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Variation in seed dispersal along an elevational gradient in Great Smoky Mountains National Park

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ABSTRACT

Seed dispersal by ants is ecologically important and geographically widespread as 20–50% of all herbaceous species in eastern deciduous forests are dispersed by ants, but we know little about how or why such interactions vary geographically. In this paper, we examined variation in seed dispersal by ants along an extensive elevational gradient (256–2025 m) in the Great Smoky Mountains National Park, USA. Specifically, we asked whether variation in ant community composition affected seed removals and seed dispersal distance of *Trillium undulatum* and *Hexastylis arifolia*, two common understory herbs found throughout the southern Appalachian Mountains. We also examined variation in myrmecochore abundance, specifically *Trillium* spp. and *H. arifolia*, along the same elevational gradient. Measures of ant community and climate variables strongly covaried with elevation, while *Trillium* species richness and abundance did not. We found that seed removals decreased with elevation, but seed dispersal distance did not depend on elevation. The most important variables predicting seed removals were average annual temperature and the abundance of *Aphaenogaster rudis*, both of which varied along the elevational gradient. Seed dispersal by ants did not depend on ant community composition, but was dominated by one species, *A. rudis,* which occurred at every site and removed the vast majority of all observed seeds in this study. Though the ant fauna in the Great Smoky Mountains National Park is diverse, dispersal of *T. undulatum* and *H. arifolia*, and likely other myrmecochores, is driven by one ant species, *A. rudis*.

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1. Introduction

Ant–seed interactions are ecologically important and geographically widespread (Giladi, 2006; Rico-Gray and Oliveira, 2007). Ants collect and transport seeds to ant nests, where they consume the nutritional reward and discard the seed virtually unharmed; the seed can subsequently germinate (Beattie and Culver, 1981). It has been estimated that ants disperse plant species from over 80 plant families (Beattie, 1985) and within the monocots, myrmecochory has evolved tens of times independently (Dunn et al., 2007a). Interactions between ants and seeds are very important to the maintenance of natural plant communities (Giladi, 2006; Kalisz et al., 1999) as seed dispersal establishes the initial template for seedling

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distribution. Though ant–seed mutualisms are ecologically important, most studies to date have focused on interactions among seeds and ants in a single location, or perhaps a few locations within a region. However, a growing number of studies have indicated that a variety of interspecific interactions can vary geographically (MacArthur, 1972; Jeanne, 1979; Bertness et al., 1981; Pennings et al., 2001). While geographic variation in certain species interactions, such as herbivory (Pennings et al., 2001; Goranson et al., 2004; Andrew and Hughes, 2005; Novotny et al., 2006; Dyer et al., 2007), seed predation (Louda, 1982; Moles and Westoby, 2003), and pollination (Thompson, 1994; Aras et al., 1996; Arroyo et al., 2005; Devoto et al., 2005), has received considerable attention, few studies to date have examined geographic variation in seed dispersal mutualisms (but see Gove et al., 2007; Manzaneda et al., 2007).

Ant–seed dispersal mutualisms might vary geographically for at least three reasons. First, the density and diversity of ant seed dispersers might vary (Gove et al., 2007; Parr et al., 2007). Second, plant density, composition, or diversity might vary, thereby influencing the relative abundance or availability of seeds to dispersers (Gorb and Gorb, 2003; Heithaus et al., 2005). Third, climatic conditions might vary and indirectly limit interactions between seeds and their ant dispersers. A critical step in understanding spatial variation in species interactions is to examine how such interactions vary along known gradients in plant or animal communities or varying climatic conditions. Elevational gradients provide an ideal context for such a study because they condense both variation in plant and animal communities and variation in climate onto a small geographic space (Fukami and Wardle, 2005). In this study, our goal was to use an elevational gradient in the southern Appalachian Mountains, where ant communities are well-characterized (Cole, 1940; Mitchell et al., 2002; Dunn et al., 2007b; Sanders et al., 2007), to understand geographic variation in ant–seed dispersal mutualisms. In particular, we examined seed removal rates and seed dispersal distance along an elevational gradient in the Great Smoky Mountains National Park, USA. By experimentally putting out seeds, we reduced the potential effects of variation in the plant community and the abundance of seed resources in order to focus on consequences of variation in the seed-dispersing ant community.

Ants are important seed dispersers in eastern deciduous forest ecosystems (Beattie and Culver, 1981; Gunther and Lanza, 1989; Heithaus and Humes, 2003; Ness, 2004), where they disperse 20 to 50% of the total herbaceous flora (Handel et al., 1981; Gaddy, 1986). Both ant communities (Sanders et al., 2007) and the abundance and diversity of ant-dispersed plants are known to vary strongly in space within this region (Mitchell et al., 2002; Webster and Jenkins, 2008). Similar to other elevational diversity gradients (Botes et al., 2006; Rahbek, 2005), both ant diversity and abundance decrease with elevation in the southern Appalachian Mountains, largely because temperature decreases with elevation (Sanders et al., 2007).

In this context, we explore two inter-related hypotheses regarding the effects of variation in the ant community on seed removals, namely that seed removal is a function of ant abundance and ant diversity. In addition, we ask whether seed

dispersal distance varies along the elevational gradient and if it does, whether that variation is a function of the ant community. If seed dispersal by ants is a diffuse mutualism, in which any of a variety of partners might suffice, then seed dispersal should track variation in ant abundance or diversity. Alternatively, if seed dispersal by ants depends on one of a few keystone mutualists, seed dispersal rates might be more closely correlated with the abundance or activity of particular ant species (Gove et al., 2007). We also examine how the presence and abundance of ant-dispersed flora vary along the same gradient.

2. Materials and methods

2.1. Site description

We carried out this study at 11 sites distributed across an elevational gradient in the Great Smoky Mountains National Park (GSMNP) in eastern Tennessee and western North Carolina, USA, in June–August 2005–2007. Approximately 80% of GSMNP is comprised of deciduous forests (Whittaker, 1956). Elevation in GSMNP ranges from 256 to 2025 m. Temperature and precipitation, as well as the number and abundance of ant species, vary systematically with elevation in this system. Moreover, there appears to be little variation among individual mountains in the GSMNP in the relationships among temperature, precipitation, and ant community composition (Dunn et al., 2007b; Sanders et al., 2007). Therefore, we treated the sites as if they occurred along a single elevational gradient, which is a common practice in many elevational gradient studies, in this system and others (e.g. Sanders, 2002; Dunn et al., 2007b; Rahbek, 2005).

We focused our study on the removal and dispersal of seeds of two common understory myrmecochores (ant-dispersed plants), *Trillium undulatum* (Willd). and *Hexastylis arifolia* (Michx.) (Berg, 1958, 1972; Gunther and Lanza, 1989; Zettler et al., 2001). We used *T. undulatum* seeds for all seed removal experiments. However, due to a late season frost and a severe regional drought in 2007, we were unable to collect any *Trillium* seeds within GSMNP. As a result, we used seeds from *H. arifolia*, a myrmecochore species with seeds morphologically similar to those of *T. undulatum* (Zettler et al., 2001), to track seed dispersal distance. Because we are interested in whether interactions between ants and seeds vary along the elevational gradient and not the details of particular plant species, such a substitution is warranted. Additionally, we compared three sites where we used both *H. arifolia* and *T. undulatum* seeds and there were no differences in seed dispersal distance between the two species ($F = 0.07$, $df = 1$, 37, $P = 0.8$). We carried out all seed removal experiments and seed dispersal observations in July and August, when ants are active throughout the elevational gradient and when *Trillium* species are fruiting at higher elevations (personal observations). While *Trillium* and *Hexastylis* are not all still fruiting at lower elevations in July and August, the high seed removal rates we report suggest that ants still remove seeds at these sites, despite the fact that natural plant populations are no longer fruiting.

2.2. Seed removal rates and dispersal distance

To examine how seed removal by ants varied along the elevational gradient at GSMNP, we established one 50 × 50 m plot at each of the 11 sites. Within each plot, we placed 15 seed depots arranged in a 3 × 5 matrix at each site, with 20 m spacing between each depot. Each seed depot consisted of a 100 × 15 mm Petri dish, which had four 5 mm holes drilled on the sides, allowing access to ants but restricting access to all other potential seed predators, such as rodents. On sunny days without precipitation, we placed 10 seeds of *T. undulatum* at each seed depot. We used 10 seeds in order to obtain results that are comparable to other published studies on seed removal by ants (Christianini et al., 2007; Ness, 2004; Rey et al., 2002) All *T. undulatum* seeds in 2005 and 2006 were collected from local populations at Chimneys picnic area in GSMNP and frozen with BHT preservative (Butylated hydroxytoluene, Fisher Scientific, Inc.) until the experiment began (Heithaus and Humes, 2003). Our preliminary observations confirmed that the use of preserved seeds did not incur any bias in ant response. We recorded the number of *T. undulatum* seeds remaining in each of the seed depots 24 h after placing them in the field. Because rain can inhibit ant activities, we repeated observations at sites where it rained between when the seeds were set out and when they were counted.

To address whether factors correlated with elevation were related to seed dispersal distance, we tracked dispersal distances of seeds by ants at elevations where seed removals exceeded 10% of seeds – at seven out of 11 sites. At each of the seven sites, we placed a 25 × 25 m grid, with seed depots at 5 m intervals. Each seed depot consisted of a 4 × 8 cm white index card, and we placed three *H. arifolia* seeds dipped in pink fluorescent powder, which allowed us to more easily observe the dispersal events. Preliminary experiments showed that the fluorescent powder did not influence seed removals by ants (personal observation; Cain et al., 1998). We used three *H. arifolia* seeds because we were unable to obtain enough seeds to include more seeds per depot and sufficiently replicate seed depots. We observed each seed depot for 2 h or until all the seeds were removed, recording each unique dispersal event. Unique events were those that involved a new depot or a new nest, regardless of how many times a single colony exploited a particular depot. For those events in which more than one seed was removed by the same colony, only one dispersal event was recorded. For each seed, we recorded the identity of the ant species dispersing the seed, dispersal distance (the distance the seed was carried by an ant), and dispersal destination (nest, drop, or lost).

2.3. Ant community

To examine how ant community structure varied along the elevational gradient, we sampled the community at each site in 16 1-m^2 quadrats, arranged in a randomly placed 50 × 50 m plot. From each 1-m^2 quadrat, we collected leaf litter, sifted it through a coarse mesh screen to separate large pieces of litter and debris, and collected fine litter. The litter from each quadrat was then suspended in Winkler sacks for 48 h at room temperature in the laboratory. All ants that were extracted from the litter were identified and stored in NJS's ant collection at the University of Tennessee. Site-level species richness was the number of species extracted from Winkler sacks at a given site, and site-level abundance of a given species was the number of 16-m^2 quadrats in which that species occurred (hereafter referred to as abundance). We chose to use species occurrences in addition to the number of workers collected as the measure of ant abundance because occurrence is a measure of species presence and is an indirect measure of colony abundance (number of ant colonies per area). Worker abundance, in contrast, is a measure of ant activity at the time of collection. Ant sampling methods were identical to those used at other sites in GSMNP (e.g. Sanders et al., 2007) and allowed for comparison of our sites with a much larger number of sites sampled in the same way.

2.4. Abiotic variation

For each of the 11 sites, we estimated mean annual temperature for each site from WorldClim 1.4 (www.worldclim.org). We focused on temperature because recent work at these and nearby sites showed temperature to be the most important determinate of ant diversity, abundance, and temporal patterns of activity (Dunn et al., 2007b; Sanders et al., 2007). WorldClim generates environmental data at a 1-km^2 resolution through interpolation of average monthly climate data from nearby weather stations. The interpolated temperature estimates from WorldClim did not differ qualitatively from temperature estimated from a series of Hobo Dataloggers at eight different sites along the same elevational gradient (Dunn et al., 2007b). Temperature data were included to test whether climate had an effect on seed dispersal via its influence on ant activity, above and beyond the effect it has on ant diversity and species composition.

2.5. Myrmecochore surveys

To determine whether the presence or abundance of ant-dispersed flora varied along the elevational gradient, we noted the presence of myrmecochores, specifically *Trillium* spp. and *H. arifolia* at each of the 11 sites where we carried out seed removal experiments. We also analyzed *Trillium* abundance data collected by volunteer groups associated with the Great Smoky Mountains Institute at Tremont, Nationals Parks Conservation Association, and Discover Life in America. These groups carried out *Trillium* surveys at 12 sites ranging from 536 to 1318 m in elevation at the GSMNP in April 2000 and 2001. At each site, groups walked a 100 m transect, recording the *Trillium* species present and counting the number of individuals located within 10 m on each side of their transect. Studies document ant seed dispersal for *T. undulatum* (Gunther and Lanza, 1989; Zettler et al., 2001), *T. erectum* (Beattie and Culver, 1981; Gunther and Lanza, 1989), *T. grandiflorum* (Beattie and Culver, 1981; Gunther and Lanza, 1989; Vellend, 2005), and *T. catesbaei* (Zettler et al., 2001), though based on fruit morphology, ants likely disperse *T. luteum* and *T. rugelii*, as well.

2.6. Data analyses

All measured variables strongly covaried with elevation (Table 1). As a result, we report the broad pattern of variation

Table 1 – Correlation matrix among all measured variables

	Elevation (m)	Temperature (°C)	% Seed removal	Species richness	A. rudis abundance per site	A. rudis worker abundance	Total worker abundance
Elevation (m)	1	−0.9859	−0.9177	−0.7959	−0.9093	−0.6982	−0.7009
Temperature (°C)	0.001	1	0.9445	0.7472	0.8474	0.6312	0.6861
% Seed removal	<0.001	<0.001	1	0.602	0.721	0.5003	0.5694
Species richness	0.003	0.008	0.05	1	0.8521	0.8944	0.891
A. rudis abundance per site	<0.001	0.002	0.003	0.008	1	0.8697	0.6954
A. rudis worker abundance	0.02	0.04	0.1	0.0002	0.01	1	0.758
Total worker abundance	0.0163	0.0197	0.0675	0.002	0.0175	0.0069	1

The upper portion of the table shows the correlation coefficients for pairwise relationships and the lower portion of the table shows the statistical significance of the relationships. Negative numbers indicate a negative relationship between variables.

in seed removal with elevation and use temperature as a proxy for variation in climate variables in subsequent analyses. To determine if seed removal rates varied with temperature and with characteristics of the ant community (ant species richness, total ant worker abundance, *Aphaenogaster rudis* abundance, and number of *A. rudis* workers) per site, we used stepwise multiple regressions (P to enter = 0.10). In our seed dispersal observations, *A. rudis* was the most common ant species removing seeds from seed depots; as a result, we included *A. rudis* abundance as a predictor of seed removal rates in the stepwise regression models. Seed removals were averaged per site and the average value was used as the response variable in the analyses. We used a linear regression to determine if log-transformed seed dispersal distances varied with elevation. Dispersal distances were log-transformed to meet the assumptions of normality and also averaged per site, using the average seed dispersal distance per site as the response variable in the analyses.

To determine if myrmecochore presence or abundance varied with elevation, we carried out two separate analyses, one including only the sites where we carried out seed removal experiments and one including only those sites where volunteer groups carried out *Trillium* surveys. These data were analyzed separately because the sites did not overlap and because they differed in data collection methods. Because site-level myrmecochore data for our sites was binary (e.g. presence or absence of *Trillium* spp. or *H. arifolia*), we ran a logistic regression to determine if presence of myrmecochores depended on elevation and also if the presence of myrmecochores correlated with measures of ant community – ant species richness, total worker abundance, and *A. rudis* abundance. We used a linear regression to determine if *Trillium* species richness and abundance varied with elevation. *Trillium* abundance (the sum of all *Trillium* individuals counted within the 100 m transect) was square-root transformed prior to analysis. We used JMP 5.1 Software (SAS Institute) for all statistical analyses.
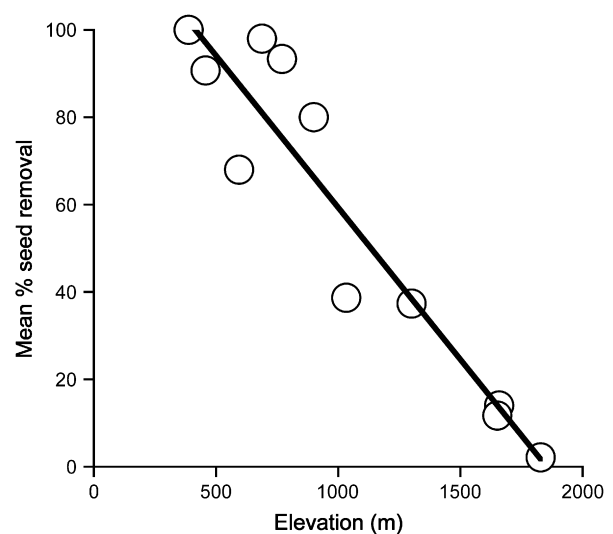
3. Results

3.1. Seed removal patterns

Seed removals declined with elevation ($n = 11$, $R^2 = 0.89$, $P < 0.0001$; Fig. 1). Elevation and temperature were strongly

correlated – as elevation increased, temperature decreased ($n = 11$, $R^2 = 0.99$, $P < 0.0001$; Table 1). Measures of ant community, ant species richness, total ant worker abundance, and the abundance of *A. rudis*, were also all strongly correlated with temperature (Table 1). Thus, when temperature entered the stepwise multiple regression model predicting seed removals, no other predictor variables entered the model (Table 2). The stepwise multiple regression excluding temperature as a predictor variable indicated that the number of *A. rudis* occurrences accounted for 65.5% of the variation in seed removal rates (Table 2, Fig. 2). The other three measures of ant community, *A. rudis* abundance, ant species richness, and total ant worker abundance, collectively accounted for an additional 2% of the variation.

3.2. Dispersal distance

Of the 600 *H. arifolia* seeds (three seeds per bait station × 25 bait stations per elevation × eight elevations) we observed during the course of the study, 303 (50.5%) were removed during our 2 h observational periods and we were able to measure the dispersal distance for 147 (48.5% of the removed) seeds. Of those 147 seeds, 146 were removed by *A. rudis* (mean dispersal distance: 73.85 cm ± SD 42.62 cm) and one was removed by

Fig. 1 – Relationship between the mean percentage of *Trillium undulatum* seeds removed in 24 h and elevation.

Table 2 – Results of stepwise multiple regressions examining the effects of temperature, ant species richness, *A. rudis* worker abundance, and *A. rudis* overall abundance per site on seed removal rates in Great Smoky Mountains National Park, USA					
Variable	Parameter	Partial r^2	Model r^2	F	P
(a) Temperature	24.89	0.885	0.885	69.28	<0.0001
(b) *A. rudis* abundance	5.71	0.65	0.65	17.07	0.0026

Model (a) included all predictor variables and model (b) included all variables except temperature. Only the significant predictor variables are included in the table.

Fig. 3 – **Mean dispersal distance of *Hexastylis arifolia* seeds per site as a function of elevation. Open circles represent mean dispersal distance (±standard error) by *A. rudis* only, including only seed depots where seeds were removed. Filled-in circles represent mean dispersal distance (±standard error) across all seed depots, including depots with no seed removals. Squares represent mean dispersal distance (±standard error) by *A. rudis*, combined with one dispersal event by *Formica subsericea*.**
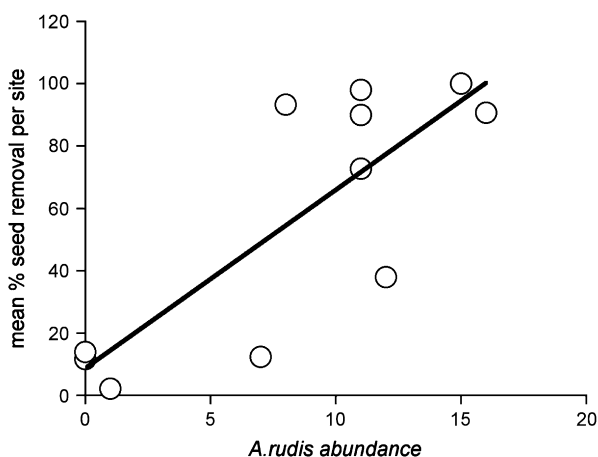
Formica subsericea (dispersal distance: 627 cm). For seeds removed by ants, we found no effect of elevation on log-transformed seed dispersal distance in GSMNP ($n = 7$, $R^2 = 0.14$, $P = 0.35$; Fig. 3). Similarly, we found no effect of elevation on log-transformed seed dispersal distance for all seeds, including uncollected seeds ($n = 7$, $R^2 = 0.21$, $P = 0.25$; Fig. 3).
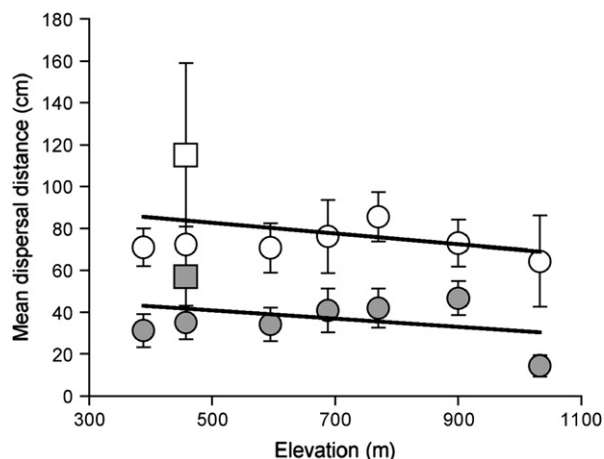
3.3. Ant community

Ant species richness ($n = 11$, $R^2 = 0.80$, $P = 0.003$, Table 1) and total ant worker abundance ($R^2 = 0.49$, $P = 0.016$) decreased with elevation. Both *A. rudis* abundance ($n = 11$, $R^2 = 0.94$, $P = 0.0003$) and the number of *A. rudis* workers ($n = 11$, $R^2 = 0.70$, $P = 0.02$) decreased as elevation increased.

3.4. Myrmecochore surveys

The presence of myrmecochores at a site did not depend on elevation ($n = 11$, $\chi^2 = 2.69$, $P = 0.10$). Measures of the ant community, ant species richness ($n = 11$, $\chi^2 = 2.0$, $P = 0.16$), total ant worker abundance ($\chi^2 = 2.7$, $P = 0.10$), and *A. rudis* abundance ($\chi^2 = 2.77$, $P = 0.10$) were not significantly correlated with the presence of myrmecochores. The *Trillium* survey recorded six species of *Trillium* within the park: T. undulatun,

Fig. 2 – **Relationship between the mean percentage of *Trillium undulatum* seeds removed and *A. rudis* abundance. Abundance is the number of 1 m² plots per site (out of 16) where *A. rudis* was collected in leaf litter.**

T. erectum, T. luteum, T. grandiflorum, T. catesbaei, and T. rugelii. The most commonly found species were *T. erectum* and *T. luteum*, which were each found at eight out of 12 sites. The *Trillium* survey at the GSMNP found that neither *Trillium* species richness ($n = 12$, $R^2 = 0.002$, $P = 0.89$) nor the square-root abundance of *Trillium* plants (pooling across species $R^2 = 0.35$, $P = 0.071$) varied with elevation in the GSMNP.

4. Discussion

We examined variation in a seed dispersal mutualism along a large elevational gradient in the Great Smoky Mountains National Park. Seed removal rates by ants, but not seed dispersal distance, varied along this elevational gradient. To our knowledge, this is the first study to document strong geographic variation in an ant–seed dispersal mutualism along an elevational gradient. But what are the possible causes of this variation? We propose three hypotheses to explain why seed removals varied along the elevational gradient examined here: (1) geographic variation in the density and diversity of seed-dispersing ants, (2) geographic variation in plant density or diversity, which may be influencing the availability of seeds to seed dispersers, and (3) variation in climatic variables, which influences the activity of seed-dispersing ants.

We found that ant species richness and total ant worker abundance varied significantly with elevation, but neither of these parameters accounted for any of the variation in seed removal rates. Our observations of seed dispersals indicate that species composition and more specifically, the relative abundance of key ant species may be more important for myrmecochores than overall ant species richness. Contrary to our expectations, seed dispersals of *T. undulatum* and *H. arifolia* at

GSMNP were not a function of the entire ant fauna but instead were driven by the activities of one species, *A. rudis*. The abundance of *A. rudis* significantly decreased with elevation. Given that this species was responsible for the vast majority of the observed seed dispersal events (99.3%), it is not surprising that the presence and abundance of *A. rudis*, accounted for much of the variation in seed removals among sites in GSMNP. Since *A. rudis* ants disperse the vast majority of seeds, and *A. rudis* nest density decreases with elevation, we would hypothesize that the distance between the seed depot and the nest should increase with elevation, leading to an increase in seed dispersal distance. Surprisingly, seed dispersal distance did not vary. This suggests that *A. rudis* seed discovery rates, not foraging ranges, change with elevation.

We found that neither *Trillium* species richness nor abundance varied with elevation in GSMNP. *Trillium* plants (Webster and Jenkins, 2008), similar to other myrmecochores (Hughes and Fahey, 1991), are patchily distributed and often occur at sites with few or no seed-dispersing ants. Mitchell and colleagues' work showed that myrmecochore species richness was positively correlated with the abundance of *Aphaenogaster* spp in their study sites (Mitchell et al., 2002). Our preliminary analysis of the distribution and abundance of *Trillium* did not confirm this trend and we found *Trillium* plants at high elevation sites, where we did not collect *A. rudis* ants. This presents the question: Why are *Trillium* found at sites without seed-dispersing ants? Other species, such as white-tailed deer (Myers et al., 2004) and yellow jackets (Jules, 1996; Zettler and Spira, 2001) can disperse *Trillium* seeds, though records of such dispersal are infrequent and the consequences of such dispersal for the seeds remain to be explored (but see Vellend, 2005; Vellend et al., 2006). Whatever the dispersal agent, it seems clear that neither the abundance nor diversity of *Trillium* in this system causes geographic variation in the ant–seed dispersal mutualism. While other studies have found a correlation between the distribution and abundance of myrmecochores and the distribution and abundance of *A. rudis* at other sites (Mitchell et al., 2002; Ness et al., in preparation), this relationship remains unresolved in GSMNP.

Though seed removals varied strongly with elevation at GSMNP, climatic variables such as temperature, in and of themselves, cannot remove seeds. Nevertheless, temperature and seed removal rate were strongly negatively correlated. In fact, temperature accounted for more variation in seed removal than did any other factor, including the abundance of *A. rudis*. Temperature strongly influences ant phenology (Dunn et al., 2007b), reproduction (Höldobler and Wilson, 1990), ant foraging behavior (Azcarate et al., 2007; Bucy and Breed, 2006) and ant distribution (Brown et al., 1997; Kaspari et al., 2004; Sanders et al., 2007). In fact, we report that all measures of the ant community varied strongly with elevation. Thus, variation in temperature along the elevation gradient likely drives the pattern of seed removals we document in our study through its effects on ant community structure and the abundance of key species.

Previous studies have documented that a variety of ant species disperse seeds in southeastern deciduous forests (Gunther and Lanza, 1989; Heithaus and Humes, 2003; Ness, 2004). However, many of these studies suggest that *A. rudis* could be among the most important seed dispersal mutualists in these forest ecosystems (Beattie and Culver, 1981; Gaddy, 1986; Heithaus, 1981; Mitchell et al., 2002; Ness, 2004). For example, Ness (2004) found *A. rudis* to be the most common disperser of myrmecochorous seeds in a southeastern deciduous forest in Georgia, USA, except in areas invaded by the red imported fire ant, *Solenopsis invicta*. Beattie and Culver (1981), also working in a southeastern deciduous forest, documented that *A. rudis* dispersed all of the myrmecochorous species that they examined. Finally, working in an eastern deciduous forest in Ohio, Heithaus and Humes (2003) found *A. rudis* to be the most common disperser, except in the most disturbed habitat they examined. Collectively, these authors also documented other ants, specifically *Myrmica americana*, *M. punctiventris*, *Prenolepis imparis*, and *F. subsericea*, dispersing seeds in their studies, but in no cases were they the main dispersers of seeds. Similarly, while these ant species occur in GSMNP (Sanders et al., 2007), we only observed *F. subsericea* dispersing a seed in one instance and did not observe any of these other species interacting with seeds. We cannot account for the fate of unremoved seeds, which may be removed by other ant species or harvested by rodents (Tallmon et al., 2003). However, a 2-h observation period is common in studies such as ours (Heithaus and Humes, 2003; Ness, 2004). Additionally, in instances in which we observed *A. rudis* ants removing seeds from seed depots, they removed all the seeds within the 2 h observational period.

What factors contribute to the disproportionate importance of *A. rudis* as seed dispersers in southeastern deciduous forests? Behaviorally, these ants are subordinate (Fellers, 1987; Mitchell et al., 2002) and do not generally out-compete other ant species at baits. However, they can exploit resources quickly, before being displaced by more dominant ant species (Fellers, 1987). Gove et al. (2007) have suggested that behaviorally subordinate ant species are more likely to be important seed dispersers as they remove seeds to nests quickly. In addition to its behavioral characteristics, *A. rudis* is geographically widespread (Gotelli and Ellison, 2002) and abundant throughout eastern North America, and nest densities can be 0.5 nests m^{-2} (Smallwood, 1982; Giladi, 2006). Their behavioral attributes, widespread distribution, and high local abundance all likely contribute to the role *A. rudis* ants play as the dominant seed dispersers of myrmecochorous fauna in eastern deciduous forests.

5. Conclusions

Combining our findings with other studies, there is strong evidence that *A. rudis* is a keystone seed dispersal mutualist in eastern deciduous forests, where this species is responsible for the majority of seed dispersals of ant-dispersed herbaceous flora (Ness et al., in preparation). All of these findings are striking given that up to 50% of all herbaceous species in the eastern deciduous forests are adapted for dispersal by ants (Handel et al., 1981; Gaddy, 1986). An important, yet unanswered question in ecology is whether seed dispersal mutualisms are diffuse or whether certain species contribute to the process more than others. Evidence is building that

myrmecochory is not a diffuse mutualism, as previously thought, but one in which key ant species play a disproportionate role in dispersing the majority of seeds where they occur (Gove et al., 2007). This is especially important in the context of geographic variation in species interactions, as the variation in the abundance of key mutualists is likely to have profound consequences for the species dependent on those mutualists.

Our findings lead to interesting questions regarding the causes of variation in myrmecochore density among sites, relatedness of ant-dispersed seeds of nearest neighbors, and vulnerability of undispersed seeds to rodent predators, to name a few. Separate studies have addressed some of these questions, though not within the same study system and not within the framework of geographic variation (but see Manzaneda et al., 2007; Rey et al., 2002). While we did not address these hypotheses, these are a few of the possible causes for variation in ant–seed dispersal mutualisms and provide exciting avenues for future research. Elevational gradient studies have much to offer in understanding variation in interspecific interactions and their underlying mechanisms. However, the fact remains that experimental manipulations of the potentially important factors, such as temperature, would be extremely useful in discerning the specific mechanisms driving the variation in species interactions, such as the one we report here.

Acknowledgements

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