distance each chasmogamous (CH) seed had traveled from the base of the parent plant. I also noted the lengths of all reproductive stalks. Reproductive stalks generally remained intact along their length, allowing stalk length to be easily measured. I fit a regression of average CH dispersal distance on average stalk length across the three sets of dispersal measures (N = 3 plants for CH dispersal distance) to determine whether stalk length could be used to predict dispersal distance.

To estimate the dispersal distance of cleistogamous (CL) seeds, I measured the distance from the base of the parent plant to each leaf node on each reproductive stalk (21-37 per plant). I collected each reproductive stalk and counted the number of CL seeds at each node. Nodes were numbered relative to their position along the inflorescence (Figure 3.1). The total number of leaf nodes on a reproductive stalk ranged

from 2 to 5. All 65 reproductive stalks had at least two leaf nodes, but only 12 stalks had a fifth node, so the sample size for estimating the distance traveled by each node number ranged from 12 to 65. The dispersal distance of CL seeds was considered to be the distance from the base of the parent plant to the position of the leaf node where they occurred. I used these measurements to determine the mean dispersal distances of CH and CL seeds and the relation to plant height. I then incorporated this information into a dispersal simulation to investigate how average dispersal differences changed as the proportion of CL seeds changed, so that I could compare dispersal in other populations based on traits I could easily measure.

Dispersal simulation

Because CH seeds are dispersed individually and CL seeds at a node are dispersed together, I used separate simulation models to predict the dispersal of CH and CL seeds. I used the relationship between CH seed dispersal distance and reproductive stalk length, determined in my dispersal experiment, to estimate average dispersal distances for CH seeds in each of my eight populations. In each of the eight populations I determined the average stalk length of 32 plants in each population, and the regression of average CH dispersal distance on stalk length in the dispersal experiment.

To estimate CL dispersal distances, I used the relationship between the node location dispersal distance of CL seeds from my dispersal experiment. I wrote a FORTRAN program to randomly select a reproductive stalk sampled from a field population (on which I had counted the number of seeds at each node) that determined the distribution of seeds along the reproductive stalk. The program then randomly chose a reproductive stalk from my dispersal experiment (for which I had measured the distance each node along the stalk dispersed) that determined the distance each node would disperse. I assumed that all CL seeds at a leaf node dispersed to the same location.

This random selection process was carried out 200 times, with replacement, for each simulation run. CL seed dispersal simulations were run five times for each population. Each population was simulated separately. For each run, I calculated the average CL dispersal distance. I also generated a frequency distribution for the proportion of seeds dispersing to different distances using 5 cm intervals. I used these simulations to determine whether different proportional allocation to CL seeds produced different dispersal patterns among these eight populations.

Combining dispersal and environmental variation

In environments where resources are highly spatially structured, seeds that disperse a short distance experience environmental conditions more similar to the environment of their mother than seeds that disperse a long distance. To examine the relationship between *D. spicata* dispersal distance and the amount of environmental variation offspring experience relative to maternal conditions, I measured the distribution of spatial variation in soil resources in the five study sites in which I had simulated dispersal distance. These five sites were selected to encompass the range of proportional allocation to CL seeds measured in my site surveys (Figure 3.2a). Two of the selected populations had a low percent of CL seeds (A_2, L_1), two had an intermediate percent of seeds that were CL (L_2, D), and one had a high percent of seeds that were CL (R; Figure 3.2a). Each of these five populations also had substantial variation in percent of CL seeds within the population (See ranges, Figure 3.2a).

I chose to examine soil moisture and the relative supply rate of ammonia-nitrogen as estimates of environmental quality because they were relatively consistent over time in these sites (Chapter 2) and often limit plant growth in the dry, nutrient-poor environments where *D. spicata* occurs. Soil moisture and ammonia supply were not correlated within these sites (Chapter 2), so I have treated them as independent measures of environmental variability. In four of these five sites (A_2, L_1, L_2, R), I measured soil

Figure 3.2: Characteristics of study populations
Percent of cleistogamous (CL) seeds (mean ± 1 s.e.; **a.**) and mean reproductive stalk length (± 1 s.e.; **b.**) of each of the eight study populations, and density of *D. spicata* plants in five of the study populations. Diamonds in **a.** indicate the highest and lowest percent of cleistogamous seeds measured among the 32 plants sampled in each population.

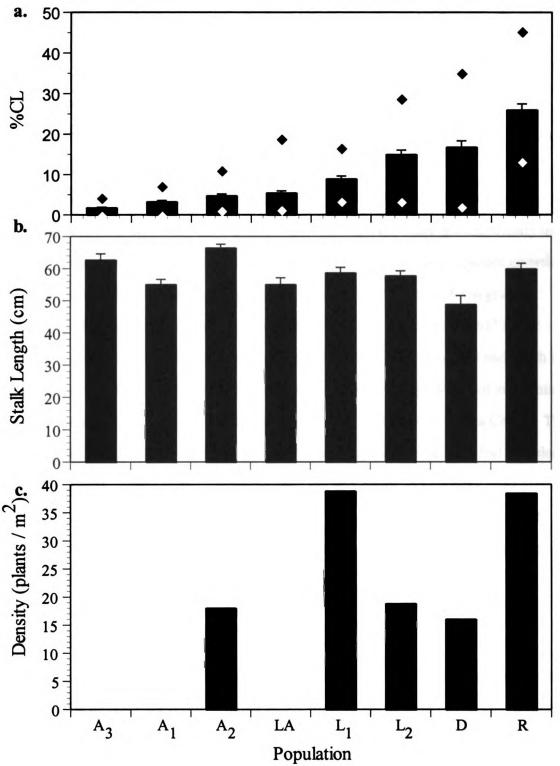


Figure 3.2: Characteristics of study populations

moisture (gravimetrically) and relative supply rate of ammonia-nitrogen (using resin bags). The supply rate of nitrate was also measured in these populations, but it was not consistent over time (Chapter 2), so its rate of supply at a maternal location could not be used to predict offspring environmental conditions. In the fifth site (D) soils were too shallow for resin bag installation or for soil cores (avg. 4.5 cm deep), so I used soil depth as an indicator of soil moisture.

I measured soil moisture and ammonia at 70 points in a 4 x 6 m plot in each site using a stratified, nested sampling design. I measured soil moisture gravimetrically in Sites A_2, L_1, L_2, and R in early June 1998, during the period of peak *D. spicata* growth. I sampled soil moisture using 10 cm deep, 2.5 cm diameter soil cores. A ten gram subsample of each soil core was passed through a 2mm sieve and dried at 65° for 72 hours before being reweighed (Chapter 2). In the fifth site (D), I measured soil depth in June 1997 as an indicator of moisture. I measured the relative supply rate of ammonia-nitrogen using bags of mixed bed ion exchange resin (Dowex MR-3, Sigma Corp.). The resin bags were placed in the field in October 1997 in the same locations I had sampled for soil moisture. These resin bags were buried under 5 cm of soil (as per Lajthe 1988) in four sites (excluding Site D) and collected in May 1998. The resin bags were rinsed, dried at 40° for 5 days, and extracted with 1M KCl (see Chapter 2) to provide an index of ammonia supply.

Calculations

I used semivariance analysis to quantify the relationship between environmental variation and distance separating sample points for soil moisture and ammonia in each site. Semivariograms summarize the average variance between pairs of points as a function of their distance apart and can be used to estimate the extent of spatial structure in variable (Rossi 1992, Robertson and Gross 1994). I calculated the semivariance (standardized by the overall level of variation) of both soil moisture and ammonia supply

using 5 cm step sizes (distance classes) from 0 to 150 cm (active lag). This active lag encompassed spatial structure at distances beyond the farthest seed dispersal distance I measured. I used standardized semivariances so that I could assess the effect of different patterns of allocation to CL seeds among populations, independent of the amount of environmental variation in each site.

I used dispersal distributions generated from my simulations of CH and CL seeds in each population as weights on the semivariograms calculated from environmental samples to estimate the impact of the percent of cleistogamy on the average amount of environmental variation encountered by dispersing seeds (Figure 3.3). On average, the seeds dispersing to a given 5 cm interval would encounter conditions whose difference from maternal conditions was predicted by the semivariance at the corresponding 5 cm step. Using the proportion of seeds dispersing to a given 5 cm interval to weight the semivariance calculated for environmental samples separated by that same interval distance allowed me to calculate an average variation from maternal conditions that would be encountered by dispersing offspring. This allowed me to estimate environmental variation as dispersing seeds would encounter it.

I calculated the average variation from maternal conditions in soil moisture and ammonia encountered by all seeds (CL and CH), in each site ($S_{CL} + S_{CH}$):

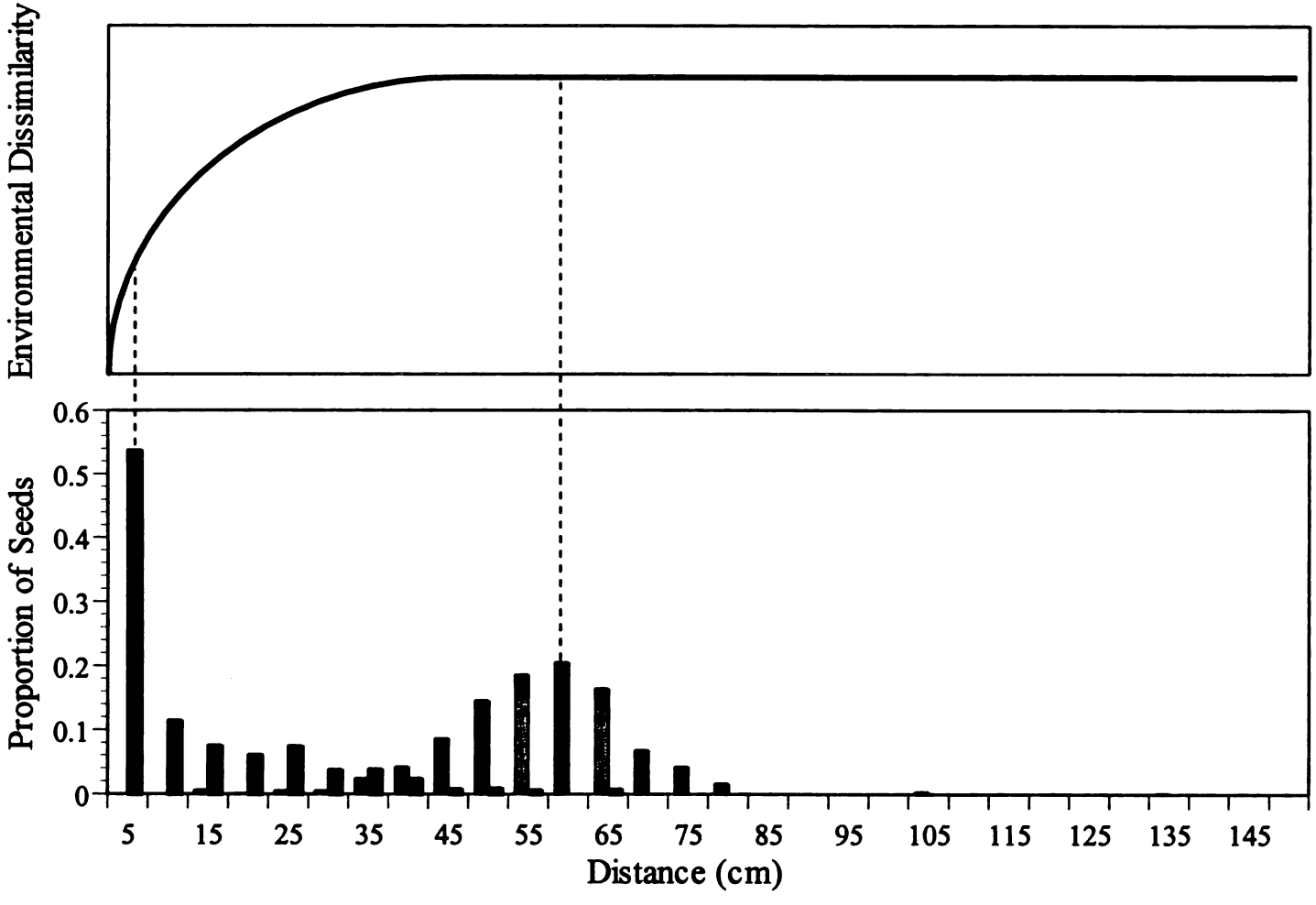
$$(S_{CL} + S_{CH}) = \sum_{n=1}^{29} (S_{5n} \times P_{CL,5n}) + (S_{5n} \times P_{CH,5n}) \qquad [1]$$

$$Where, \sum_{n=1}^{29} P_{CL,5n} + P_{CH,5n} = 1$$

Where S_{5n} is the standardized semivariance calculated for the nth distance and $P_{CL,5n}$ and $P_{CH,5n}$ are the proportion of CL and CH seeds, respectively, dispersing in distance class n, 5n cm from parent plants. I used equation [1] to calculate variation from maternal conditions that would be encountered by offspring of plants allocating low, moderate, high, and the population average proportion of their seeds to cleistogamy for

Figure 3.3: Relating seed dispersal to environmental variation
Diagram demonstrating use of the simulated proportion of cleistogamous (CL; black bars) and chasmogamous (CH; gray bars) seeds in each 5 cm interval as weights on variation from maternal environmental conditions. The semivariogram describes the similarity between pairs of points. Using seeds dispersing different distances from the maternal plant to weight semivariance allowed estimation of the similarity between maternal and offspring environmental conditions.

Figure 3.3: Relating seed dispersal to environmental variation



each population. I used the seed distribution simulated for Population A_2 to estimate the amount of variation from maternal conditions that would be encountered by seeds from a parent with low allocation to CL seeds, Population D for a parent with moderate allocation to CL seeds, and Population R for a parent with high allocation to CL seeds. These calculations allowed me to determine how changing allocation to CL seeds would affect the average similarity to maternal conditions encountered by dispersing seeds across all five populations.

I also calculated an equal weighting of the similarity to maternal conditions encountered by the two seed types ($S_{100\%CL}$, $S_{100\%CH}$):

$$S_{100\%CL} = \sum_{n=1}^{29} \left(S_{5n} \times P_{CL,5n} \right) \qquad [2]$$

$$S_{100\%CH} = \sum_{n=1}^{29} \left(S_{5n} \times P_{CH,5n} \right. \qquad [3]$$

$$Where, \sum_{n=1}^{29} P_{CL,5n}, \sum_{n=1}^{29} P_{CH,5n} = 1$$

Where S_{5n} is the semivariance calculated for the nth distance and P_{CL5n} and P_{CH5n} are the proportion of CL and CH seeds, respectively, dispersing in distance class n, 5n cm from parent plants. Each equation is summed over all distance intervals to which simulated seeds dispersed. For all populations, the farthest distance class containing seeds was class 29 (145 cm). This equal weighting of the two seed types was used to indicate differences in the amount of variation from maternal environmental conditions encountered by CH and CL seeds as a function of differences in their dispersal distance, independent of the proportion of seeds allocated to each seed type across different populations.

Predictions

If allocation to CL seeds in *D. spicata* is a result of the scale of spatially structured environmental variation, then populations with different amounts of allocation to CL seeds would occur in environments in which CL seeds were differently effective at reducing the amount of variation from maternal conditions that offspring encountered. Specifically:

1) If shorter dispersing CL seeds increase the average similarity between maternal and offspring environmental conditions, then increasing the percent of CL seeds would decrease the average variation encountered by dispersing seeds across populations, increasing similarity to parent conditions. This would produce a negative correlation between the average environmental variation encountered ($S_{CL} + S_{CH}$) and simulated allocation to CL seeds (high, moderate, or low).

2) Because CL seeds disperse shorter distances than CH seeds, increasing allocation to CL seeds decreases average dispersal distance. Decreased dispersal distance will only increase similarity to maternal conditions, if environmental variation is spatially structured. If increasing similarity to maternal conditions is beneficial, then I would expect spatially structured environmental variation to select for increased allocation to CL seeds. I predict that populations of *D. spicata* in environments where decreased dispersal distance increases the similarity between maternal and offspring conditions would have higher allocation to CL seeds than those occurring in environments where decreased dispersal distance was less effective at increasing the similarity between maternal and offspring conditions (i.e. environments with little spatial environmental structure). This hypothesis predicts a negative correlation between population average percent of CL seeds and the effectiveness with which a population can increase the average similarity between maternal and offspring conditions by shifting allocation between CH and CL seeds ($S_{100\%CH} - S_{100\%CL}$). I calculated the extent to which changing allocation to CL seeds increases similarity between maternal and offspring conditions

($S_{100\%CH}$ - $S_{100\%CL}$) for each population under simulated conditions of allocation to CL seeds (low, 4.6%; moderate, 16.7; and high, 25.9%).

Interpretation testing

Using the similarity between maternal and offspring conditions as an estimate of the amount of spatial environmental variation encountered by dispersing seeds assumes that the parent location is a good microhabitat relative to random locations in a site. This may not be a bad assumption for *D. spicata*, which grows in low productivity habitats where there is often substantial open ground. If a maternal location were better for *D. spicata* performance than a location chosen at random in a site, then decreasing the variance between offspring environment and maternal environment would be analogous to increasing the rate at which seeds encounter good environments. Schoen and Lloyd (1984) specifically predicted that a difference in the rates at which CH and CL seeds encounter good environments would determine the proportional allocation to the two seed types (q/(1-q)) that would be favored:

$$\frac{q}{1-q} = \frac{e1}{e2} \times \frac{[(e2-e1)\times(1-\frac{1}{2N_2})]}{\{e1\times[1-\frac{1}{2N_2}]-[e2\times(1-\frac{1}{N_1})]\}} \qquad [4]$$

In equation [4], modified from Schoen and Lloyd (1984), I have simplified the terms e1 and e2 to the rates at which CL and CH seeds, respectively, encounter good environments or 'safe sites' by assuming that factors other than the environment (e.g. differential provisioning of the two seed types, differential maternal effects) did not differ among populations. N_1 and N_2 are the number of other maternal plants within the dispersal range of CL and CH seeds, respectively. In Schoen and Lloyd's (1984) equation, the number of plants reflects the number of other plants producing seeds with which seeds of the target plant must compete for good sites. I used plant density measures from surveys of each population (see Figure 3.2c) and average CL and CH

dispersal distances from my dispersal simulations (Figure 3.4) to estimate N_1 and N_2. N_1 = average plant density x π x (average CL dispersal distance)^2. N_2 = average plant density x π x (average CH dispersal distance)^2.

The proportional resource investment in CL seeds was represented by q. The proportional resource investment in CH seeds was represented by 1 - q. Although the amount of resource needed for *D. spicata* to produce a CL seed was not necessarily the same as amount needed to produce a CH seed (i.e. equal resource investment may not produce equal numbers of CH and CL seeds), I assumed that CL seeds required the same resource investment, relative to CH seeds, in all five of my populations. Because I did not measure actual investment in the two seed types, this assumption allowed me to use the number of CL and CH seeds as an estimate of differences in allocation among populations. I solved equation [4] for e1 in terms of e2 and calculated e1 / e2, the ratio of the rate at which CL seeds encounter good environments to the rate at which CH seeds encounter good environments.

I used this ratio as an estimate of the difference in the amount of environmental variation encountered by CL and CH seeds that would be needed to explain the differences in proportional allocation to CH (q) and CL (1-q) seeds that I measured among my populations. If increased allocation to CL seeds increased the rate at which seedlings encountered good environments, then the ratio of environmental variance encountered by CL seeds to variance encountered by CH seeds ($S_{100\%CL}$ / $S_{100\%CH}$) would be negatively correlated with Schoen and Lloyd's ratio of CL to CH rates of encounter with good environments (e_1 / e_2).

RESULTS

Population and site characterizations

The eight populations of *Danthonia spicata* that I surveyed for this study had different percentages of CL seeds (Figure 3.2a) but had little difference in their

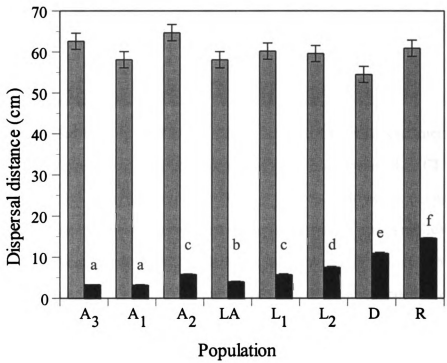


Figure 3.4: Simulated seed dispersal distances
Simulated dispersal distances (mean ± 1 s.e.) of cleistogamous (black bars) and chasmogamous (gray bars) seeds. Letters above cleistogamous dispersal bars indicate significant differences among populations for simulated CL seed dispersal. Dispersal distances of CH seeds did not differ significantly among populations.

reproductive stalk lengths (Figure 3.2b). In the five populations that were resampled for percent allocation to CL seeds, few of the plants that were sampled in 1996 reflowered in 1997 (6-18 of 32 plants in each population). Therefore I had limited power to assess the consistency of percent allocation to CL seeds by individual plants. However, both Population and 1996 %CL were significant predictors of 1997 %CL in an ANOVA (each $p < 0.001$), suggesting that the five populations differed consistently in their percent allocation to CL seeds.

Measuring dispersal distance

The dispersal distances of CL and CH seeds in the dispersal experiment were markedly different; on average CH seeds dispersed ~4.5 times farther than CL seeds (CL distance = 12.25 $\pm$ 0.74 cm; CH distance = 56.58 $\pm$ 0.39 cm). However, the magnitude of the difference in dispersal distances of the two seed types varied across replicate plants (Seed Type x Replicate, $p < 0.001$). Much of this variation was attributable to differences in reproductive stalk length and the distribution of CL seeds among nodes. Across the three replicate plants, average reproductive stalk length was a good predictor of average CH dispersal distance (Figure 3.5a). Although stalk length was a good predictor of average CH seed dispersal distance, there was substantial variance about the mean in the distance traveled by individual CH seeds (Figure 3.2b). Because of the variance about the mean CH dispersal distance populations did not differ in simulated CH dispersal distance (Figure 3.5a).

CL seed dispersal distance was strongly correlated with the node number at which CL seeds occurred (Figure 3.5b). Taller reproductive stalks had more nodes at which CL seeds could be produced, so variation in stalk length was included in node number and was not an independent variable. Although the plants used in the dispersal experiment produced most of their CL seeds at lower leaf nodes, plants in other populations had

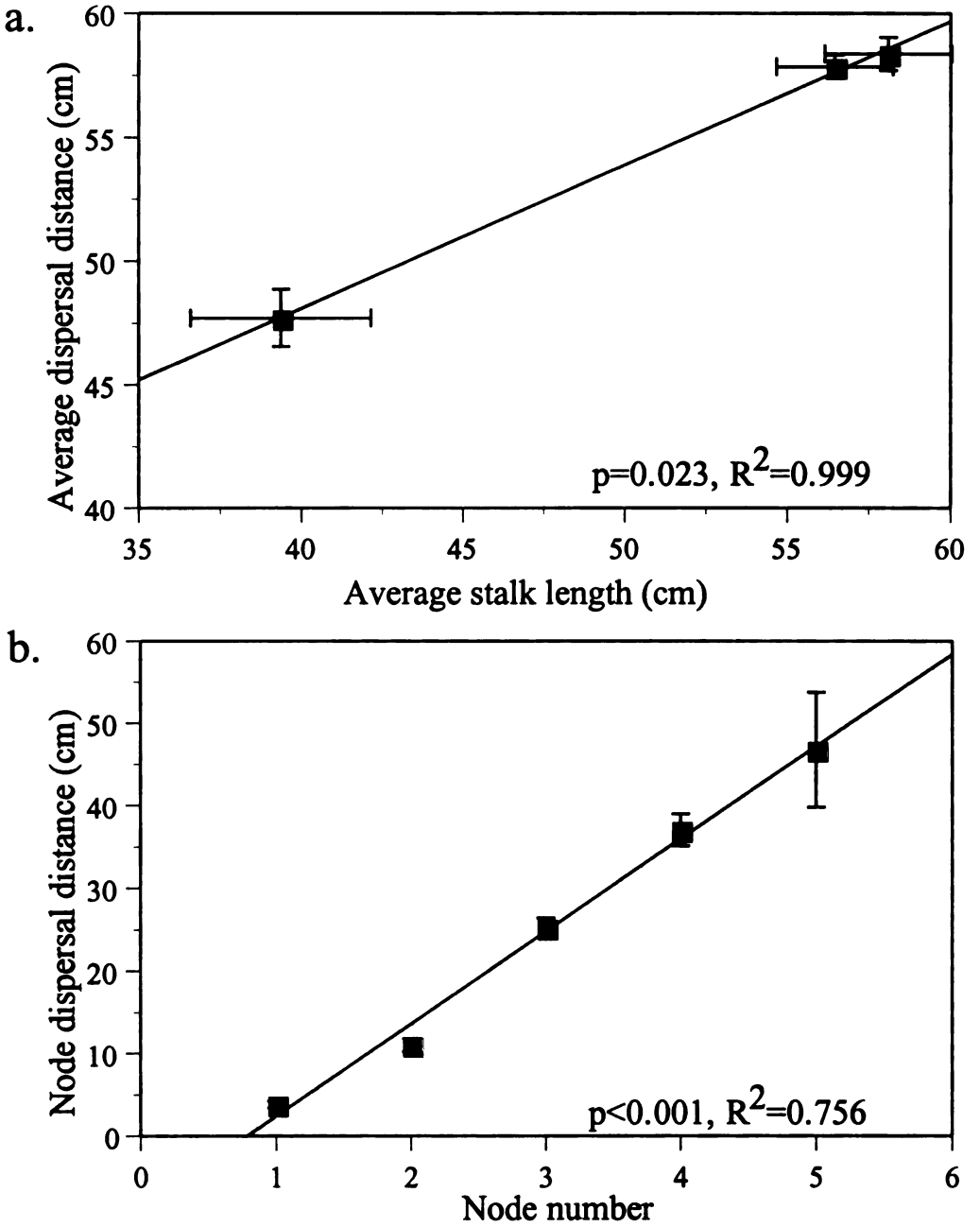


Figure 3.5: Dispersal of CH and CL seeds
Relationship between CH seed dispersal distance and reproductive stalk length (N = 3 plants, each mean ± 1 s.e.; **a.**) and the relationship between CL seed dispersal distance (mean ± 1 s.e.) and node number at which the seeds were produced (N = 12 to 65 reproductive stalks per node; **b.**). P-values indicate the significance of each regression and R^2 values indicate regression fit.

many CL seeds at leaf nodes higher up on the reproductive stalk. Thus I used node number in the dispersal simulation rather than actual CL dispersal distance measures.

Dispersal simulation

There was no difference among the eight populations in the simulated dispersal distance of CH seeds (Figure 3.4). The distribution of individual CH seed dispersal distances about the mean distance (measured in the dispersal experiment) was so large that a 95% confidence interval ($\pm$ 22 cm) for the average dispersal distance of any of the eight populations encompassed the full range of average CH dispersal distances. However, the simulated average dispersal distances of CL seeds were significantly different among populations (Figure 3.4). Differences among populations in CL seed dispersal distance were largely explained by percent allocation to CL seeds. Increases in the percent of CL seeds occurred predominantly by increasing the number of CL seeds at the farther-dispersing, upper leaf nodes. Consequently, populations that had a larger proportion of CL seeds also dispersed their CL seeds further (Figure 3.6).

Combining dispersal and environmental variation

Prediction 1: Increasing allocation to CL seeds significantly decreased the variation from maternal conditions encountered by dispersing seeds ($S_{CL} + S_{CH}$) across the five populations (Figure 3.7). This result indicates that there is the potential for increased allocation to cleistogamous seeds to reduce environmental variation encountered by dispersing seeds in these populations.

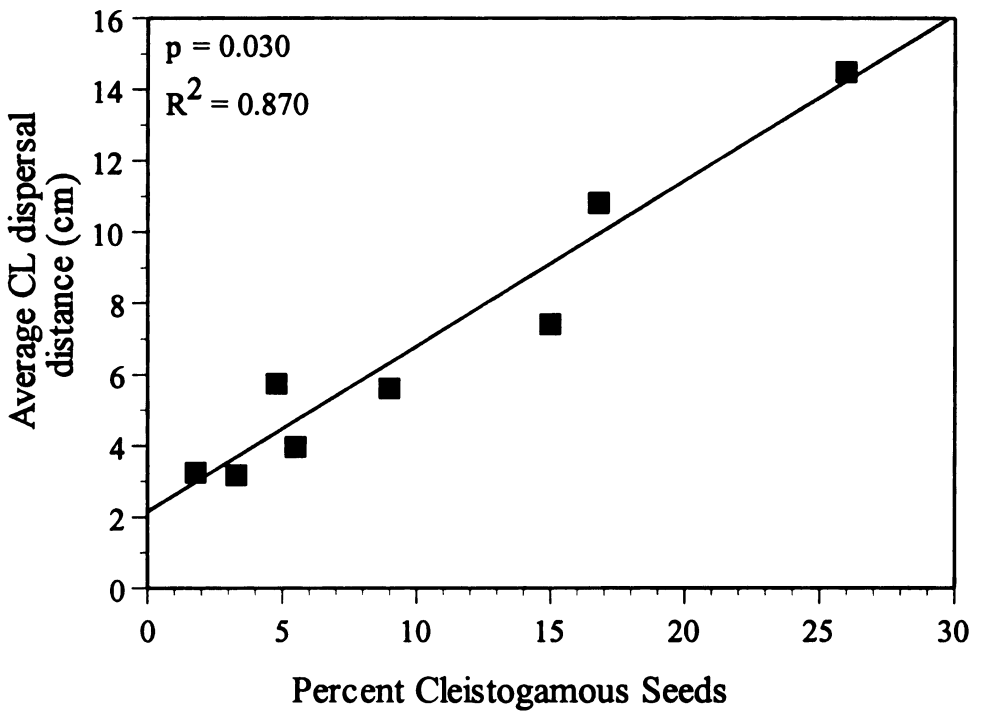


Figure 3.6: Relationship between CL dispersal distance and percent of CL seeds P-value and R^2 value indicate significance and fit of the regression of simulated average CL dispersal distance for each population on the population average percent of CL seeds. N = 8 populations.

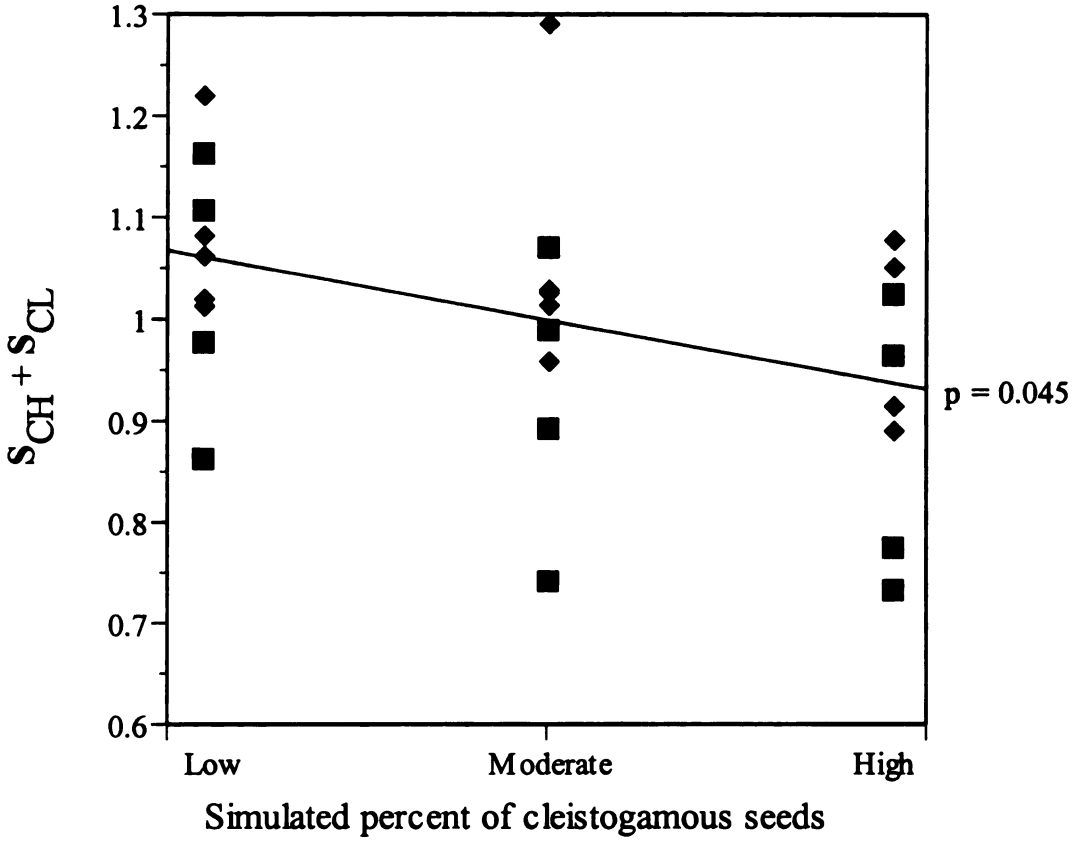


Figure 3.7: Effect of CL seed allocation on variation encountered by seeds
The relationship between average variance from parental conditions encountered by dispersing seeds and simulated proportional allocation to CL seeds. Variance from maternal conditions was estimated for each of five populations based on measured variation in soil moisture (◆) and ammonia supply (◇). P-value denotes significance of the regression.

Prediction 2: In four of these five populations (L_1, L_2, D, R), increased allocation to CL seeds was associated with increased spatial environmental structure. In sites with more spatially structured variation in soil resources, CL seeds were effective at increasing the similarity between parent and offspring environments (high S_{CH}-S_{CL}). In contrast, lower allocation to CL seeds was associated with sites that had little spatial environmental structure, in which increased allocation would be ineffective at increasing the similarity between parent and offspring environments (low S_{CH}-S_{CL}). The positive correlation between S_{CH}-S_{CL} and percent of CL seeds was significant for high and moderate, but not low, allocation to CL seeds in these four populations (Figure 3.8). Population A_2, differed substantially from all other populations in the relationship between percent of CL seeds and S_{CH}-S_{CL}. Soil resources in Population A_2 were strongly spatially structured (high S_{CH}-S_{CL}), but the population had very few CL seeds. This may be due to the effect that a very short growing season has on the allocation to later-maturing CL seeds in this site.

Interpretation testing

In four of five populations (L_1, L_2, D, R) there was a significant negative correlation between my estimate of similarity to maternal conditions at CL and CH dispersal distances ($S_{100\%CL}/S_{100\%CH}$) and Schoen and Lloyd's (1984) predicted rates of encountering good sites by CL and CH seeds (e1/e2). This result indicates that variation (i.e. average semivariance) from maternal conditions was an accurate indicator of variation from good environmental conditions. The negative correlation between my index ($S_{100\%CL}$ / $S_{100\%CH}$) and Schoen and Lloyd's index (e1/e2) was significant for both soil moisture and relative ammonia supply at high and moderate, but not low, allocation to CL seeds among these four populations (Figure 3.9).

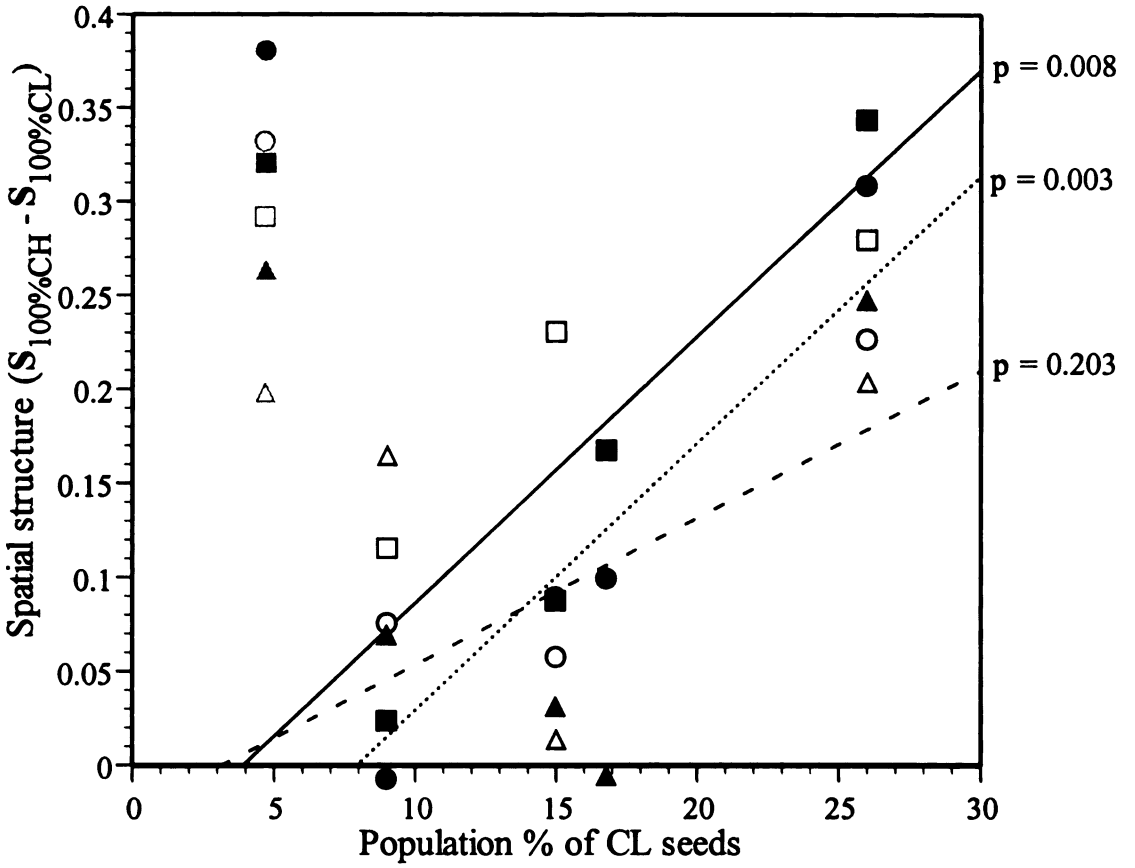


Figure 3.8: Relationship between population %CL and environmental structure Correlation between spatial environmental structure in each field site and the actual percent of cleistogamous seeds in each population. Spatial environmental structure ($S_{100\%CH}$ - $S_{100\%CL}$) refers to the extent to which changing allocation to CL seeds (changing dispersal distance) changes the variation in soil moisture (solid symbols) and ammonia supply (open symbols), relative to maternal environmental conditions, encountered by dispersing chasmogamous (CH) and cleistogamous (CL) seeds. In environments with substantial spatial structure the difference between CH and CL conditions is large. In unstructured environments the difference is small. Spatial structure (as encountered by dispersing seeds) was simulated in each population for low (solid line, ■), moderate (dotted line, ●), and high (dashed line, ▲) percent allocation to CL seeds. As %CL increases, average CL dispersal distance approaches CH dispersal distance, so the difference between the two seed environments decreases. Gray symbols indicate measurements for Population A_2, which were excluded from regressions. P-values to the right of the graph indicate significance of each regression of effectivenss on population % CL.

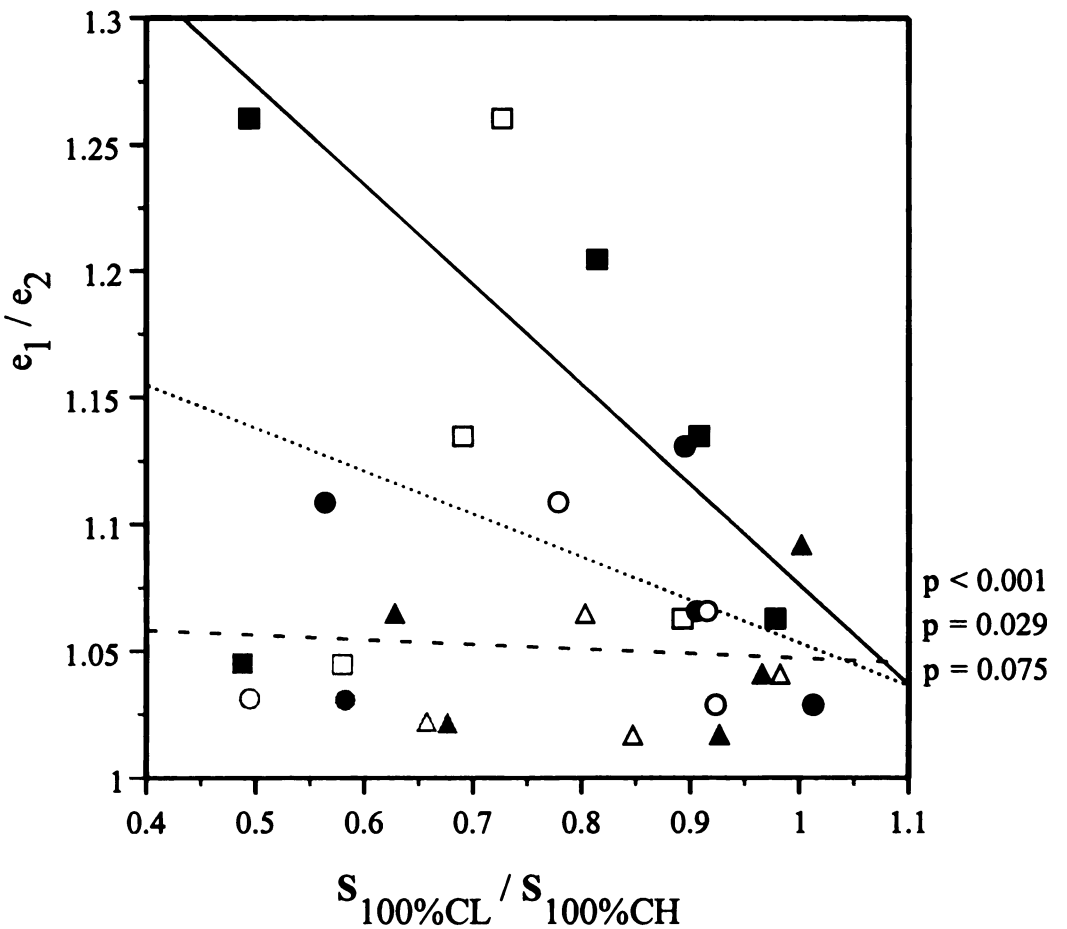


Figure 3.9: Comparison to Schoen and Lloyd's rates of encounter with good sites Relationship between the ratio of variance from parental conditions encountered by cleistogamous ($S_{100\%CL}$) and chasmogamous ($S_{100\%CH}$) seeds with Schoen and Lloyd's (1984) ratio of the predicted rate of encounter with good conditions by cleistogamous (e1) and chasmogamous (e2) seeds for a low (solid line, ■), moderate (dotted line, ●), and high (dashed line, ▲) percent of simulated allocation to CL seeds. Soil moisture is indicated by solid symbols and ammonia supply is indicated by open symbols. P-values indicate significance of the regression of e1/e2 on $S_{100\%CL}/S_{100\%CH}$. The significance of this relationship tests whether it is reasonable to interpret similarity to maternal conditions as similarity to good environmental conditions. Gray symbols indicate values for Population A_2, which were not included in regressions.

DISCUSSION

Spatial environmental variation has often been proposed to influence gene flow evolution (e.g. Spieth 1979, Gillespie 1981, Hedrick 1986, Venable and Brown 1988), but has rarely been examined in natural systems (but see Venable and Brown 1993). In this study, I found that the amount of spatial environmental structure in soil resources was correlated with differences in population gene flow distance among populations of *D. spicata*.

In four of the five populations of *D. spicata* that I surveyed, the percent allocation to CL seeds was higher in populations where the environment close to parent locations was more similar to maternal conditions. In populations with very little spatially structured environmental variation, increasing the proportion of short-dispersing (CL) seeds would not be very effective increasing the similarity between offspring and maternal conditions. These patterns are consistent with those predicted of selection by spatially structured environmental variation on allocation to CL seeds, where allocation to CL seeds serves to increase the number of dispersing seeds that encounter locations similar to their mother's.

In one population that I surveyed (A_2), the percent allocation to CL seeds was very different than what I would predict based on the pattern of spatial environmental variation. Soil moisture was strongly spatially structured in Population A_2, which meant that shorter dispersing seeds would effectively increase the similarity between offspring and maternal conditions, but plants in this population produced very few CL flowers (and hence few CL seeds). CL seeds in *D. spicata* are fertilized before, but matured after, CH seeds, and so seasonal truncation of the growing season by early season drought could limit the ability of *D. spicata* in to mature CL seeds. *Danthonia spicata* commonly occurs in dry environments, but grows and matures seed during the spring, when moisture

is available (a second growth period occurs in late fall). Population A_2 was the most chronically dry population studied and plants in this population were often unable to complete seed maturation in the spring, before the soil became too dry and caused the plants to senesce (McCormick, personal observation). In the long term, energy allocated to the development of CL seeds would be wasted and thus be disadvantageous. This may explain the limited CL production by plants in Population A_2. Although the other four populations I studied were all quite dry, they were not subject to the early season droughts that occurred predictably in Population A_2. In these four populations, percent of CL seeds did not correlate with soil moisture.

In three of the study populations (L_1, L_2, R) some *D. spicata* plants were infected by the epiphytic fungus *Atkinsonella hypoxylon*. Because *A. hypoxylon* causes abortion of CH seeds (Clay 1984), infected plants do not have the potential to alter their proportional allocation to CL seeds (all seeds produced are CL). However, while this fungal infection could retard a population's ability to respond to selection for altered dispersal distance, it does not appear to act as a direct selective force on allocation to CL seeds in these populations. Plants with different patterns of allocation to CL seeds do not differ in the number of seeds produced when infected (McCormick, unpublished data).

Quantifying spatial environmental variation at scales that are perceived by plants and dispersing seeds is notoriously difficult. The correlation between measured and perceived variation is generally unknown and even measured variation cannot be measured at all points in a population. McCormick and Gross (Chapter 2) found that differences in soil moisture and nitrogen supply rate of the magnitude measured in these five field populations can affect the performance of *D. spicata* plants in the field. These factors are also likely to be major factors limiting growth in natural populations of *D. spicata*, so it is reasonable to interpret measured differences in soil moisture and nitrogen supply rate as impacting plant growth and fitness.

I used semivariance analysis to obtain estimates of the similarity of pairs of points based on their distance apart from relatively few sample points in each population. Although interpreting the similarity of offspring to maternal conditions as an estimate of the quality of environments encountered by dispersing offspring is unconventional, it may be reasonable in environments that are largely unsuitable for plant growth (Levin et al. 1984). This interpretation of predicted similarity between offspring and maternal conditions was consistent with independent estimates of relative environmental quality that would allow the observed patterns of allocation to two differently dispersing seed types to be an evolutionarily stable strategy for *D. spicata* (Schoen and Lloyd 1984). Other studies of local adaptation have also found that offspring performance decreases with increasing distance from the maternal location (e.g. Schmitt and Gamble 1990, Miller and Fowler 1995).

Spatial environmental heterogeneity may have particularly strong selective effects on species like *D. spicata*, which produces heteromorphic seeds with different dispersal potentials. Species with heteromorphic seeds may be especially powerful tools for detecting the effects of spatially structured environmental variation on dispersal distance (Venable 1984). Schoen and Lloyd (1984) argued that spatially structured environmental variation can be an evolutionary force favoring differently-dispersing dimorphic seeds.

However, many plants have genetic variation for seed dispersal distance that is not related to seed heteromorphism. Venable and Brown (1988) suggested that spatially structured environmental variation may select more generally on dispersal distance polymorphisms, of which dimorphic seeds are only one example. In plants with a continuous dispersal distribution, selection for increased dispersal distance by density-dependent effects, such as competition with siblings and parents, may be stronger than in a bimodal disperser. Thus continuous dispersers may have longer mean dispersal distances than bimodal dispersers in similar environments. However, in environments where decreasing dispersal distance could substantially increase the probability of

offspring encountering good environments (such as Populations D and R studied here), I would predict that spatial environmental variation would also select for decreased dispersal distance in species with unimodal dispersal, forming a balance with the density dependent forces selecting for increased dispersal distance.

In this study, I found that the pattern of allocation to short-dispersing CL seeds among populations of *D. spicata* was consistent with predictions based on the degree to which variation in soil moisture and ammonia in those populations was spatially autocorrelated. Specifically, allocation to CL seeds was higher in populations where short dispersal would be most effective at reducing spatial environmental variation encountered by dispersing seeds. Because gene flow distance in *D. spicata* is largely determined by seed dispersal, these measured differences in seed dispersal distance also reflect differences in gene flow distance. Even though the pattern of variation in soil moisture and ammonia within these sites was relatively consistent over years, they were still seasonally variable. Although temporal environmental variation may have strong effects on the evolution of many traits (e.g. Sultan and Bazzaz 1993, Stratton and Bennington 1998), these results suggest that a trait that explicitly affects the spatial distribution of plants or their offspring (e.g. seed dispersal) might be responsive to spatially structured environmental conditions, despite substantial temporal variation. If traits like gene flow distance can respond evolutionarily to spatial environmental heterogeneity despite temporal variability, then the effect of spatial environmental variation in maintaining genetic variation and structuring interactions within and among populations may be greater than previously thought.

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Chapter 4

Plasticity for nitrogen availability in *Danthonia spicata*: phenotypic plasticity in response to spatial environmental heterogeneity?

INTRODUCTION

Spatial environmental heterogeneity is well known to affect plants ecologically (e.g. Grime 1994, Fitter 1994, Chapter 5), but it has received less attention for its ability to affect them evolutionarily. Phenotypic plasticity has been primarily considered an evolutionary response to temporal variation (e.g. Sultan 1987 and refs. therein), while local adaptation has been considered the predominant response to spatial variation (e.g. Bell, Lechowicz and Schoen 1991, Bell and Lechowicz 1994). In addition to being the only mechanism by which sessile organisms, such as plants, can respond to temporal environmental heterogeneity that occurs within generations (Sultan 1987, Schlichting and Levin 1990, Schlichting 1993), adaptive phenotypic plasticity, or appropriate phenotypic changes in specific traits in response to particular environmental conditions (Sultan 1987), may also be important means by which plants can respond to spatial environmental variation encountered among generations. However, few studies have looked for an explicit relationship between amount of spatial environmental variation and amount of phenotypic plasticity (but see Miller and Fowler 1994).

Prevailing wisdom suggests that temporal variation in environmental conditions often greatly exceeds spatial variation and thus should dominate selection on phenotypic plasticity (e.g. Scheiner and Teeri 1986, Sultan and Bazzaz 1993). However, if the range of conditions experienced within a generation differs consistently among locations, then that range of conditions will select on a trait's norm of reaction (Galloway 1995). For example, one location might be consistently drier than another, but wet conditions at a dry location could overlap dry conditions at a wet location. Plants in dry locations would experience conditions that were much drier than those in wet locations. Plants in both

locations would need to be able to withstand temporal fluctuation in moisture levels, but plants in dry locations would experience greater selection for ability to withstand long-term dry conditions than plants in wet locations.

Local adaptation to spatial environmental variation (e.g. Bell et al. 1991, Schmitt and Gamble 1990) and differences in phenotypic plasticity (e.g. MacDonald and Chinnappa 1989, Schlichting and Levin 1990, Scheiner 1993) have repeatedly been demonstrated, but the two have rarely been explicitly examined together (but see Miller and Fowler 1994). I used a greenhouse experiment to test for differences in phenotypically plastic responses to nitrogen availability among *Danthonia spicata* seedlings from near- and far-dispersing families from five populations in Michigan. Because the five sites had different mean nitrogen availabilities (Chapter 2), populations from different sites might have different mean nitrogen acquisition traits. However, because differences in population mean dispersal distance can equalize the amount of spatial environmental variation encountered, plants from different populations would not necessarily encounter different amounts of spatial nitrogen variation (Chapter 3).

Within these populations, offspring from far-dispersing families encounter more spatial variation in nitrogen availability (Chapter 3). Thus, I predicted that offspring from far-dispersing families would have greater phenotypic plasticity than those from near-dispersing families in response to varied nitrogen supply in all five source populations.

I measured plasticity in three different modes of response to nitrogen levels, changes in growth rate (biomass accumulation), shifts in allocation (root:shoot), and physiological changes in nitrogen uptake (nitrogen concentration). In the low nutrient, nitrogen limited, habitats where *D. spicata* grows, nitrogen acquisition is likely to be an important determinant of plant fitness. If differences in spatial nitrogen variation selected for differences in amount of phenotypic plasticity, then the plasticity of these three traits should differ among near- and far-dispersing families.

MATERIALS AND METHODS

Study System

Danthonia spicata (Poaceae) is a native, perennial, C_3 bunch grass that commonly occurs in dry, nutrient-poor oak savannas, old-fields, and openings throughout the northern and eastern United States and southern Canada (Clay 1982). *Danthonia spicata* produces two seed types with different dispersal potential. Obligately self-fertilized cleistogamous (CL) seeds remain within leaf sheaths and are dispersed near the parent plant (avg. 10 cm; Chapter 3). Potentially outcrossed, wind-pollinated chasmogamous (CH) flowers are produced at the tip of the reproductive stalk and disperse farther from the maternal parent (avg. 60 cm; Chapter 3). Chasmogamous seeds may also have secondary dispersal that further increases their average dispersal distance (Clay 1983a). Although these seed types differ in potential for outcrossing, they differ little in genetic variation and inbreeding depression is not apparent (Clay 1983b, Clay and Antonovics 1985).

The proportion of seeds that are allocated to cleistogamy in *D. spicata* is variable both within and among populations. Clay (1982) found that there is a strong genetic basis for the proportion of cleistogamous seeds (heritability 52.6 %), producing genetic variation for dispersal distance. In habitats where there is substantial temporally consistent spatially structured environmental variation, families that have different proportions of short-dispersing CL seeds differ in how much spatial variation is encountered by dispersing offspring. Families that disperse all of their seeds far from the maternal plant (high CH) would encounter more spatial environmental variation than families that disperse many of their seeds close to the maternal plant (high CL; Chapter 3).

The five sites I selected for these studies had *D. spicata* populations that differed in their average proportion of short-dispersing, CL seeds (Figure 4.1; Chapter 3). Within the sites where these populations occurred there was spatially structured variation in

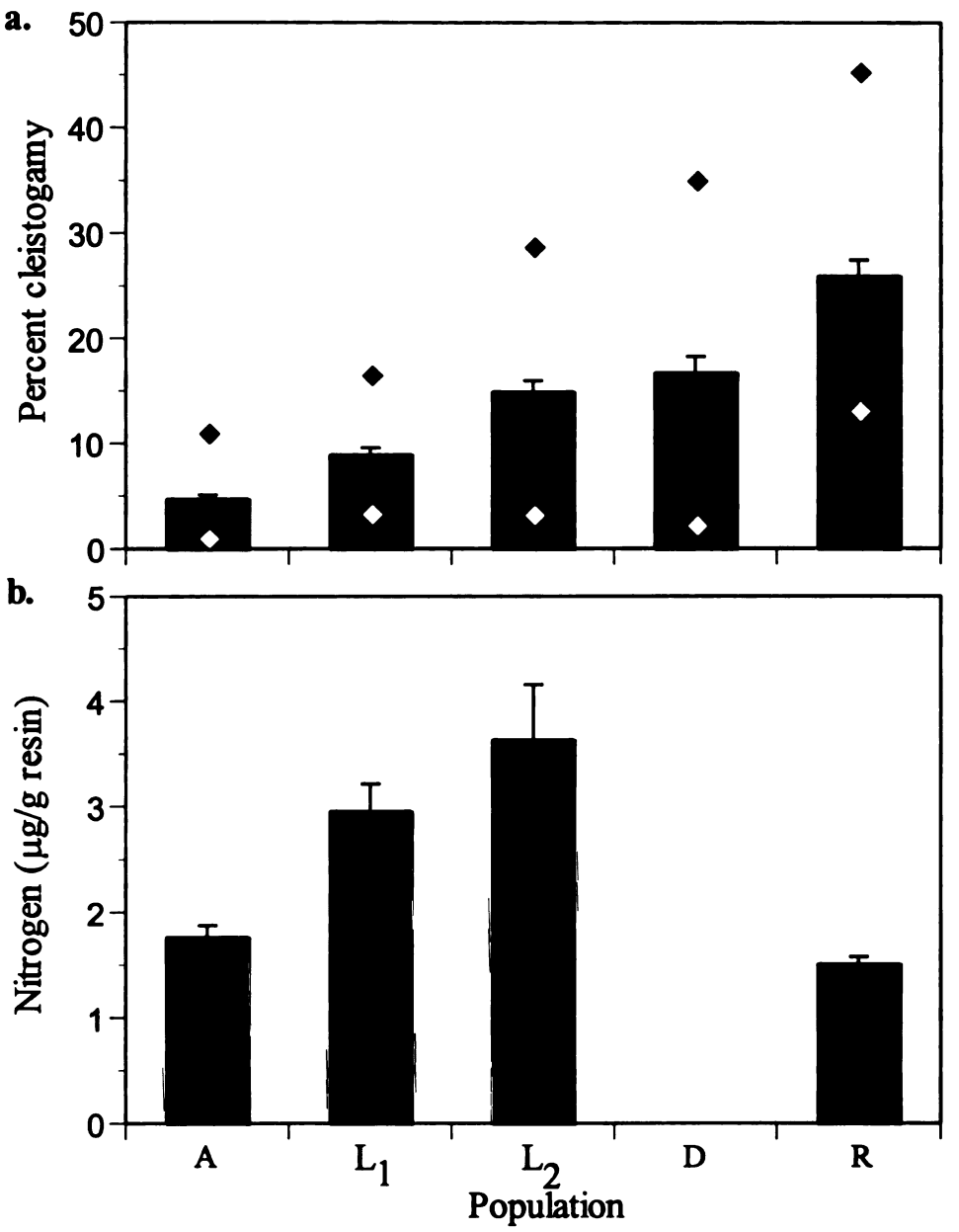

Figure 4.1: Characteristics of five study sites and populations
a. Average percent of cleistogamous seeds (± 1 s.e.) produced in each population (A_2, L_1, L_2, D, R) and **b.** average nitrogen supply (nitrate + ammonia nitrogen; ± 1 s.e.). Diamonds (◆) indicate highest and lowest allocation to cleistogamous seeds measured in each population. Soil nitrogen was not measured in Population D, because soils were too shallow. N = 32 plants for %CL; N = 58 to 68 for nitrogen (see Chapter 2).

nitrate and ammonia supply rates (Chapter 2), producing a correlation between parent and offspring conditions that depended on dispersal distance (Chapter 3). *Danthonia spicata* plants have a median lifespan of 2 to 2.5 years (Scheiner 1987) and spatial environmental variation at this time scale was temporally consistent in these sites (Chapter 2). Allocation to CL seeds among these populations differed in a way that suggested population average seed dispersal distance was adapted to the scale of variation in soil moisture and ammonia supply rates (Chapter 3). Study sites differed in both the mean availability of nitrogen and the degree to which it was spatially variable (Chapter 2). Differences in population average dispersal distance somewhat equalized the amount of nitrogen variation encountered by dispersing seeds among populations (Chapter 3), but variation in dispersal distance among families within populations produced differences in the amount of spatial variation in nitrogen their seeds would encounter. Dispersal distance differences among populations that are concordant with selection by the distribution of spatial environmental variation suggest that differences in spatial environmental variation can select on
population characteristics. I hypothesized that spatial variation in nitrogen supply rates could also select on the amount of phenotypic plasticity in these populations.

Greenhouse experiment

In July 1997, I collected CH seeds from four maternal parents that differed in their percent allocation to short dispersing CL seeds, two with many CL seeds (near-dispersing families) and two with few (far-dispersing families), in each of these five populations (Figure 4.2). The seeds were stored at room temperature for five months and then treated with 70% sulfuric acid for 35 minutes, rinsed and placed on moist, sterile sand in petri dishes under ambient light in the greenhouse. Seeds began to germinate after seven days. Two families from Population A_2 and one from Population R had no seed germination (Figure 4.2). To keep sample sizes among dispersal types as balanced as possible, I

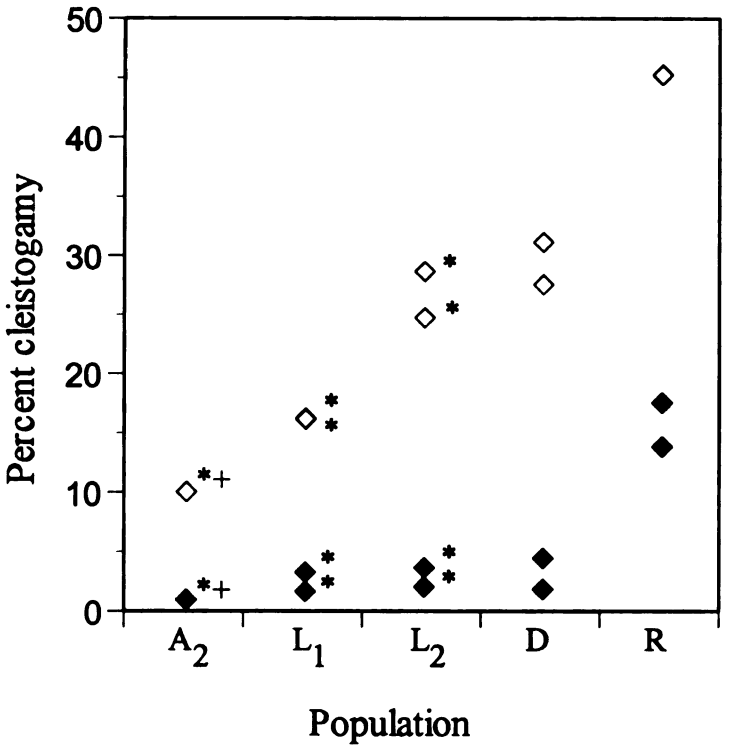

Figure 4.2: Percent cleistogamy of *Danthonia spicata* families
Percent allocation to short dispersing (cleistogamous) seeds by families used as high (◇) and low (◆) CL families from each population. Asterisks (*) designate families that also received medium (80 ppm) nitrogen fertilizer. Plus signs (+) designate families with additional seeds used to replace families with no germination.

increased the number of seedlings in the experiment from the surviving family in the dispersal type. After one month of growth, I transplanted eight to ten seedlings from each family (14 - 18 from increased families) into 6 x 6 x 20 cm paper pots filled with silica sand. Pots were placed in 6 baskets of 36 pots each. Each seedling was randomly assigned a basket and pot location within the basket, with two seedlings from each family in each of four or five of the six baskets. I fertilized all seedlings with a moderately high nitrogen (90 ppm N) fertilizer solution for two weeks after transplanting to encourage establishment.

After the two week establishment period, I washed water through each pot to rinse remaining fertilizer out of the sand and established the nitrogen level treatments. Within each block, I randomly chose one of each pair of seedlings per family to receive high (150 ppm N) or low (30 ppm N) nitrogen fertilizer (modified Hoagland's solution; Appendix A.1). All seedlings received the same background levels of other nutrients. Fertilizer was applied to each individual seedling at a rate of 10 ml per day, three times per week. I chose these nitrogen levels to approximate the minimum and maximum of nitrogen supply rates measured in these sites (Chapter 2).

Because of uncertainty of the shape of the reaction norms of response to nitrogen level, I established an additional treatment with a total of 20 seedlings from three populations (A_2, L_1, L_2) that were fertilized with an intermediate level of nitrogen (80 ppm N). This third treatment allowed me to determine whether the reaction norms of these nitrogen access traits (biomass accumulation, root:shoot ratio, and shoot percent nitrogen) to increasing nitrogen were non-linear, and whether my high nitrogen treatment was too high relative to other nutrients in the fertilizer solution. All seedlings were grown for four months at 18 to 30°C with 12 hours of supplemental light (762 mmol m^{-2} s^{-1} PAR).

At the end of the experiment, all plants were harvested, and I measured total biomass (indicative of growth rate), root:shoot ratio, and shoot nitrogen concentration of

each plant. I harvested plants by peeling away the paper pot and washing sand from the roots with water. I separated the root and shoot tissue, dried them for 72 hours at 38°C, and weighed them. A subset of the dried green shoot tissue from each plant was then clipped very finely and analyzed for tissue nitrogen concentration (g N g plant tissue^{-1}) using an elemental analyzer (Nitrogen Analyser 1500 Series 2, 1990, Carlo-Erba Instruments, Milan, Italy).

I examined differences in phenotypic plasticity (biomass, root:shoot ratio, shoot nitrogen concentration) in response to nitrogen treatment (high vs. low) among populations and differently dispersing families using an ANOVA for each trait (Systat 8.0 for Windows, 1997. SPSS, Chicago Il.). Biomass and shoot nitrogen concentration were log-transformed to improve normality. There was a significant negative correlation between log biomass and log shoot nitrogen concentration (-0.198; $p < 0.028$), but all other pairs of traits were not correlated. Nitrogen treatment, Population, and Dispersal type (near or far) were tested as main effects. The presence of plasticity for each trait was tested by the significance of the nitrogen treatment main effect. My hypothesis specifically predicted that there would be differences among populations and differently dispersing families in the degree of plasticity in response to nitrogen treatment. I tested this hypothesis by examining the significance of the Population x Nitrogen treatment and Dispersal type x Nitrogen treatment interactions, respectively. Both biomass and nitrogen concentration were log-transformed to improve normality for these tests.

Plants in three of the baskets became infected with an unknown fungus, differed significantly in all traits measured from those that were not infected. The unidentified fungus invaded over 90% of the pots in these three baskets, but less than 5% of pots in the other three baskets. Because there were no significant differences in plant traits among baskets within the infected or uninfected baskets, I grouped the six baskets into two blocks, defined by presence or absence of fungus. To account for effects of the

fungal infestation in my analyses, I tested for Block main effects and interactions in the ANOVA for each trait.

I visually examined the linearity of plastic responses to three levels of nitrogen treatment in the three populations that received all three nitrogen treatments. Substantial non-linearity of reaction norms would necessitate caution in interpreting differences in responses among populations in plasticity to nitrogen level. These three populations (A_2, L_1, L_2) and differently dispersing families were not significantly different in any of the traits examines and were combined for this analysis.

RESULTS

Plants from different populations had similar mean biomass and shoot nitrogen concentration, but significantly different root:shoot ratios (Table 4.1). Biomass and shoot nitrogen concentration were phenotypically plastic in response to nitrogen availability, but root:shoot ratio was not (Table 4.1). Plants from all five populations had similar amounts of plasticity in the three traits I examined. Among-population differences in mean root:shoot ratio and a lack of plasticity in this trait suggest that populations differed genetically in root:shoot ratio, although difference resulting from maternal effects cannot be ruled out. Root:shoot ratio may respond to mean nitrogen availability, which differed among the sites, rather than to its spatial variation.

Not all plants produced sufficient leaf tissue to analyze for shoot nitrogen concentration (3 to 5 mg). Some combinations of Block x Population x Dispersal type had no plants large enough for nitrogen analysis. The Population main effect and interactions were substantially non-significant when analyzed in an ANOVA without Dispersal type ($p \geq 0.391$). Because the test of my hypothesis specifically involved tests of the Dispersal type x Nitrogen interaction and because the Block effect was very substantial, I tested for differences in shoot nitrogen concentration among nitrogen levels,

Table 4.1: ANOVA tables presenting variation in biomass, root:shoot and shoot nitrogen Effects of Population (P), Dispersal class (high or low allocation to CL seeds; DC), Nitrogen treatment (30 or 150 ppm N; N), and Block (B) and their interactions on biomass (**a.**), root:shoot ratio (**b.**), and shoot nitrogen concentration (**c.**) in the greenhouse experiment. Populations used as seed sources were A_2, L_1, L_2, D, and R.

a. Biomass (log transformed)

N = 165 Squared multiple R = 0.646

Source:	DF	Mean Square	F-ratio	P
Population	4	1.275	1.703	0.153
Dispersal class	1	0.630	0.842	0.361
Nitrogen	1	61.871	82.673	< 0.001
Block	1	35.132	46.943	< 0.001
P x DC	4	0.398	0.532	0.712
P x N	4	0.634	0.848	0.498
P x B	4	0.799	1.067	0.376
DC x N	1	0.286	0.383	0.537
DC x B	1	0.459	0.613	0.435
N x B	1	2.837	3.790	0.054
P x DC x N	4	2.407	3.216	0.015
P x DC x B	4	0.327	0.437	0.782
P x N x B	4	0.523	0.698	0.595
DC x N x B	1	0.315	0.421	0.518
P x DC x N x B	4	0.394	0.527	0.716
Error	125	0.748		

Table 4.1 (cont'd)

b. Root : Shoot ratio

N = 163 Squared multiple R = 0.285

Source:	DF	Mean Square	F-ratio	P
Population	4	0.392	2.919	0.024
Dispersal class	1	0.202	1.509	0.222
Nitrogen	1	0.121	0.904	0.344
Block	1	0.741	5.524	0.020
P x DC	4	0.085	0.631	0.641
P x N	4	0.071	0.528	0.716
P x B	4	0.186	1.385	0.243
DC x N	1	0.007	0.049	0.824
DC x B	1	0.044	0.326	0.569
N x B	1	0.014	0.105	0.747
P x DC x N	4	0.058	0.431	0.786
P x DC x B	4	0.088	0.659	0.622
P x N x B	4	0.099	0.741	0.565
DC x N x B	1	0.049	0.367	0.546
P x DC x N x B	4	0.058	0.434	0.784
Error	123	0.134		

c. Shoot nitrogen concentration (log %)

N = 123 Squared multiple R = 0.512

Source:	DF	Mean Square	F-ratio	P
Dispersal class	1	0.016	0.867	0.354
Nitrogen	1	0.074	3.985	0.048
Block	1	1.602	86.172	< 0.001
DC x N	1	0.001	0.065	0.799
DC x B	1	< 0.001	0.017	0.898
N x B	1	0.002	0.083	0.774
DC x N x B	1	0.001	0.066	0.797
Error	115	0.019		

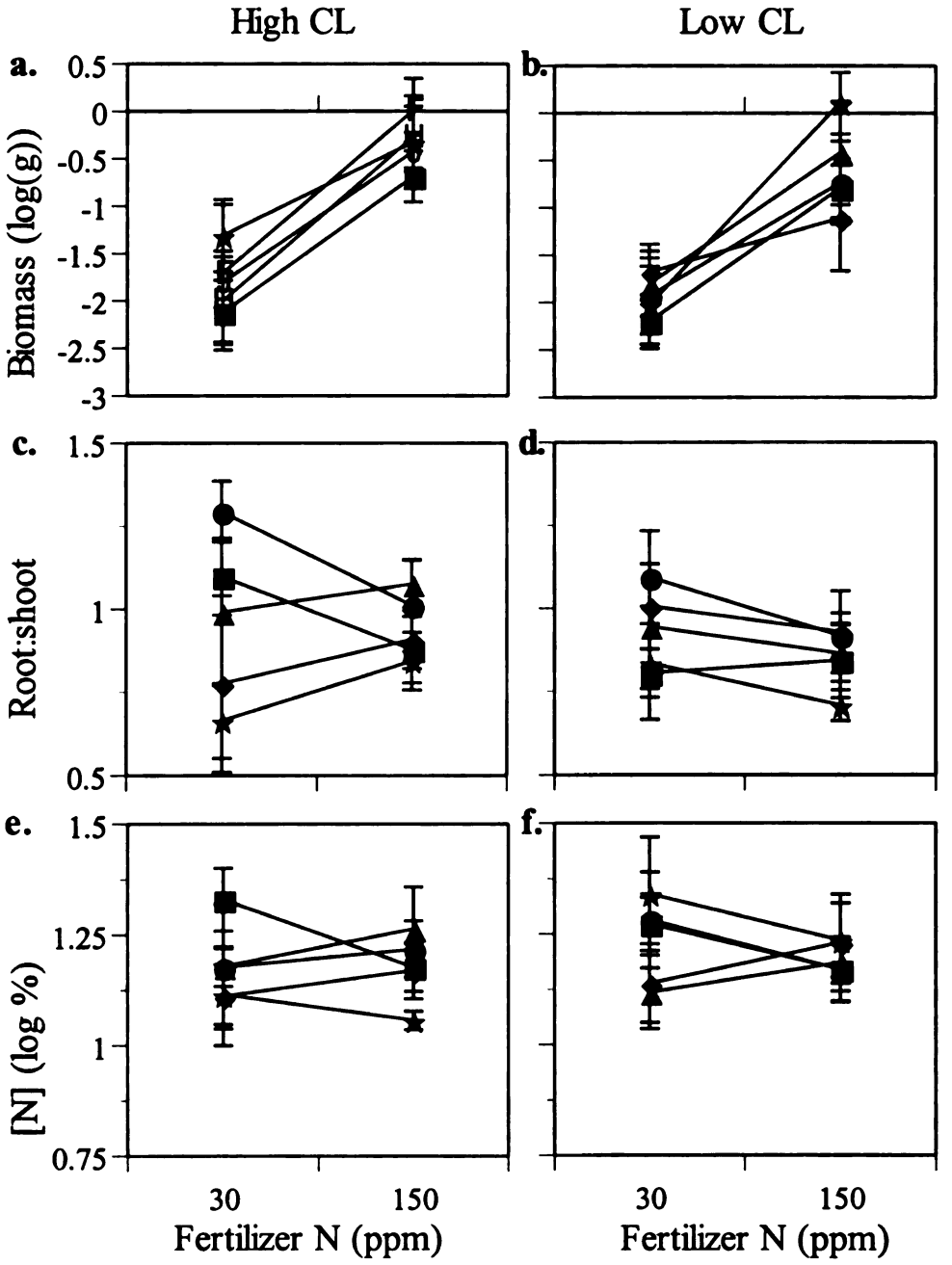

Figure 4.3: Reaction norms across two nitrogen levels
Mean (± 1 s.e.) biomass (**a.**, **d.**), root:shoot ratio (**b.**, **e.**), and shoot nitrogen concentration (**c.**, **f.**) norms of reaction across two nitrogen levels by families with high (**a.-c.**) and low (**d.-f.**) allocation to cleistogamous seeds. Each symbol represents a different population: A_2 =■, L_1 =●, L_2 = ▲, D = ◆, R = ★.

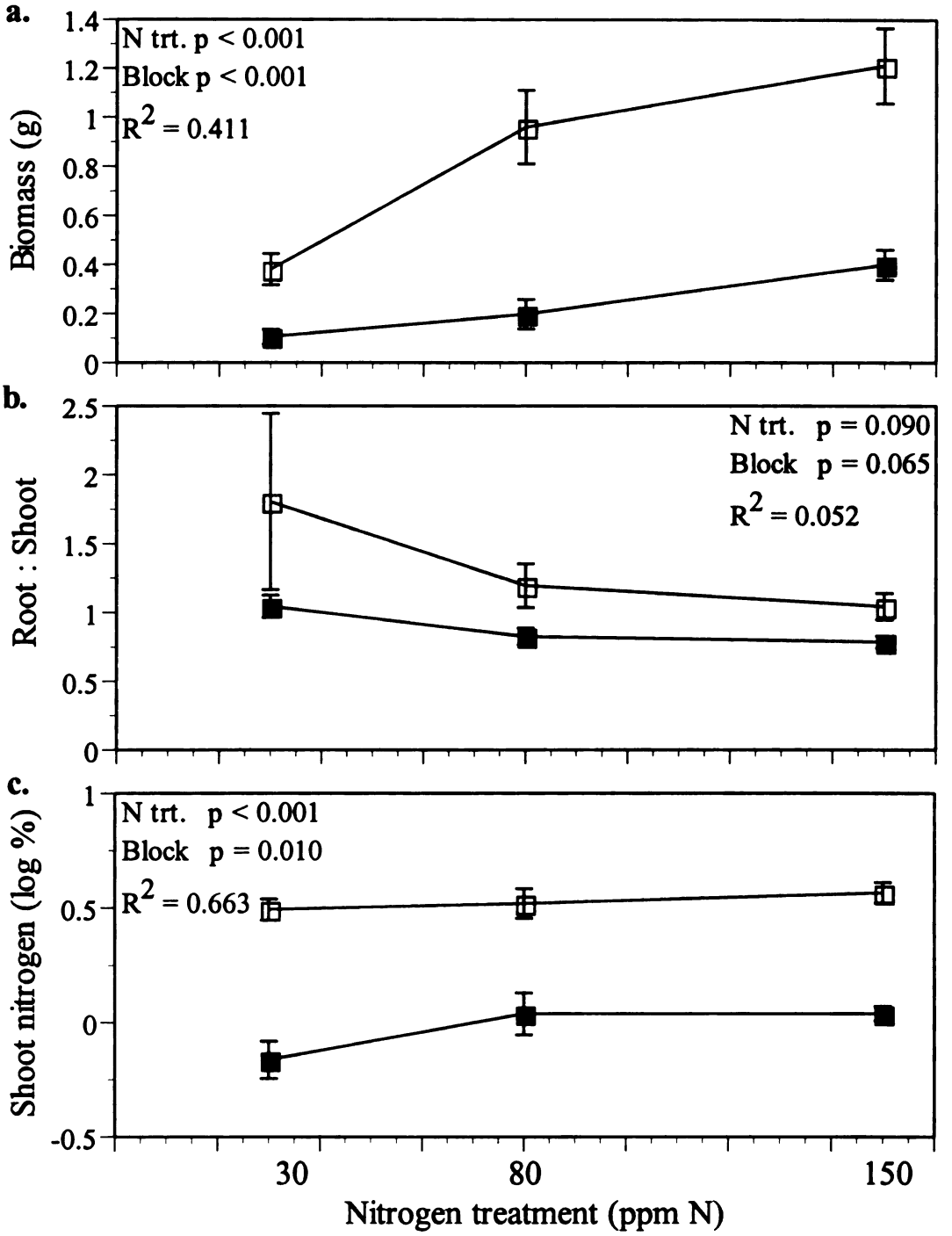

Figure 4.4: Reaction norms of response to nitrogen treatment across three levels Norms of reaction across three nitrogen levels in **a.** biomass, **b.** root:shoot ratio, and **c.** shoot nitrogen concentration in the block group uninfected (■) and infected (□) by an unidentified fungus.

near and far dispersing families, blocks, and the interactions of these effects without Population as a factor.

Within populations, differently dispersing families had similar total biomass, root:shoot ratio, and shoot nitrogen concentration (Figure 4.3). Contrary to my predictions, differently dispersing families also had similar amounts of phenotypic plasticity in the three traits I examined (Figure 4.3, Table 4.1). For biomass, the Nitrogen x Dispersal type x Population interaction was significant ($p < 0.015$), indicating that high and low CL

seedlings had different amounts of phenotypic plasticity in some populations. However, seedlings from low CL families did not have consistently greater plasticity than those from high CL families (Figure 4.3). No other interactions were significant for any of the traits I examined.

Reaction norms for the populations receiving all three nitrogen treatments appeared linear for both the infected and uninfected blocks (Figure 4.4). This suggests that the two nitrogen levels that I used to measure phenotypic plasticity were adequate to describe the reaction norms of biomass, root:shoot ratio, and shoot nitrogen concentration in response to nitrogen levels over the range from 30 to 150 ppm N, which is comparable to the range of nitrogen supply in these field sites.

DISCUSSION

Studies of phenotypic plasticity in plants have rarely attempted to explicitly examine plastic responses to spatially variable environmental factors. In this study, I tested for differences in amount of phenotypic plasticity in response to nitrogen availability among and within five populations of *Danthonia spicata*. These five study sites differed in

mean availability of nitrogen and also in amount of spatially structured variation in nitrogen availability (Chapter 2). Consequently, within each population, differently dispersing families were expected to encounter different amounts of variation in nitrogen availability (Chapter 3), which I expected would select for differences in plasticity of nitrogen acquisition traits. Although population differences in average dispersal distance equalized the average amount of spatial variation encountered by dispersing seeds among populations, I expected that plants from sites with higher nitrogen levels would respond differently to variation in nitrogen levels than plants from sites with low nitrogen availability.

Despite differences in nitrogen availability among field sites, I found that *D. spicata* plants from different populations responded similarly to greenhouse nitrogen treatments. Plants from different populations had similar mean biomass and shoot nitrogen concentration and also had similar amounts of plasticity in these traits. The similarity in plasticity among populations with very different mean nitrogen availabilities suggests that biomass and shoot nitrogen concentration in *D. spicata* may respond to factors other than spatial nitrogen variation or that other factors, such as temporal variation, may act to maintain uniformly high levels of plasticity in these traits. Plants from these five populations had significantly different root:shoot ratios, but did not exhibit plasticity for this trait.

Within each population, I predicted that plants from far-dispersing families would be more phenotypically plastic than plants from near-dispersing families. Differently dispersing families had similar mean biomass across nitrogen treatments, but some populations had different amounts of biomass plasticity. However, far-dispersing families were not consistently more plastic than near-dispersing families. Plants from differently dispersing families had similar shoot nitrogen concentrations, root:shoot ratios, and plasticity for both of these traits.

Substantial block effects in this experiment were most likely due to invasion of many pots in one block by an unidentified fungus. Plants in uninfected pots responded substantially differently to nitrogen treatments than those in pots infected by fungus. However, block did not interact significantly with any of the design factors.

One possible explanation for the similarity of plasticity in two of the three nitrogen acquisition traits I examined is a very small or negligible cost of maintaining plasticity for nitrogen acquisition. Sultan and Bazzaz (1993) proposed that because the mechanisms contributing to phenotypic plasticity in response to nutrient availability may allow individual genotypes to perform well in a wide range of soil conditions, there may never be sufficient costs of maintenance to outweigh the benefits of plasticity. Grime (1994) suggested that nutrient acquisition in low fertility habitats consistently require an ability to access nutrients available in short-lived pulses. If this is true, then plants that typically occur in low fertility habitats, such as *D. spicata*, may be especially subject to temporal variation in soil nutrients, which may select for high levels of phenotypic plasticity. If there were a consistent benefit of plasticity for nutrient access, then populations would be fixed for uniformly high levels of plasticity. The generality of plasticity costs and constraints is currently a subject of substantial research, but the likelihood of very limited costs in nutrient access traits is still unknown (Dewitt 1998).

Selection by temporal variation in environmental conditions has often been proposed to dominate selection for phenotypic plasticity, equalizing phenotypic plasticity among even extremely different, genetically isolated populations (e.g. Sultan and Bazzaz 1993, and references therein). Scheiner and Goodnight (1984) found high, but invariable, amounts of phenotypic plasticity in multiple biomass allocation traits in response to soil moisture and light availability in five *D. spicata* populations of different successional age in northern Michigan. Scheiner and Teeri (1986) proposed that selection for plasticity in *D. spicata* was dominated by short-term temporal, rather than spatial, variation in moisture and light conditions.

However, in these five field sites in Michigan, the spatial patterns of nitrate and ammonia availability were relatively consistent within the lifetime of *D. spicata* plants, despite substantial temporal variation within years in all sites (Chapter 2). Hence in these sites, selection by nitrogen conditions should be dominated by spatial variation.

Despite different amounts of spatial heterogeneity in nitrogen supply (Chapter 2) and possible locally adaptive responses in dispersal distance (Chapter 3), I found little evidence for differences in phenotypically plastic responses to nitrogen supply rate among differently dispersing families from these five populations of *D. spicata* in Michigan. Similarity among populations of nitrogen acquisition plasticity could not be fully explained by the comparative strengths of temporal and spatial variability within the average lifespan of *D. spicata*, but might be explained by limited costs or consistent benefits of plasticity for nutrient access among and within these five populations.

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Chapter 5

DANTHONIA SPICATA AND *ATKINSONELLA HYPOXYLON*: ENVIRONMENTAL DEPENDENCE OF A SYMBIOSIS

(Manuscript co-authored with K.L. Gross and R.A. Smith)

INTRODUCTION

Much attention has been focused recently on the role of fungal symbiotes in structuring interactions within and among plant populations and communities (Clay 1990b, Dobson and Crawley 1997, Thrall and Burdon 1997). Many plant species, particularly grasses, commonly form symbiotic associations with epiphytic or endophytic fungi that have the potential to strongly alter the host plant's morphology, physiology, and response to environmental conditions (e.g., Bacon et al. 1986, Read and Camp 1986, Belesky et al. 1987, Carroll 1988, Marks and Clay 1990). These fungi can be beneficial to their host plant in some environments, but may be parasitic in others (Clay 1990a).

Plants that are infected by otherwise advantageous fungi often show growth disadvantages when available nutrients are very low, because nutrient requirements of active fungal biomass and toxin production may outstrip nutrient availability (Bacon 1993). Soil fertility-dependent growth advantages of fungal infection have been demonstrated for endophyte-infected *Lolium perenne* and *Festuca arundinacea* (Cheplick et al. 1989, Marks and Clay 1990, Cheplick 1997). In both of these species, the growth advantage of infected plants in the greenhouse was either small or negative at the lowest fertility levels and increased with fertility.

Most plant species that have epiphytic or endophytic fungal associations have both infected and uninfected individuals mixed within and among populations (Bradshaw 1959, Clay 1990a). In mixed populations, infected and uninfected plants are often patchily distributed, with some areas predominantly infected and other areas predominantly uninfected. Soil resources can also vary spatially within and among environments at scales from plant populations to communities (Robertson and Gross 1994, Gross et al. 1995) and differential growth of infected and uninfected plants under these heterogeneous conditions could produce patches of infected and uninfected plants.

If infected and uninfected plants respond differently to soil conditions that are patchily distributed, then patches of infection would correspond to patches of environmental conditions that are favorable to infected plants. To determine whether patchy distributions of soil nitrogen and percent water were related to observed spatial variation in a plant-fungal symbiosis, we examined the correlation between *Atkinsonella hypoxylon* infection and soil environmental factors (relative soil ammonia supply and percent water) in three populations of *Danthonia spicata* in southern Michigan. We then conducted a greenhouse experiment to test whether growth and survival of *D. spicata* plants infected by *A. hypoxylon* differed from that of uninfected plants under high and low moisture levels and high and low fertility conditions. A common garden planting of infected and uninfected culms was used to determine how the growth and survival of infected and uninfected plants differed in the field.

MATERIALS AND METHODS

Study Species:

Danthonia spicata (Poaceae) is a native perennial C_3 bunch grass that commonly occurs in dry, nutrient-poor oak savannas, old-fields, and openings throughout the eastern and northern United States and southern Canada (Clay 1982). Throughout much of its range, *D. spicata* is infected by the epiphytic fungus *Atkinsonella hypoxylon* (Balansiae), which is specific to the genus *Danthonia* (Clay 1994). Uninfected *D. spicata* produce two flower types. Potentially outcrossed, wind-pollinated chasmogamous flowers are produced at the tip of the reproductive stalk. Obligately self-fertilized cleistogamous flowers are produced in the axils of the leaf sheaths along the reproductive stalk. In infected plants, a fungal sclerotium, or 'choke', is produced at the initiation of host plant flowering and causes abortion of all but a few infected cleistogamous seeds at the base of the much-reduced reproductive stalk. Because plants infected by *A. hypoxylon* often have higher growth rates than uninfected plants, this symbiosis is often considered mutualistic (Diehl 1950, Clay 1984, Clay 1990a). The proportion of plants infected by *A. hypoxylon* varies among populations in Michigan (McCormick, unpublished data). Some populations contain a high proportion of plants infected by *A. hypoxylon*, but others are entirely free of infection (Scheiner 1989, McCormick, unpublished data).

Study Sites

The three populations of *D. spicata* and *A. hypoxylon* we examined in this study were growing in low productivity old-fields in central and southern Michigan that had been abandoned at least 45 years ago. Populations L_1 and L_2 were located near the W.K.

Kellogg Biological Station in Hickory Corners, MI. Population R was located in the Rose Lake Wildlife Research Area near East Lansing, MI. These three sites were all dominated by herbaceous perennials, simplifying the identification of limiting resources. They also had similar proportions of infected plants. Density (number of *D. spicata* plants m^{-2}) was measured along two randomly located belt transects (12 x 0.25 m) in each population.

Field Patterns

In each population, we established a 4 by 6 m permanent plot where we quantified soil moisture and nitrogen supply. Seventy soil samples were taken from each plot, using a 10 cm deep, 2.5 cm diameter soil core, in a stratified, nested sampling design. At each location, where a soil core was removed, an ion exchange resin bag (Dowex MR-3, Sigma Corp.) was inserted and buried under 5 cm of soil to measure the relative supply of ammonia-nitrogen in the soil. Soil moisture and relative supply of ammonia were chosen to characterize environmental quality because these resources often limit plant growth in the dry, nutrient-poor environments where *D. spicata* occurs. We also sampled nitrate-nitrogen in these samples, but found that the availability of nitrogen in these sites was dominated by ammonia and nitrate supply was rarely consistent among years (Chapter 2). Because relative nitrate-nitrogen supply was not spatially consistent, we felt that it could not be used as a reliable determinant of micro-habitat conditions to be related to infection distribution.

We measured gravimetric soil moisture in all three populations (L_1 and L_2) in mid-May 1998, corresponding to the time of maximum growth by *D. spicata*. The relative ammonia supply was estimated in all three populations for four months after sampling for infection (June 1997-October 1997). Soil moisture and ammonia supply were also measured at other times using identical methods (see Chapter 2), but in reference to the distribution of infected and uninfected plants measurements from other times were used only to assess temporal consistency in infected and uninfected quadrats. We chose to use 1998 measures of soil moisture and 1997 measures of ammonia supply because they were taken at all sites, thus allowing comparison across sites, and were the closest in time to when we measured the distribution of infected and uninfected plants.

To determine whether the pattern of infection incidence in each population was correlated with these two environmental factors, we surveyed the incidence of *A. hypoxylon* infection in each population after the plants had bolted in June 1997. Infected plants are easily distinguished from uninfected plants at this stage by the presence of a gray fungal sclerotium ('choke') on aborted reproductive stalks. We randomly selected 75 quadrats (25 x 25 cm) from a 16 x 24 quadrat grid within each of our three permanent plots to survey for incidence of infection. This small quadrat size was chosen to allow assessment of plant density without integrating across substantial variation in spatially heterogeneous environmental conditions. In each quadrat we noted the number and location of infected and uninfected *D. spicata* plants. We then used semivariance analysis and kriging interpolation (GS^+ v.3.11.6. 1999. Gamma Design Software, Plainwell, MI) to estimate percent water and ammonia supply for the center of each quadrat that we sampled for infection incidence. Kriging interpolation uses the variance-

distance relationship, summarized in a semivariogram, to assign weights to sample points as a function of their distance from a point for which an estimate is desired. Kriging allowed us to use the spatial structure of variation in each factor (moisture and ammonia) in each population to estimate the levels of soil moisture and ammonia for each quadrat in which we sampled plant infection.

Additional measurements of soil moisture and ammonia supply were taken in each site using the same methodology as described for the 1998 samples above. To assess the temporal consistency of estimated soil moisture and ammonia conditions in the quadrats, we examined the correlation between 1998 and 1996 (L_1, L_2) or 1997 (R) estimates of soil moisture and between 1997 and 1998 estimates of ammonia supply for each quadrat. We assessed the significance of temporal consistency using an ANOVA for each factor, where Population and the earlier soil estimate (1997 for ammonia and 1996 or 1997 for soil moisture) were used as the predictors of later soil estimates (1998 soil moisture and ammonia).

Statistical Analysis

We designated each quadrat as infected if it contained at least one infected plant. We then used logistic regression to analyze the distribution of infected quadrats over the range of soil moisture and ammonia conditions across the three populations (Systat 8.0 for Windows. Systat Inc. 1998. Evanston, IL.). Because of the possible confounding of spatial structure with point of infection introduction or other processes within a population, we can only draw conclusions about the pattern of association between environmental factors and infection incidence across all three populations. A significant

population effect in the regression would indicate that patterns were not the same across the three populations.

Because more favorable environments might promote higher plant densities and plant density could affect contagious spread of the fungus, we needed to address the possibility of indirect environmental effects on the distribution of infection acting through plant density. To evaluate this effect, we estimated plant density across all infected and uninfected quadrats. We used a t-test to compare the average plant density in infected and uninfected quadrats.

Greenhouse Experiment

To determine whether the variation observed in the field in soil fertility and moisture levels could affect the performance, and thus influence the differential distribution of infected and uninfected plants, we conducted a 2 x 2 factorial greenhouse experiment in which watering regime and fertilizer were manipulated. We collected cleistogamous seeds from 21 infected and 28 uninfected individuals selected randomly from two *D. spicata* populations. The two populations from which we collected seeds were Population L_1 from the field patterns survey and a second, unsurveyed, population, which was dominated by woody vegetation and may have experienced very different environmental conditions.

Infected and uninfected seeds were surface sterilized with bleach and ethanol according to the methods of Leuchtmann and Clay (1988) and nicked with a sterile razor blade to stimulate germination. Seeds were then germinated in moist, sterile sand in a growth chamber with 14 hour days at 29°C and 10 hour nights at 24°C. Of the 21

infected and 28 uninfected plants from which we collected seeds, 12 infected and 17 uninfected plants had sufficient germination for experimental replication. After nine days, 109 seedlings from infected and uninfected families were each divided into four treatment groups, assigned at random to positions in 70-hole Conetainers™ (each hole was 2.5 cm diameter by 15 cm deep) filled with sterile silica sand and placed in the greenhouse under ambient light. Each infected family was represented by nine or ten seedlings, two in each treatment with the additional one or two seedlings randomly assigned to treatments. Each uninfected family was represented by six or seven seedlings, one per treatment with the other two or three randomly assigned to treatments. After a four day stabilization period with daily watering to saturation, we established the four treatments (2 x 2 factorial) in which fertility and watering regime were varied. Seedlings from each infected and uninfected family were grown under all combinations of high and low fertilizer and high and low moisture.

The moisture and fertility levels used in the greenhouse experiment were chosen to represent the range of conditions observed in the field, without imposing extremely high mortality. Fertilizer levels consisted of 0.015 g L^{-1} (low fertility) or 0.500 g L^{-1} (high fertility) of Peter's Peat-lite Special Fertilizer (20-10-20) applied at a rate of 4.5 ml per planting location 2-3 times per week. The nitrogen levels in these fertility treatments corresponded to nitrogen mineralization rates of 0.01 and 0.45 mg N g^{-1} dry soil day^{-1}, the approximate range of fertility found in field populations of *D. spicata* (Chapter 2). Plants in high moisture treatments were watered daily, while plants in the low moisture treatments were watered every second or third day when approximately half of the plants showed leaf rolling, symptomatic of water stress. Average greenhouse temperatures were

approximately 30°C from June-September and approximately 24°C from October-April with ambient light. The positions of the 70-hole Conetainers™ on the greenhouse bench were rotated randomly each week to minimize position effects.

Plants were monitored at regular intervals over nine months for size and survival. Plant size was measured as the number of living leaves on each plant. After six months, plants in high fertility treatments were sufficiently large that individuals in adjacent locations began to shade plants each other. Therefore, we transplanted the plants in these treatments into new 70-hole Conetainers™ and placed individuals in staggered locations with one empty location on all sides to prevent shading.

Statistical Analysis

Plant growth data were log transformed to minimize heterogeneous variances produced by substantial growth differences between the high and low fertility treatments. Transformed data were analyzed using a repeated measures ANOVA (Systat 8.0 for Windows. Systat Inc. 1998. Evanston, IL.). We also calculated performance by using the percent survival as a weight on the size of each plant in each treatment (log number of leaves). We compared performance among treatments using a repeated measures ANOVA.

Common garden experiment

To assess differences in growth of infected and uninfected plants under field conditions, we established a common garden adjacent to our permanent plot in Population L_1. We collected nine uninfected and three infected adult *D. spicata* plants

from field Populations L_1 and L_2 in April 1995. The plants were divided into culms and each culm was planted into a 10 x 10 cm pot filled with sterile sand in the greenhouse. Culms that propagated were again divided into individual culms and planted into new 10 x 10 cm pots. The culms were grown without fertilizer until June 1995, when we used them to establish the common garden experiment.

We established the common garden by tilling a 2 x 2 m area near Population L_1. We planted four culms of each genotype (individual parent) into randomly selected locations on a 10 x 10 cm grid established in the central 1 x 1 m of the tilled area. Remaining planting locations in the grid were occupied by *D. spicata* culms from another experiment. We planted additional randomly selected culms around the perimeter of the common garden to avoid edge effects on the study plants. We measured the initial size of planted culms by counting the number of living leaves at the time of planting.

Planted culms were allowed to grow in the common garden for two years. In June 1997 (peak seed production) we harvested above-ground biomass from all surviving culms. From the harvested biomass we measured final plant size by counting the number of tillers, leaves, and inflorescences produced. All clipped material was dried at 45°C for 48 hours and then weighed. We ground random sub-samples of leaf material from each planted culm to determine the tissue nitrogen concentration. We also ground reproductive stalks, with all seeds removed, from all infected culms and from three randomly selected uninfected culms, each from a different parent. For infected reproductive stalks we included the fungal sclerotium, which was intimately associated with the inflorescence, in tissue to be ground so we could assess the overall nitrogen content of the inflorescence. Percent nitrogen content of this tissue was analyzed using

an elemental analyzer (Nitrogen Analyser 1500 Series 2, 1990, Carlo-Erba Instruments, Milan, Italy).

RESULTS

Field patterns

Plant density and relative supply of ammonia were not statistically different among the three sites, but Population R had significantly lower soil moisture than Populations L_1 and L_2. The three sites also had similar percentages of infected plants and soil moisture (Figure 5.1). Over all three sites, plant density in infected quadrats was not significantly different from that in uninfected quadrats (40.8 ± 0.79 plants m^{-2} versus 35.6 ± 3.65 plants m^{-2}, respectively; [mean $\pm$ 1s.e.] $p>0.2$ from t-test). Within each site, 1996 or 1997 soil moisture estimates were significantly predictive of 1998 soil moisture ($p < 0.04$) and 1997 ammonia estimates were significantly predictive of 1998 ammonia supply in two of the three populations (L_1, R, $p < 0.004$; L_2, $p = 0.504$), suggesting that soil conditions where infected or uninfected plants were growing were generally temporally consistent among years.

Across the populations, infected quadrats were drier and more ammonia-rich than quadrats with no infected plants (Figure 5.2). There was a significant interaction between soil moisture and ammonia supply in determining infection prevalence (logistic regression; $p < 0.001$). Because our three populations did not overlap in their mean conditions, they reveal substantial information about the distribution of *A. hypoxylon* across a wide range of conditions, but they can not be treated as replicates. Populations L_1 and L_2 had substantial overlap in their soil moisture and ammonia conditions and both

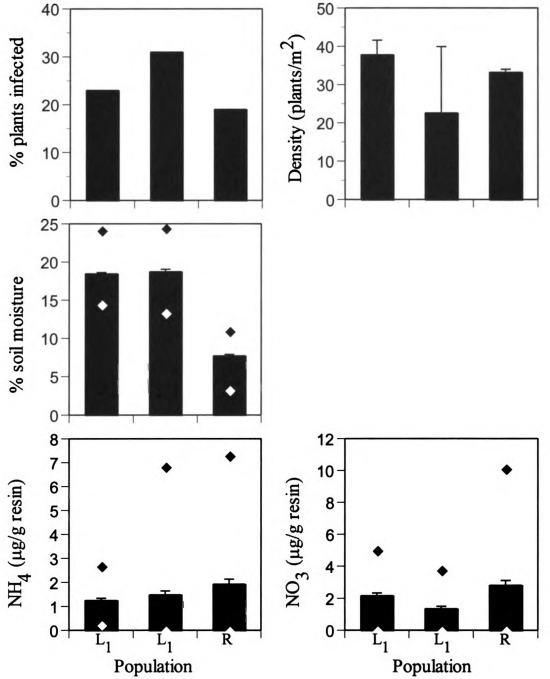
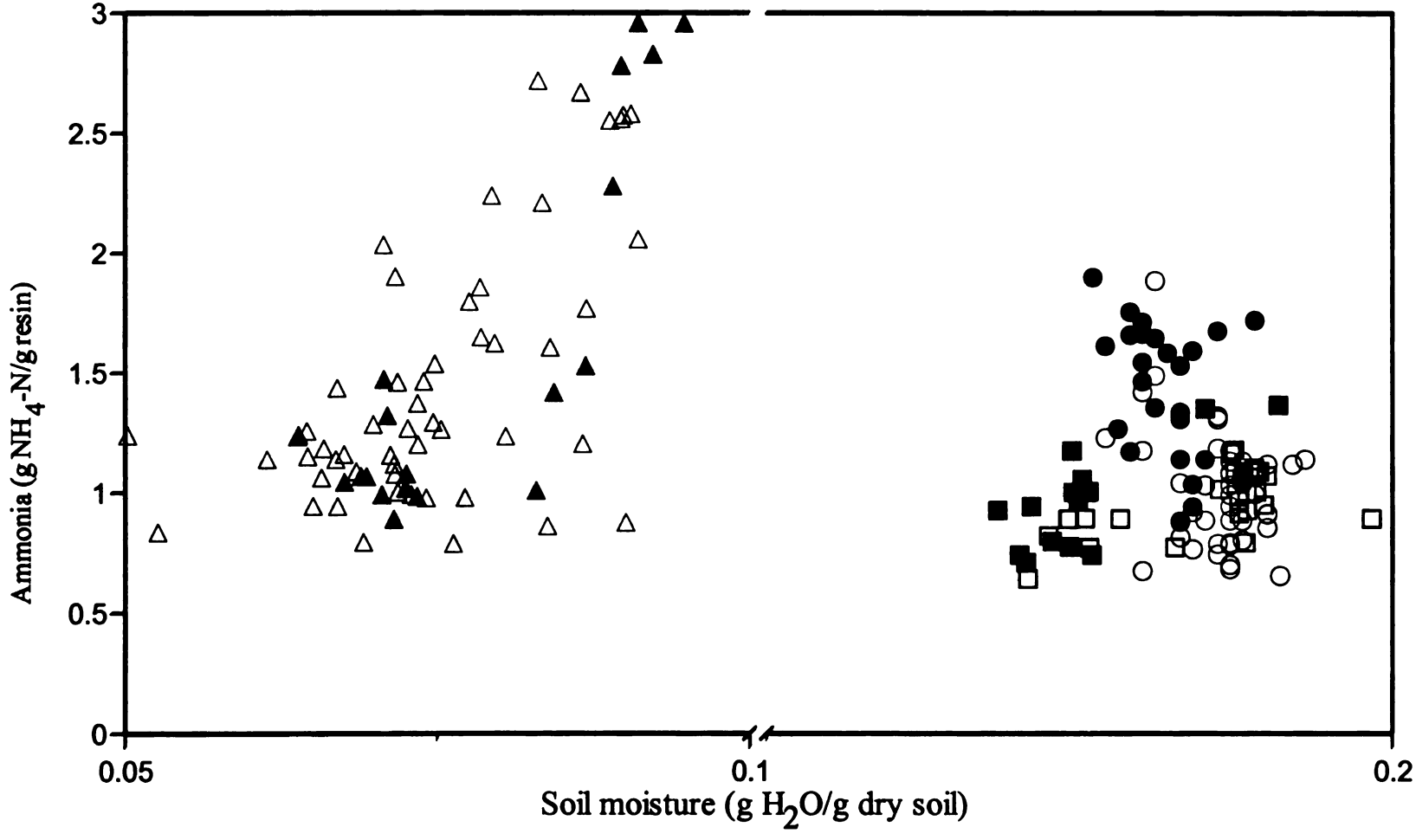


Figure 5.1: Characteristics of three study populations
Percent of plants infected, mean plant density, 1998 soil moisture, 1997 ammonia-nitrogen, and 1997 nitrate-nitrogen (+ 1 s.e.). N = 2 for density measures, and ranges from 58 to 68 for soil moisture and nitrogen measures. Diamonds (◆) indicate minimum and maximum soil moisture and nitrogen measures.

Figure 5.2: Field distribution of infected and uninfected *Danthonia spicata* plants
The distribution of *Danthonia spicata* plants infected (solid symbols) and uninfected (open symbols) by *Atkinsonella hypoxylon* relative to soil moisture and ammonia supply in Population L_1 (●), Population L_2 (■), and Population R (▲). The axis break in the soil moisture axis runs from 0.1 to 0.15 g H_2O / g dry soil.

Figure 5.2: Field distribution of infected and uninfected *Danthonia spicata* plants



had infected plants only in lower moisture, higher ammonia conditions (Figure 5.2). In contrast, Population R had somewhat higher ammonia and substantially lower soil moisture than Populations L_1 or L_2, placing the entire population in conditions suitable for infected plants. Accordingly, infected plants were distributed throughout Population R without regard to soil moisture or ammonia conditions (Figure 5.2).

Greenhouse Experiment

In the greenhouse experiment, the direction and magnitude of growth and survival differences between infected and uninfected plants depended on both the fertility and moisture treatments. All plants grew more slowly at low fertility than at high fertility (Figure 5.3), resulting in a significant Fertility effect ($p < 0.001$). However, no other between subject effect was significant. Within subjects, Time x Fertility and Time x Infection x Fertility effects were significant ($p < 0.001$), indicating that infected and uninfected plants grew differently in response to fertility treatment. In contrast to high fertility, where high moisture uninfected plants grew the least, at low fertility, the low moisture infected plants had lower growth rates than other low fertility plants (Figure 5.3). This suggested that the differential growth of infected and uninfected plants depended on both fertility and watering regime, but the Time x Fertility x Water x Infection interaction was not significant ($p = 0.165$).

Plant performance, calculated as the product of average treatment group survival and plant size (log number of leaves) was significantly different among the experimental treatments (Figure 5.4). Using performance as an index of relative group success, uninfected plants under high moisture, high fertility conditions outperformed all other

Figure 5.3: Growth of *Danthonia spicata* in greenhouse treatments
Growth of *D. spicata* plants infected (closed symbols) and uninfected (open symbols) by *Atkinsonella hypoxylon* in the high (◆) and low (●) fertility treatments at high (black symbols, solid lines) and high (gray symbols, dashed lines) moisture. Sample sizes for high fertility vary from 27 to 28 at week 0 to 16 to 22 at week 37. Sample sizes for the low fertility treatment vary from 26 to 27 at week 0 to 11 to 14 at week 37. Values are mean log number of leaves $\pm$ 1 s.e. P-values indicate significant effects from a multivariate repeated measures ANOVA.

Figure 5.3: Growth of *Danthonia spicata* in greenhouse treatments

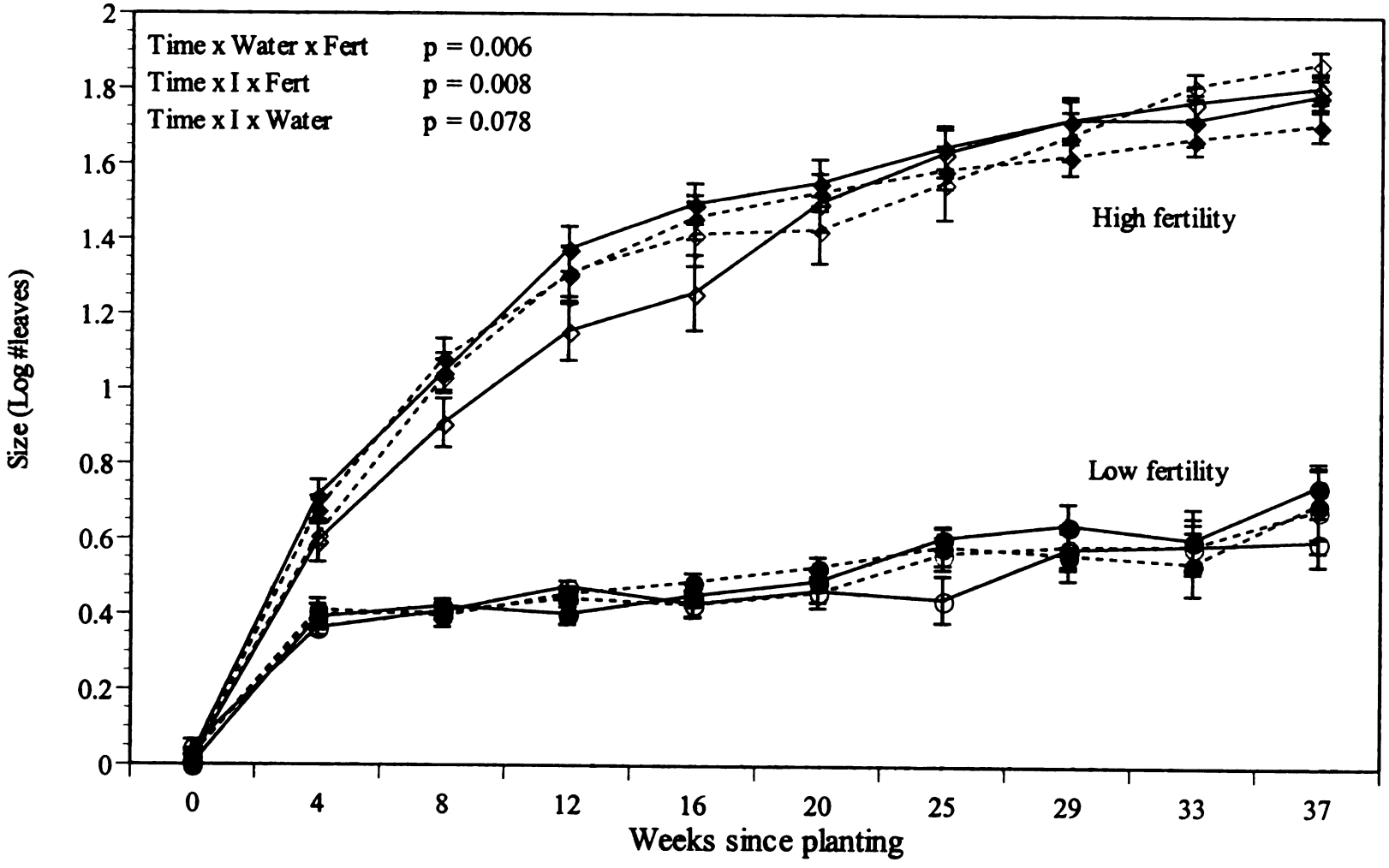
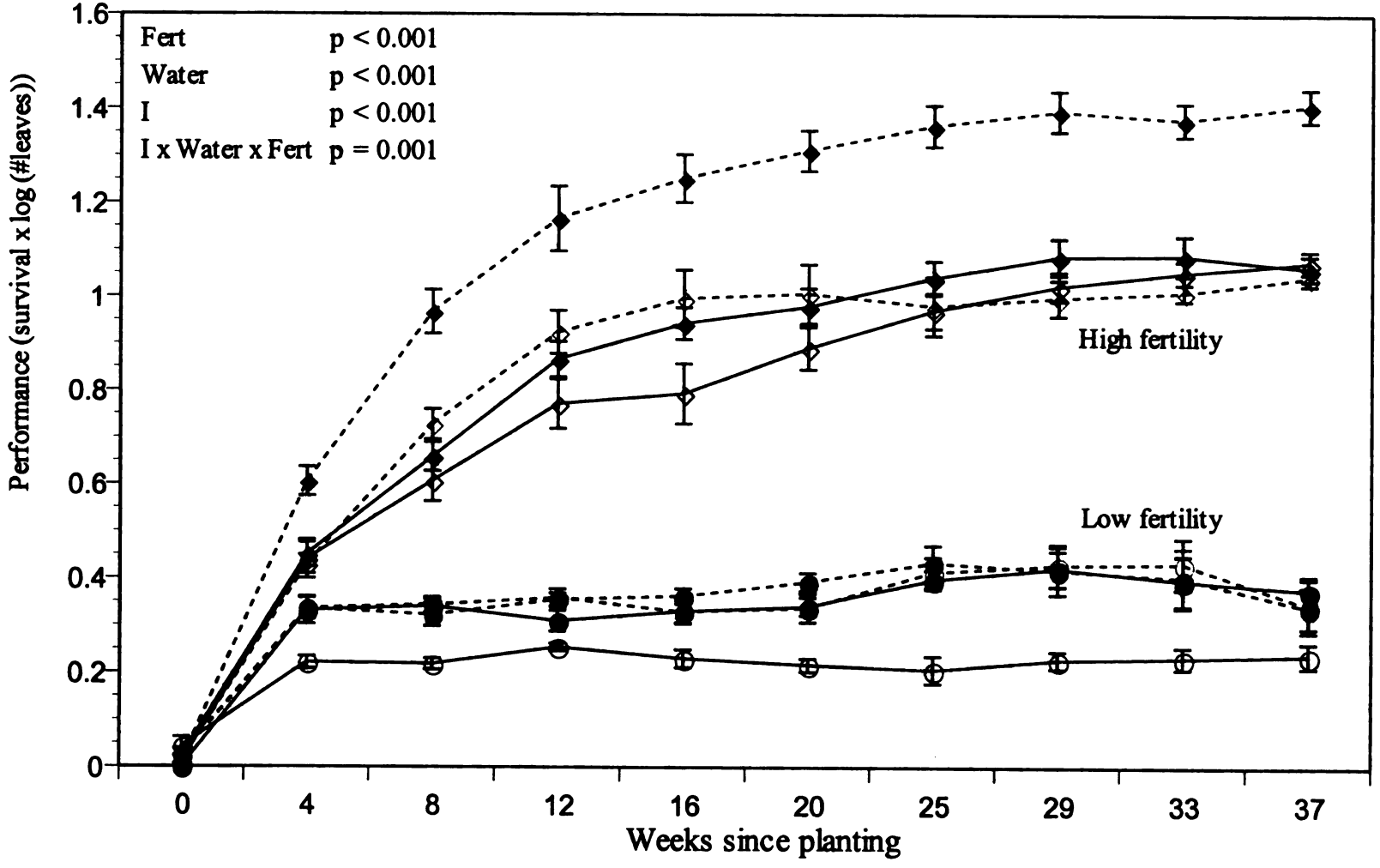


Figure 5.4: Performance of *Danthonia spicata* in greenhouse treatments
Performance (survival x log number of leaves) of *D. spicata* plants infected (closed symbols) and uninfected (open symbols) by *Atkinsonella hypoxylon* in the high (◆) and low (●) fertility treatments and low fertility treatment at high (black symbols, solid lines) and low (gray symbols, dashed lines) moisture. Sample sizes for high fertility vary from 27 to 28 at week 0 to 16 to 22 at week 37. Sample sizes for the low fertility treatment vary from 26 to 27 at week 0 to 11 to 14 at week 37. Values are mean performance + 1 s.e. P-values indicate significant between subject effects from a repeated measures ANOVA.

Figure 5.4: Performance of *Danthonia spicata* in greenhouse treatments



high fertility plants (Figure 5.4). These uninfected plants were the smallest high fertility plants (Figure 5.3), but had substantially higher survival than other treatment groups. Infected plants under low fertility, low moisture conditions performed significantly less well than all other low fertility treatment groups. These infected plants were the smallest low fertility treatment group (Figure 5.3) and also had the lowest survival (Table 5.1).

Table 5.1: Survival of infected and uninfected plants in the greenhouse treatments Survival (%; N = 26 to 28 plants) of infected (I) and uninfected (U) *Danthonia spicata* plants under high and low fertility and high and low moisture treatments in the greenhouse.

			FERTILITY	
			High	Low
		Infection		
	High	U	79	48
MOISTURE		I	56	46
	Low	U	59	52
		I	59	39

Survival in the greenhouse appeared size-dependent. Plants that died often had few living leaves in the previous months, although their maximum size may have been quite large. In the high fertility, high moisture treatment many more small uninfected plants survived than infected plants (Figure 5.5a). It is possible that, small uninfected

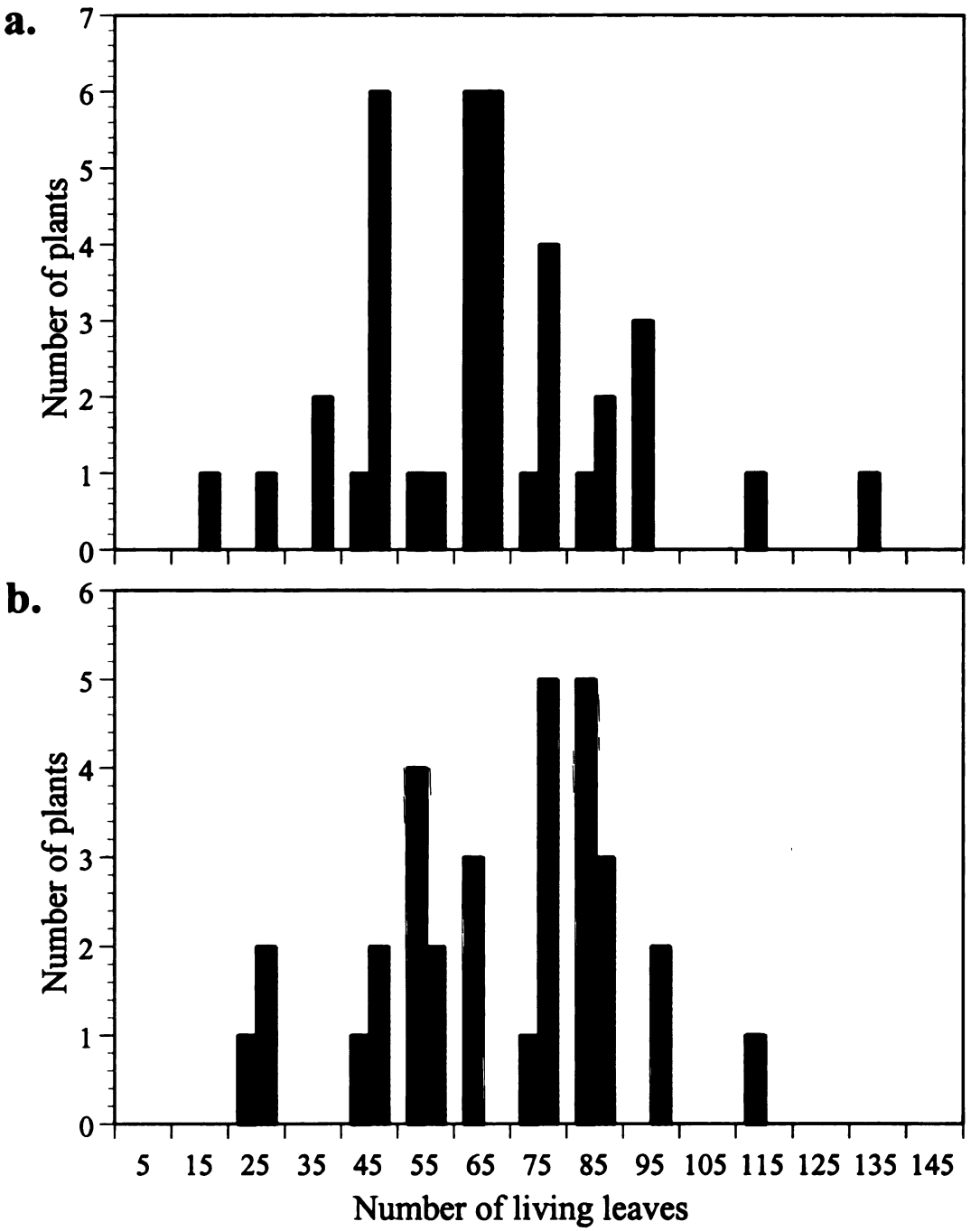


Figure 5.5: Final size distributions of *Danthonia spicata* plants
Final size distributions of *Danthonia spicata* plants infected (black bars) and uninfected (gray bars) by *Atkinsonella hypoxylon* in the high fertility, high moisture treatment (**a.**) and in the high fertility, low moisture treatment (**b.**) in the greenhouse experiment. The number of living leaves is the median value of the category (e.g. 0 to 10 leaves is written as 5).

plants were able to survive in the relatively benign high fertility, high moisture growing conditions, while the nutrient demand by *A. hypoxylon* on small infected plants caused them to die. In contrast, in the low moisture treatment where neither survival nor plant size differed, the size distributions of infected and uninfected plants were similar (Figure 5.5b). Thus, the small average size of uninfected plants may be due to their higher survival under high fertility and moisture conditions compared to infected plants in this same treatment (Figure 5.3). Because differences in mean size are counteracted by differences in survival, the larger size and higher growth rates of infected plants in the high fertility, high moisture treatment does not result in a performance advantage under these conditions.

Common Garden

In the common garden, size at planting had no significant effect on final size (Pearson correlation, $p>0.3$), so only final size was considered in these analyses. Infected and uninfected plants did not differ significantly in total biomass in the common garden ($p = 0.310$). Because reproductive stalks of infected plants were aborted by the fungal sclerotium at an early stage of growth, they were much smaller than reproductive stalks of uninfected plants. As a consequence, infected plants had significantly more vegetative biomass, but less reproductive biomass than uninfected plants (Figure 5.6a).

Infected plants had significantly higher nitrogen concentrations in their vegetative tissues than uninfected plants. Inflorescences of infected plants (with the fungal sclerotium) had almost twice the nitrogen concentration of uninfected plants (Figure 5.6b). The substantial increase in nitrogen concentration in infected inflorescences

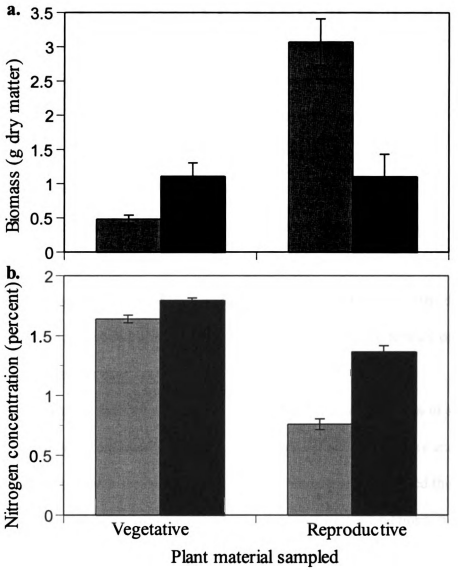


Figure 5.6: Biomass and nitrogen concentration of plants grown in the common garden Plant biomass allocation (**a.**) and tissue nitrogen concentration in vegetative and reproductive tissue (**b.**) for *Danthonia spicata* plants infected (gray bars) and uninfected (black bars) grown in a common garden. Tissure nitrogen of infected plants included fungal hyphae on vegetative surfaces and the fungal stroma on the reproductive tissue. All values $\pm$ 1 s.e.; N = 3 genotypes for infected plants and uninfected reproductive tissue. N = 10 genotypes for uninfected biomass and vegetative tissure nitrogen measurements. Infected and uninfected plants were significantly different in biomass distribution and nitrogen concentration of both vegetative and reproductive tissue at $p \leq 0.002$.

relative to vegetative material was probably due to the much larger concentration of fungal biomass in the sclerotium. This result suggests that the higher nitrogen concentrations of infected plants was due to the presence of nitrogen-rich fungal biomass, rather than from the plant tissue having a higher nitrogen concentration.

DISCUSSION

In two of three field populations, quadrats where *D. spicata* was infected by *A. hypoxylon* had lower soil moisture and higher relative ammonia supply. This suggests that in these Michigan sites soil resource heterogeneity influences the distribution of *A. hypoxylon* infected *D. spicata* plants. It is unknown how the fertility of their field sites compares to our sites.

Several studies have addressed the role of fungal symbiotes in altering growth requirements of infected plants (e.g., Antonovics et al. 1987, Marks and Clay 1990, Bacon 1993, Latch 1993). However, none of these studies assessed the importance of environmental heterogeneity in structuring plant-fungal interactions (but see Thrall and Burdon 1997). Our greenhouse results demonstrate that infected and uninfected plants respond differently to fertility and moisture. We also found that ammonia supply was correlated with the incidence of fungal infection in our field populations. The uniqueness of our study is that we linked growth differences in the greenhouse and common garden to the distribution of fungal infection in natural field populations.

Results from our greenhouse study suggested that infected plants had a performance disadvantage when grown under low moisture, low fertility and high moisture, high fertility conditions (Figure 5.4). Both watering regime and fertility

differentially affected the performance of infected and uninfected plants. Consequently, there was no overall performance (survival x fecundity) advantage for infected plants under our greenhouse conditions.

In our field surveys, we found that infected plants were most common in areas with high ammonia supply, while areas with high soil moisture and low ammonia supply had very few infected plants. This result is in contrast with studies in Indiana and North Carolina that have found that infected plants grew consistently better than uninfected plants in both field plantings and in the greenhouse (Clay 1984, Kelley and Clay 1987, Leuchtmann and Clay 1988). The soil used in these other experiments was quite fertile compared to our field sites and this difference may explain the dominance of infected plants in their greenhouse studies.

In the greenhouse experiment infected plants grew less well than uninfected plants in the low moisture treatments. In the greenhouse, soil moisture in the low frequency watering treatments was maintained at approximately 6%, while in the high frequency watering treatments soil moisture was approximately 25%. In our three field populations, *Danthonia* was rarely found in sites where soil moisture was above 20%. Consequently, the low moisture treatments in the greenhouse were more relevant to understanding the distribution of infected and uninfected plants in our three field populations than the high moisture treatments were. Further comparisons among *D. spicata* populations with and without *A. hypoxylon* may yield a better understanding of the role played by soil moisture in mediating the effect of *A. hypoxylon* on *D. spicata*.

When both fertility and moisture were low in the greenhouse experiment, infected plants grew and performed less well than uninfected plants. A possible reason for the

poor performance of infected plants in the low fertility, low moisture treatment was suggested by the nitrogen content data from plants grown in the common garden experiment. Higher nitrogen concentration in fungal biomass may put a high nitrogen acquisition demand on infected plants. An increased nitrogen demand on infected plants could limit them to areas of the field with high relative supply of ammonia. This may also have been what caused them to grow less well than uninfected plants in the low fertility, low moisture treatment in our greenhouse experiment. In southern Michigan fields, *D. spicata* rarely occurs in areas of the field with both high moisture and high ammonia, as these sites are dominated by other plant species (McCormick, unpublished data). In a greenhouse study, Kelley and Clay (1987) found that *D. spicata* was a relatively poor competitor for light with other grass species, suggesting that other plant species might exclude *D. spicata* from richer areas of the field. Thus if *D. spicata* only occupies areas of the field with either high moisture OR high ammonia, then a high nitrogen demand might also limit infected *D. spicata* to lower moisture areas of the field.

Infected *D. spicata* plants are common and often dominate low moisture, high ammonia areas of fields in southern Michigan. However, in the greenhouse they did not have a clear performance advantage under these or any conditions. The high incidence of infection in some areas of the field, despite no indication of advantage in the greenhouse or common garden, suggests that other factors may be influencing the field distribution of *A. hypoxylon*-infected *D. spicata* in these sites. Competition (intra- and inter-specific) and herbivory were not included in our greenhouse study, but could influence the distribution of infected plants in the field. Kelley and Clay (1987) found that infected *D. spicata* were better interspecific competitors than uninfected *D. spicata*. Infected *D.*

spicata may also be less susceptible to herbivory, as has been shown for several endophyte-infected species (e.g., Prestidge et al. 1982, Bacon et al. 1986, Read and Camp 1986, Clay et al. 1993). We saw no evidence of herbivory in our common garden study even though herbivores (e.g. rabbits, deer, mice) are common in this field and were not excluded from the plots. Infected plants also did not differ from uninfected plants in total biomass, but they did have greater leaf biomass. Increased competitive ability or herbivory resistance could convey a performance advantage to infected plants under conditions of sufficient fertility.

We have found that *D. spicata* plants infected by *A. hypoxylon* performed less well under low moisture, low fertility and high moisture, high fertility conditions than uninfected plants. These performance differences and the spatial heterogeneity of soil resources in the field may explain some of the variation in the distribution of infected and uninfected plants we observed in the populations we surveyed. The absence of infected plants under low ammonia conditions in the field may have resulted from reduced survival or growth caused by a high nitrogen demand by *A. hypoxylon* from infected plants. Our observation that infected plants have higher nitrogen concentration may also suggest a mechanism for the exclusion of infected plants from dry, low fertility field locations. Additionally, if fungal tissue has a higher nitrogen content than plant tissue, it may leak nitrogen to host plant leaf tissue. The increased leaf nitrogen content could increase photosynthetic efficiency and possibly the competitive ability of infected plants under high nitrogen conditions, allowing them to dominate uninfected plants in higher fertility areas of the field. However, this hypothesis remains to be tested.

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Chapter 6

CONCLUSIONS

Although it has long been recognized that spatially structured variation in soil resources has the potential to affect plant populations and communities ecologically and evolutionarily, population and community effects of spatially structured resource variation have rarely been examined in field populations (Robertson et al. 1988, Ehrenfeld 1997). This limited attention is partly because of difficulty in relating measured heterogeneity in resources to plant-perceived heterogeneity, and partly because it was unknown whether patterns of spatial variation would persist long enough to produce consistent directional selection on plant populations (e.g. Stratton and Bennington 1998). The studies presented in this dissertation demonstrate that spatially structured soil resource variation can have strong ecological and evolutionary effects on plant populations and communities.

The five field sites that I studied differed in the degree to which soil resources were spatially structured and temporally consistent. More importantly, I found that *D. spicata* populations in these sites responded ecologically to the measured differences in spatially structured soil resources within sites. Plant response to these differences in spatially structured variation in soil resources was largely through differences in survival. There was little evidence for growth differences among surviving plants that could be attributed to spatial variation in soil resources. This suggests that spatial variability in soil resources influences the recruitment distribution of *D. spicata* plants in these sites, but not their growth and production. The lack of effect of small scale heterogeneity in

soil resources on the growth of surviving plants may result from the very high levels of phenotypic plasticity in *D. spicata*. In greenhouse studies, I found that there were similar levels of growth plasticity to variation in nitrogen availability in plants from all five study populations.

Differences in average population dispersal distance among these five *D. spicata* populations suggest that this trait may be responding to selection to reduce the amount of spatial environmental variation encountered by offspring. In spatially structured environments, decreasing dispersal distance decreases the average environmental variation encountered by offspring, while decreasing dispersal distance in unstructured environments has little effect on the amount of environmental variation encountered by offspring. I found that populations in sites with structured variation in soil resources allocated a greater proportion of their seeds to short dispersing seeds than those in unstructured environments. However, the observation that these study populations have similar, high, amounts of phenotypic plasticity in three traits that affect nitrogen acquisition suggests that dispersal and nitrogen acquisition traits respond differently to selection by spatially structured environmental variation.

Selection for phenotypic plasticity of many traits may be influenced more by temporal variation in environmental conditions than spatial variation (e.g. Sultan 1987, Sultan and Bazzaz 1993). Although I found significant temporal consistency of spatially structured variation for soil moisture, nitrogen availability was less consistent in my study sites. For plants such as *D. spicata* that are limited to low resource environments, the benefits of having high plasticity in traits that affect resource acquisition may outweigh the cost of maintaining that plasticity (Sultan and Bazzaz 1993). However, to

clearly distinguish the relative importance of selection by temporal versus spatial environmental variation on any plant species would require long-term experiments that followed the success of genotypes with different dispersal distances and levels of phenotypic plasticity under different levels of independently manipulated temporal and spatial environmental variation.

I also examined the way that resource heterogeneity influenced the interaction between *D. spicata* and *A. hypoxylon*. Soil moisture and nitrogen conditions affected the performance of *D. spicata* plants infected by *A. hypoxylon* differently than they affected uninfected plants. Uninfected plants out performed infected plants under low moisture, low fertility and high moisture, high fertility conditions in the greenhouse. Because of these different effects, I also expected that spatially structured soil resources would alter the spatial distribution of infected and uninfected plants in the field. I found that infected and uninfected *D. spicata* were distributed differently with respect to soil moisture and ammonia conditions in three field populations. Field conditions in these three sites corresponded largely to the low moisture treatment in the greenhouse and, consistent with the results of the greenhouse experiment, infected plants were not found in dry, low fertility areas of the field.

The results from these studies with *D. spicata* suggest that spatial environmental structure within plant populations may be more consistent and have greater ecological and evolutionary effects than previously thought. The effect of spatially structured environmental variation on population characteristics and species interactions has wide-ranging implications for processes at levels of ecological organization from genes to ecosystems. If population evolution in response to spatial heterogeneity is a general

phenomenon, then additional attention to the spatial structure of environmental variables may be warranted in studies of ecology and evolution at all levels of ecological organization.

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APPENDIX

Table A.1: Composition of modified Hoagland's solution used in this experiment

CHEMICAL	Concentration (g/L)
Micronutrients:	
KCl	0.003728
H_3BO_3	0.001546
$MnSO_4 \cdot H_2O$	0.000338
$ZnSO_4 \cdot 7H_2O$	0.000575
$CuSO_4 \cdot 5H_2O$	0.000125
MoO_3	0.00007197
FeEDTA	0.00742
Macronutrients:	
$CaSO_4 \cdot H_2O$	0.3443
$MgSO_4$	0.2465
K_2SO_4	0.1046
K_2HPO_4	0.0871
KH_2PO_4	0.0476
Nitrogen:	
NH_4NO_3 (low N)	0.0429
NH_4NO_3 (mod N)	0.1144
NH_4NO_3 (high N)	0.2135

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