Overall seed dispersal effectiveness is lower in endemic *Trillium* species than in their widespread congeners

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PREMISE OF THE STUDY: Comparing ecological attributes of endemic species with related, widespread species can reveal differences accounting for rarity. Forests of the southeastern United States are home to many range-restricted endemic and widespread species of *Trillium*, a genus of ant-dispersed herbs. Evidence suggests that aspects of seed-related life history stages are often correlated with plant rarity, but few studies have tested whether the process of seed dispersal differs for endemic and widespread species. To address this question, we compared aspects of seed dispersal effectiveness (SDE) for three sympatric, widespread endemic *Trillium* species pairs.

METHODS: We observed seed dispersal for *Trillium* species pairs by ants at eight sites, recorded numbers of seeds dispersed and dispersal distances, and described disperser interactions. To test disperser preference, we presented seeds of each pair to captive colonies of *Aphaenogaster picea*, a keystone disperser. Seeds were assigned scores based on worker behavior, and we recorded proportions of seeds dispersed after 1 h and 24 h.

KEY RESULTS: Field observations yielded some significant within-pair differences. Ants dispersed more seeds of widespread species for all pairs, although dispersal distances did not differ. In laboratory experiments, after 24 h, ants dispersed more seeds of widespread species into nests.

CONCLUSIONS: Endemic *Trillium* species had lower overall SDE than did their widespread congeners. These findings add to the list of ecological and demographic challenges that face endemic plants when compared to common congeners. Lower SDE may negatively impact reproductive rates and the colonization of new habitats, which may contribute to patterns of endemism.

KEY WORDS *Aphaenogaster*; ant–plant interaction; Melanthiaceae; myrmecochory; rarity; southeastern United States; spring ephemeral.

Endemic species are key components of global biodiversity and have been used in conservation prioritization (Myers et al., 2000; Lamoreaux et al., 2006). Of the characteristics noted by Rabinowitz (1981), restricted geographic ranges (i.e., endemism) are of great interest in the field of rare species biology (Morrone, 1994). Rare species have been shown to disproportionately support the functional structure of species assemblages and are thus crucial in maintaining ecosystems (Leitao et al., 2016), so understanding the processes affecting rarity is critical for successful conservation efforts. Furthermore, determining why some species are rare while others are common is central to our understanding of the dynamics of species and communities (Brown et al., 2003) and is a fundamental question in ecology.

In flowering plants, ecological traits have been shown to correlate with species rarity. Some studies suggest that the reproductive and recruitment stages of a species' life history are most likely to explain rarity (Brown et al., 2003), while others have implicated specific factors such as limited fecundity, lack of seed-soil banks, and reduced seed dispersal (Osunkoya and Swanborough, 2001). Theoretical work suggests that the success of seed dispersal might be related to relative commonness when seed availability is in short supply, such as in species with few large seeds, in the context of a scarce seed bank, or in species with little to no clonal abilities (Bond, 1994). Indeed, direct comparisons of narrow endemic and widespread congeners have shown that endemic species generally have lower seed availability (Fiedler, 1987; Byers and Meagher,

1997), and classic work in the field of rare species biology indicates that seed dispersal syndromes are likely correlated with aspects of relative rarity and commonness (Rabinowitz, 1978; Rabinowitz and Rapp, 1981; Pirie et al., 2000). This body of work has established that seed-related life history stages play important roles in plant rarity, but that this hypothesis is in need of further investigation.

The use of demographic comparisons of related rare and common taxa is an established method for examining factors regulating rare plant populations (Karron, 1987; Walck et al., 1999). This method has been used to compare functional traits (Hand et al., 2017), seed production and germination (Brown et al., 2003), reproductive and ecophysiological attributes (Osunkoya and Swanborough, 2001), responses to herbivory and invasive species removal (Leege et al., 2010), predispersal seed predation (Combs et al., 2011, 2013), and the effects of invasive competitors (Combs et al., 2011) for rare and common taxa. In these studies, many of which compare endemic and widespread congeners, the noted demographic differences highlight the challenges that rare species face. Such a comparative framework can provide a better understanding of species characterized by narrow endemism, those that occur in one or a few populations and thus are confined to a single domain or a few localities (Kruckeberg and Rabinowitz, 1985). Furthermore, the use of a framework that examines rare–common differences in sympatric congeneric populations may be especially insightful because confounding factors such as differences in phylogeny, ecology, and local environmental factors can be controlled or reduced (Combs et al., 2013). Despite the applicability of this method, few studies have directly tested whether the process of seed dispersal differs for rare and common species (Pirie et al., 2000).

The framework of seed dispersal effectiveness (SDE; Schupp et al., 2010) can be used to compare dispersal-related outcomes of different plant species. This model specifies that poor seed dispersal may stem from ineffective quantitative and qualitative aspects of the seed dispersal process. For a given plant species, quantitative aspects of seed dispersal include numbers of seeds moved away from parent plants, while qualitative aspects pertain to survival through the dispersal process and facets of the deposition site that promote successful germination and establishment (Schupp, 1993). In the context of animal-mediated seed dispersal, the quantitative portion of SDE can be measured by recording numbers of seeds removed by partners during dispersal events, while qualitative aspects can be approached by identifying disperser preferences, the identity of disperser assemblages (knowing that some are better than others), the strength and resiliency of disperser assemblages (single vs. multiple effective dispersal agents), and potentially through quantification of dispersal distances. When shown to be ineffective, any of the above can contribute to lower overall SDE (Schupp et al., 2010). Comparing quantitative and qualitative aspects of SDE for sympatric rare and common congeners may reveal meaningful differences in aspects of seed dispersal, especially in cases where seed dispersal for both species is mediated by the same animal partners.

In this study, we employed field observations and laboratory experiments to investigate whether four aspects of seed dispersal effectiveness—numbers of seeds dispersed, dispersal distance, disperser preference, and interactions with dispersers—differed between sympatric populations of narrow endemic and widespread species of ant-dispersed (myrmecochorus) *Trillium* (Liliales, Melanthiaceae). Our focus on seed dispersal metrics is relevant given that seed dispersal connects the reproductive phase of adult plants to the establishment of the next generation (Wang and Smith, 2002) and that the seed life history stage is important to population growth rates of myrmecochores (Horvitz and Schemske, 1995). We hypothesized that metrics for endemic trilliums would more consistently indicate less-effective seed dispersal reflecting the quantitative and qualitative aspects of SDE than their sympatric widespread congeners. Specifically, we predicted that widespread trilliums would (1) have more seeds dispersed, (2) be dispersed longer distances, (3) be preferred by the keystone seed-dispersing genus of ants in eastern North America (*Aphaenogaster*; Ness et al., 2009), and (4) have more observed interactions than sympatric, narrow endemic trilliums with effective seed dispersers.

MATERIALS AND METHODS

Study species

Plants of the genus *Trillium*, a group of long-lived perennial understory herbs, encompassed our study system. Trilliums are perennial monocot rhizomatous herbs found in temperate deciduous forests of eastern Asia, western North America, and eastern North America (Freeman, 1975). Like other myrmecochores, *Trillium* seeds are characterized by the presence of elaiosomes: fleshy seed coat-borne appendages rich in lipids and other nutrients (see Turner and Frederickson, 2013). Trilliums flower from March to June, with flowering lasting 2-4 weeks, followed by the production of a single fruit containing a few to dozens of diaspores (collective unit of a seed with its attached elaiosome). Ripe fruits dehisce and drop mature diaspores beneath the maternal plant. Members of *Trillium* are among dozens of plant species in the southern Appalachian region that share a mutualism with *Aphaenogaster* ants, a keystone seed-dispersing genus in the mesic forests of eastern North America (Ness et al., 2009; Schultz, 2014).

At least 29 species of *Trillium* are native to eastern North America (Ohara, 1989), including 15 that are characterized as rare and are ranked as G3 or higher conservation priority (NatureServe, 2017). Some trilliums are also endemic in the sense that they inhabit restricted geographic regions or specific habitat types, and many of these co-occur with widespread congeners. For the purposes of this study, we used definitions of widespread species as those found throughout deciduous forests of the southeastern Appalachian region, and endemic species as those found only in restricted geographic localities or in specific habitats. Endemic/widespread status of the study species was determined using the NatureServe (2017) conservation status system coupled with maps of endemism generated by the Biota of North America Program (Kartesz and BONAP [Biota of North America Program], 2015), and was confirmed by *Trillium* experts (E. Schilling, University of Tennessee, Knoxville; T. Patrick, Georgia Department of Natural Resources, personal communications).

The widespread species used in this study, *Trillium cuneatum* Raf. and *Trillium catesbaei* Elliot., are globally and locally secure (NatureServe, 2017) and considered common in the counties in which they are found (see Kartesz, 2015). The endemic species, *Trillium decumbens* Harbison., *Trillium lancifolium* Raf., and *Trillium discolor* Wray ex Hook., are considered rare in the counties in which they are found, or, in the case of *T. lancifolium*, have patchy distributions throughout each state in their range (see Kartesz, 2015). The endemic species range from globally secure (*T. decumbens* and *T. discolor*) to globally vulnerable (*T. lancifolium*), and are

vulnerable, imperiled, or critically imperiled in the states in which they occur (NatureServe, 2017).

A number of ant species are known to disperse seeds of myrmecochores in eastern North America, including the trilliums that comprise our study species (Gaddy, 1986; Zelikova et al., 2011). Ants of the genus *Aphaenogaster* are the primary seed dispersal vector for most temperate ant-dispersed flora in eastern North America, approximately responsible for 74% of myrmecochore seed collection in eastern forests where encounters with *Aphaenogaster* have been reported (Ness et al., 2009). In the summer months, *Aphaenogaster* nests can be found in soil, decaying wood, under rocks, and in leaf litter. Nests are generally compact and shallow, and colony numbers range from 100 to 1000 workers, with a single queen (Lubertazzi, 2011). While *Aphaenogaster* are general scavengers, primarily subsisting on insects and other invertebrates, members of this genus collect and move the seeds of dozens of myrmecochorous plants in eastern North America (Lubertazzi, 2011). Such movement is especially likely in the early spring when other food items are less abundant, as has been noted for many temperate myrmecochorous systems (Thompson, 1981; Handel and Beattie, 1990; Ohkawara et al., 1997; Oberrath and Böhning-Gaese, 2002; Gorb and Gorb, 2003), including those in eastern North America involving *Aphaenogaster* (Warren et al., 2014). Workers collect diaspores by grasping the elaiosome in their mandibles and carry the diaspores to nests. Within nests, workers remove the elaiosome from the seed and feed it to the developing brood. Seeds that have had the elaisome removed are then moved outside the nest, either to seemingly random locations (Canner et al., 2012) or to a refuse pile (Rico-Gray and Oliveira, 2007; C. Kwit, personal observation). *Aphaenogaster* species are considered effective seed dispersers due to their high abundance; subordinate nature and ability to rapidly discover and retrieve seeds before superior competitors interfere; predictable foraging schedules, corresponding with myrmecochore seed release; and utilization of the elaiosome without harming seeds (Warren and Giladi, 2014).

Study sites and pairwise framework

We compared seed dispersal effectiveness of endemic and widespread trillium for three species pairs: Species Pair 1, *T. cuneatum* (widespread) and *T. decumbens* (endemic); Species Pair 2, *T. cuneatum* (widespread) and *T. lancifolium* (endemic); and Species Pair 3, *T. catesbaei* (widespread) and *T. discolor* (endemic). *T. cuneatum* was used as the widespread congener in Species Pairs 1 and 2 because it is ubiquitous throughout the southern Appalachian region and was sympatric with endemic species at multiple study sites. We located eight study sites in the southern Appalachian region containing sympatric populations of the three species pairs. Sites were unique patches roughly 50 m² and were separated from each other by at least 3 km. Seed dispersal metrics for Species Pair 1 was compared at three sites ranging in elevation from 210 to 282 m a.s.l., all in northwestern Georgia. Species Pair 2 was compared at three sites ranging in elevation from 218 to 252 m a.s.l., all of which were located in northwestern Georgia. Species Pair 3 was compared at two sites with elevations of 204 and 803 m a.s.l. on the western border between North Carolina and South Carolina. Only two sites were used to compare SDE for Species Pair 3 because we were unable to locate a third accessible site. See Fig. 1 for site map and coordinates.

All study sites are cove hardwood habitats, located in low-lying, mesic, deciduous forests with moderate to thick canopy cover. Study
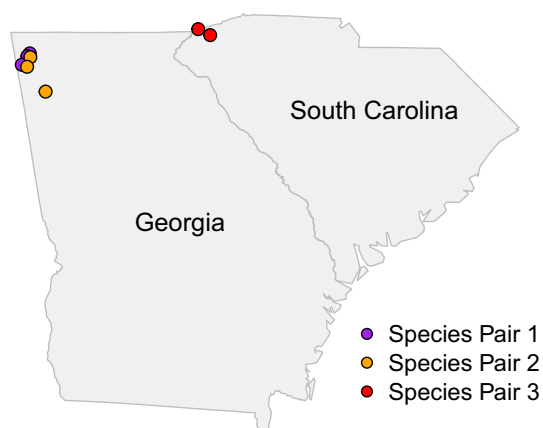

FIGURE 1. Study area and site map for the three *Trillium* species pairs. Purple indicates Species Pair 1 (N = 3 sites. Old Mine: 34.746400, −85.346250; Pocket Branch: 34.714230, −85.378670; Cave: 34.613990, −85.456680). Orange indicates Species Pair 2 (N = 3 sites. Butler: 34.30227, -85.12740; Blue Hole: 34.702330, −85.339170; Dallas: 34.59097, −85.38359). Red indicates Species Pair 3 (N = 2 sites. Jocassee Gorges: 34.95887, −82.85210; Whitewater Falls: 35.02855, −83.01711).

sites for Species Pairs 1 and 2 are located in the Limestone Valley soil province of Georgia, and sites for Species Pair 3 are located in the Blue Ridge soil province. Both soil provinces are characterized by mildly acidic soils high in phosphate. Sites for Species Pairs 1 and 2 are dominated by tulip poplar (*Liriodendron tulipifera*), American beech (*Fagus grandifolia*), and oak species (*Quercus* spp.). Sites for Species Pair 3 are characterized by tulip poplar, American holly (*Ilex opaca*), and eastern hemlock (*Tsuga canadensis*). All sites have understory layers characterized by red buckeye (*Aesculus pavia*), goldenseal (*Hydrastis canadensis*), and jack-in-the-pulpit (*Arisaeama triphyllum*), and other myrmecochorous herbs such as trout lily (*Erythronium americanum*), violets (*Viola* spp.), bloodroot (*Sanguinaria canadensis*), wood anemone (*Anemone quinquefolia*), and sharp-lobed hepatica (*Anemone acutiloba*).

Field observations and laboratory experiments

For the three species pairs, we recorded observational metrics of seed dispersal in the field, including numbers of diaspores (hereafter referred to simply as "seeds") dispersed and dispersal distances. We collected specimens of ants observed dispersing seeds from pairs at all sites, which we identified and used to qualitatively characterize interactions with disperser assemblages for each trillium species. Following field observations, we conducted "cafeteria" experiments in the laboratory with captive *Aphaenogaster picea* Wheeler colonies to determine disperser preference for seeds of endemic and widespread species.

Number of seeds dispersed and dispersal distances—In early May 2016, we opportunistically located and flagged 30–50 flowering individuals of each trillium species at respective sites. In late May–July 2016, we randomly selected 15 flagged individuals of each species for use in our observational study. At individual plants, we removed and manually dehisced the single ripe fruit. Fruits of species pairs at each site were generally at the same stage of ripeness at the time of these experiments, although the phenological window for peak ripeness may differ marginally for different species and individual

plants even at the same environmental locations. For logistical purposes, we removed fruits near or at peak ripeness in an effort to control when seeds were first exposed to the external environment and to identify definitive start times for our observations. Despite the possible marginal differences in ripeness, we conducted this work at a time of year when trilliums are known to start dropping or dehiscing their fruits in the southeastern United States (early summer), such that within 2 weeks of our observations, fruits of maternal plants of the same species at the same sites had naturally dehisced and dropped diaspores (C. Miller, personal observation). Before starting observations, we counted and recorded the number of seeds produced per fruit (and per plant, as trilliums produce one fruit per individual). We did not use this as a response variable to test the hypothesis that endemic trilliums experience lower SDE than widespread congeners, but we included it as a model covariate for assessing our response variables to account for variability in the sizes of seed piles available to ants. We then placed seeds under the maternal plant in the leaf litter and initiated an observational period of 1 h. This period was chosen because seed-dispersing ants, especially *Aphaenogaster,* exhibit the ability to remove myrmecochore seeds rapidly in nature and are adept at detecting such seeds (Ness et al., 2009), so it represented both a logistically feasible observational period and a sufficient timeframe to observe ant-seed dispersal.

During the observational period, we recorded the total number of seeds removed by ants, and the distances that ants carried seeds (for up to five seeds). Distances were then averaged per individual plant. We capped distance measurements at five seeds per individual because when ants removed more than one seed from a pile, they often did so simultaneously, which made tracking logistically challenging. Seeds were also almost always taken to the same location by the same ant colony (C. Miller, personal observation), making additional measurements redundant. When ants were observed carrying seeds into confirmed nests or when ants abandoned seeds in the leaf litter, the linear distance was measured in centimeters from the seed depot to the final location. If ants with seeds disappeared into the leaf litter, distance measurements were not recorded. During observations, we collected ant specimens observed interacting with seeds for use in recording trillium interactions with the ant disperser community (see below in the *Disperser communities* section). At the conclusion of each observational period, we left any remaining seeds at the base of the parent plant and removed all flagging.

To test whether there were significant differences in numbers of seeds dispersed by ants and in average distances of dispersal, we ran quasi-poisson generalized linear mixed effects models (PQL-GLMMs) for each species pair. PQL-GLMMs were used to deal with over-dispersion of the data (Ver Hoef and Boveng, 2007). Data from all study sites associated with a species pair were pooled, yielding sample sizes of 45 plants per species for Species Pairs 1 and 2 across three sites each and 30 individuals per species for Species Pair 3 across two sites (Fig. 1). A power analysis required at least 30 experimental replicates, but based on permit restrictions we were unable to sample more than 15 individuals of each species at each site, so we observed plants at multiple sites to increase overall sample size. Species (endemic vs. widespread) was the fixed effect in each model. We included site as a random effect in each model to account for environmental variability in the data, although it is important to note that because there is some association between sites and species pairs, this factor also includes the identity of a species within its respective category (endemic/widespread). PQL-GLMMs were conducted using the package MASS (Venables and Ripley, 2002) in R (v. 3.4.0; R Core Team, 2017). Variable significance was assessed using type II Wald chi-square tests in the package car (Fox and Weisberg, 2011) in R, which were run on full models. Variables were considered significant predictors if they yielded *P*-values less than $a = 0.05$.

We tested whether the number of seeds produced differed for endemic and widespread paired species using linear mixed effects models (LMEs) in R package lme4 (Bates et al., 2015). We included site as a random effect in each model as above. Variable significance was assessed using type II Wald chi-square tests as above. All subsequent statistical analyses were conducted using these analyses and R packages unless specified otherwise.

Disperser preference—We conducted cafeteria experiments in the laboratory to determine whether disperser preference for widespread or endemic seed species was apparent by presenting seeds of species pairs simultaneously to seed-dispersing ants. Cafeteria experiments enabled us to control for spatial heterogeneity, size of seed pile, and distance to ant nests. We collected fruits from five maternal plants of each species pair at peak dehiscence times from field sites, which we transported to the University of Tennessee, Knoxville (UTK) and froze at −20°C for 1–1.5 months. *Aphaenogaster picea* colonies were collected from the University of Virginia's Mountain Lake Biological Station (MLBS) grounds atop Salt Pond Mountain in Virginia in July 2016. This area did not contain populations of any of the *Trillium* species used in this study, so *A. picea* colonies were naïve to our study pairs; however, at least one trillium, *T. undulatum* Willd., is found at MLBS. We collected colonies using weathered nest boxes consisting of two pine boards loosely screwed together with grooves carved into the bottom board to allow for colonization. Nest box dimensions were 20.32 × 10.16 cm, with a width of 8.89 cm. We buried nest boxes in the leaf litter and checked them after 2 weeks. We then moved the colonies from the nest boxes to 25.40 × 15.24 cm plastic containers, with fine-mesh fabric glued into lids to provide airflow. We provided the colonies with artificial nests consisting of test tubes and cotton wrapped in aluminum foil for darkness. A petri dish was placed in each container, holding moistened cotton for a water source and ~0.5 g of standardized ant diet, modified from the Bhatkar–Whitcomb diet (Dussutour and Simpson, 2008). We kept colonies in artificial nests indoors from July to September, under ambient light and temperature conditions. Twice a week, we replaced dried or moldy nests with fresh test tubes and cotton and resupplied colonies with fresh water and food (Turner and Frederickson, 2013).

In cafeteria experiments in September 2016 at MLBS, all colonies were starved for 4 d before the experiment to ensure equal hunger levels. To control for colony size, which we anticipated might impact dispersal behaviors of individual ant colonies, we pruned each colony 4 d before the experiment by haphazardly selecting 30 workers and one queen (eliminating additional gynes and larvae), which were transferred into new plastic containers (Turner and Frederickson, 2013). Of 35 captive colonies, we presented seeds from Species Pair 1 to 15 colonies; seeds from Species Pair 2 to 12 colonies; and seeds from Species Pair 3 to eight colonies. To increase statistical power, we pooled all trials and categorized the endemic species in each pair as "endemic" and the widespread species in each pair as "widespread" so that we assessed the effect of seed identity, either endemic or widespread, on ant preference.

Experiments began by setting up seed grids that were presented to colonies. One seed of either an endemic species or its widespread congener was placed at each grid point in an alternating pattern for a total of 10 seeds each (Fig. 2) to eliminate spatial clumping and to control for numbers of seeds available. Grids were drawn on the top surface of petri dish lids using a permanent marker and ants were able to access grids by climbing on top of the lids. To differentiate endemic from widespread seeds, which are similar in appearance, we marked endemic seeds with a dot of yellow paint and widespread seeds with a dot of silver paint (Elmer's Painters Yellow and Silver Paint Markers, medium tip; Elmer's Products, Westerville, OH, USA). Paint marks were preliminarily tested for effects on ant seed dispersal and were shown to have no effect (C. N. Miller and C. Lash, unpublished data).

Seed grids were placed into colony containers 20 cm from the nest opening. In an observational period of 1 h (chosen to resemble our field observations), we recorded numbers of endemic and widespread seeds taken into nests and numbers of endemic and widespread seeds removed from grids but not taken into nests. Following the observational period, we calculated the proportions of endemic and widespread seeds (of 10) that fell into each of these categories. Colonies were revisited after 24 h, which enabled colonies to fully explore and discover seed piles, and the above measurements were repeated. Binomial generalized linear models (GLMs) were performed for data collected after 1 h and 24 h to determine whether seed identity was a significant predictor of either the proportion of seeds dispersed into nests or the proportion of seeds removed from the grid but not taken into nests. The effects of seed identity were assessed using type II Wald chi-square tests run on GLMs, and effects were considered significant if tests yielded a *P*-value less than $\alpha = 0.05$.

Following the initial 1 h observational period, individual seeds were assigned a behavioral score from 0 to 3, according to the level of ant interaction the seed experienced (Culver and Beattie, 1978). A score of 0 meant the seed was never discovered by ants; a score of 1 indicated discovery and handling, but no movement; a score of 2

FIGURE 2. Design for placement of endemic and widespread seeds on grids for cafeteria experiments. Gray dots and odd numbers indicate widespread seed species; white dots and even numbers indicate endemic seed species. Grids were drawn on the back of petri dish lids.

indicated removal from the grid, but not dispersal into the nest; and a score of 3 indicated dispersal into the nest. We counted the number of observed behaviors expressed by ants in each behavioral category for each seed and compiled these counts into a contingency table with *n* = 700 seeds (350 endemic, 350 widespread). Observed values were compared to expected values using a likelihood ratio test. Significance was considered at the $\alpha = 0.05$ level. The contingency table was created and analyzed using SAS Enterprise Guide (v.7.1; SAS Institute, Cary, NC, USA).

During the initial observation, numbers of *A. picea* workers handling endemic and widespread seeds was also recorded. At the end of 1 h, we calculated the average number of workers handling widespread and endemic seeds for each colony, and averages were rounded to the nearest whole number. We rounded averages because seeds cannot be handled by a fraction of a worker. This resulted in each colony receiving a single average worker integer for widespread and endemic seeds. Because of the relatively low number of ant–seed interactions observed during the initial 1 h (see *Disperser preference* section in Results below), the data were heavily skewed, and transformations did not yield normality. We therefore used a Poisson-distributed GLM to determine whether seed identity was a significant predictor of average number of workers handling seeds. Effects were assessed for significance using a type II Wald chi-square test as above.

Disperser communities—To qualitatively describe and assess whether the endemic and widespread *Trillium* species pairs interacted differently with disperser assemblages, we haphazardly collected specimens of ants witnessed dispersing seeds of each trillium species at each study site during field observations. Specimens were collected based on the criteria that they were among several workers observed removing diaspores from a given seed pile. We collected at least one specimen at each maternal plant we observed in this study, given that the plant experienced ant activity during the observational period. We attempted to collect at least one specimen of each distinguishable ant species visiting each plant. Mouth aspirators were used to collect specimens, which were then transferred to microcentrifuge vials and stored in 95% ethanol. Specimens (*n* = 54) were stored at room temperature for 5 months and then identified to species using the Mississippi State University Entomology Museum dichotomous key (MacGown, 2014). Identifications were independently confirmed by a second observer.

RESULTS

Number of seeds dispersed and dispersal distances

Our observations of numbers of seeds dispersed by ants in the field yielded results consistent with lower quantitative SDE for endemic *Trillium* species. We found that the number of seeds dispersed differed significantly for Species Pair 1 [$\chi^2(1) = 18.780$, $P < 0.001$], with the widespread *T. cuneatum* having greater numbers of seeds dispersed than the endemic *T. decumbens* (Fig. 3). The number of seeds available to ants (i.e., the number of seeds produced by an individual parent plant) was included as a covariate in each model, but was not a significant predictor of number of seeds dispersed for Species Pair 1, nor was the interaction between number of seeds available and species (*P* > 0.05, respectively). Species Pair 2 had
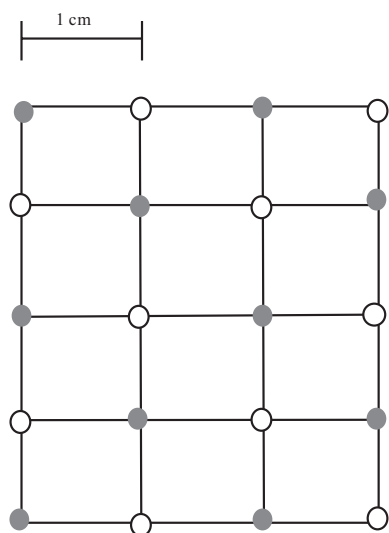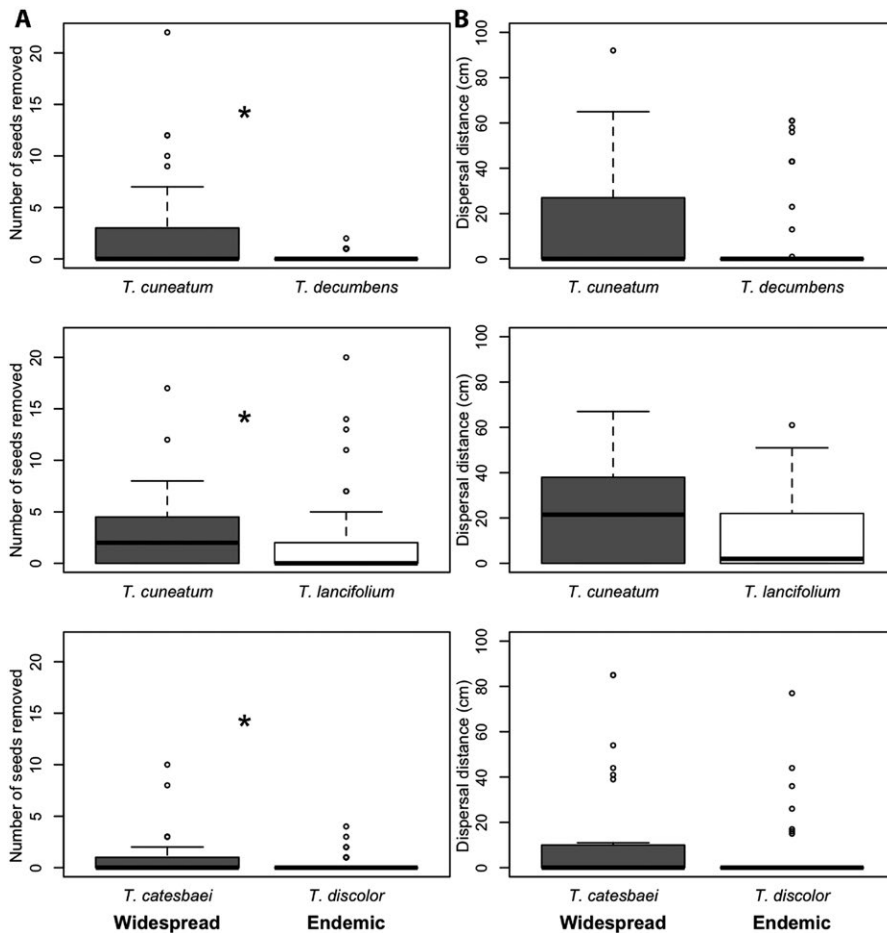
FIGURE 3. Box plots illustrating median, interquartile range, and outliers for (A) number of seeds removed and (B) dispersal distance (cm) for three *Trillium* species pairs. Columns indicate response variables; rows indicate pairs. Widespread species are gray; endemic species are white. Asterisks denote significant species effects. Widespread species had significantly more seeds removed by ants than endemic congeners did for all species pairs. Average linear dispersal distances were not significantly different for any species pair.

The number of seeds produced per maternal plant differed for endemic and widespread species in all three pairs. For Species Pairs 1 and 2, the widespread species (*T. cuneatum*) produced significantly more seeds than the endemic species (*T. decumbens* and *T. lancifolium*; $\chi^2(1) = 28.038$, $P < 0.001$; $\chi^2(1) = 11.055$, $P < 0.001$, respectively). For Species Pair 3, the endemic species, *T. discolor*, produced significantly more seeds than the widespread species, *T. catesbaei* [$\chi^2(1) = 18.717$, $P < 0.001$].

Disperser preference

We found no significant effect of seed identity (endemic vs widespread) on the proportion of seeds taken into nests after 1 h [$\chi^2(1) = 0.891$, $P = 0.345$; Fig. 4A]. There was also no significant difference in the proportion of endemic and widespread seeds removed from the grid, but not taken into the nest after 1 h [$\chi^2(1) = 0.005$, $P = 0.941$; Fig. 4B]. It should be noted that we observed very little ant activity of any kind during initial 1 h observational periods in the laboratory. We attribute the discrepancy between ant activity during 1 h observations in the field and laboratory to several possible factors. First, we conducted field observations during peak diurnal and annual foraging times in a natural setting, so ants were highly active in the field; the same was not necessarily true in the lab. Second, ants from many different colonies were active in the field, whereas in the lab, we were observing workers from one pruned colony per trial.

After 24 h, which provided captive colonies enough time to discover and interact with seeds in an artificial environment, we found that workers dispersed significantly greater proportions of the seeds of widespread trilliums into nests than endemic congeners [$\chi^2(1) = 12.380$, $P < 0.001$; Fig. 4A]. We did not find a significant difference in the proportion of seeds removed from the grid but not taken into the nest after 24 h for widespread and endemic trilliums [$\chi^2(1) = 1.070$, $P = 0.301$; Fig. 4B], although a trend shows that ants removed a higher proportion of widespread trillium seeds from the grid than endemic congeners. Results after 24 h suggest that *Aphaenogaster picea* prefer the seeds of widespread trillium species over their endemic congeners, a finding that is consistent with lower quantitative and qualitative SDE for endemic species.

Lower SDE for endemic species is also supported by the results of seed behavior scores recorded after the 1 h observations, which indicated that seed identity (endemic vs widespread) was significantly related to numbers of seeds scored for each of the four behavioral categories [$\chi^2(3) = 12.150$, $P = 0.007$]. Endemic seeds were more likely to be scored with a "0," indicating no discovery by ants, as indicated by relative row percentages ("0" made up 74.29% of total behavioral scores for endemic seeds, whereas "0" made up only 71.14% of total behavioral scores for widespread seeds). More widespread seeds were

significantly greater numbers of seeds dispersed for the widespread *T. cuneatum* than the endemic *T. lancifolium* (Fig. 3), with significant effects of both the number of seeds available and the interaction between number of seeds available and species [$\chi^2(1) = 8.344$, $P < 0.001$; $\chi^2(1) = 14.157$, $P < 0.001$, respectively]. Species identity alone was not a significant predictor [$\chi^2(1) = 1.790$, $P = 0.181$], so we attribute the difference in the number of seeds dispersed for the two species primarily to the effects of the number of seeds available, in conjunction with an additive species effect. For Species Pair 3, we found significantly greater numbers of seeds dispersed for the widespread *T. catesbaei* than the endemic *T. discolor* (Fig. 3). Species identity and the number of seeds available were significant predictors [$\chi^2(1) = 8.694$, $P < 0.001$; $\chi^2(1) = 5.0312$, $P = 0.025$, respectively], but their interaction was not ($P > 0.05$).

Our measurements of seed dispersal distances by ants in the field were not consistent with qualitative differences in SDE for widespread and endemic *Trillium* species pairs. None of the models indicated significant differences in linear seed dispersal distance from maternal plants for endemic and widespread species in any of the pairs ($P > 0.05$ in all cases; Fig. 3).

scored with a "1", suggesting that ants discovered and handled widespread seeds more often (22.57% of total seed scores and 21.43% of total seed scores, respectively). Endemic seeds were more likely to be moved from the seed grid and abandoned in a location other than the ant nest ("2" made up 3.43 and 1.71% of total behavioral scores for endemic and widespread seeds, respectively), and widespread seeds were more likely to be taken directly into nests than endemic congeners (4.57 and 0.86% of total behavioral scores for widespread and endemic seeds, respectively).

We found a nonsignificant effect of seed identity on average numbers of ant workers handling seeds [$\chi^2(1) = 0.022$, $P = 0.882$], suggesting no difference in average numbers of workers handling seeds of endemic and widespread species.

Disperser communities

Among 54 ant specimens collected and identified for our study system, 12 ant species were identified, representing seven genera in the family Formicidae across study sites. *Aphaenogaster rudis* had the most interactions with four of the five *Trillium* species (35), with *Aphaenogaster picea* having the second highest number (6), followed by the invasive Asian needle ant, *Brachyponera chinensis* Emery (3). All other ant species were represented by a single observed interaction. *Trillium cuneatum* had the most interactions in the network (31), followed by *T. lancifolium* (10), *T. catesbaei* (7), *T. decumbens* (5), and *T. discolor* (1). See Table 1 for a complete list of identified ant species and the number of interactions we observed for each *Trillium* species.

Because members of *Aphaenogaster* are the most effective dispersers in eastern North America and are thus linked to higher quality SDE, we emphasize here the observed number of interactions between widespread and endemic species and *Aphaenogaster.* Widespread trilliums had a higher number of interactions with members of *Aphaenogaster* than endemic trilliums (32 interactions and 9 interactions, respectively). Although interactions with ant dispersers were primarily characterized by *Aphaenogaster,* widespread species also were observed to interact with other non-keystone dispersing ants (6 of 38 total interactions), including *Camponotus chromaoides* Bolton*, Formica pallidefulva* Latreille*, and Nylanderia faisonensis* Trager. In one instance, the widespread *Trillium cuneatum* was found to interact with the invasive *B. chinensis,* which is an ineffective seed disperser that is known to disrupt the structure of native ant assemblages in eastern North America, and specifically has been shown to displace native *Aphaenogaster* species in invaded sites (Rodriguez-Cabal et al., 2012). The widespread *T. catesbaei* was observed to only interact with members of *Aphaenogaster.*

Roughly half of the interactions observed for endemic *Trillium* species were with non-*Aphaenogaster* species (7 of 16 total interactions), including members of *Camponotus* and *Crematogaster,* which are less-effective seed dispersers than *Aphaenogaster* species (Culver and Beattie, 1978). The endemic *T. decumbens* interacted
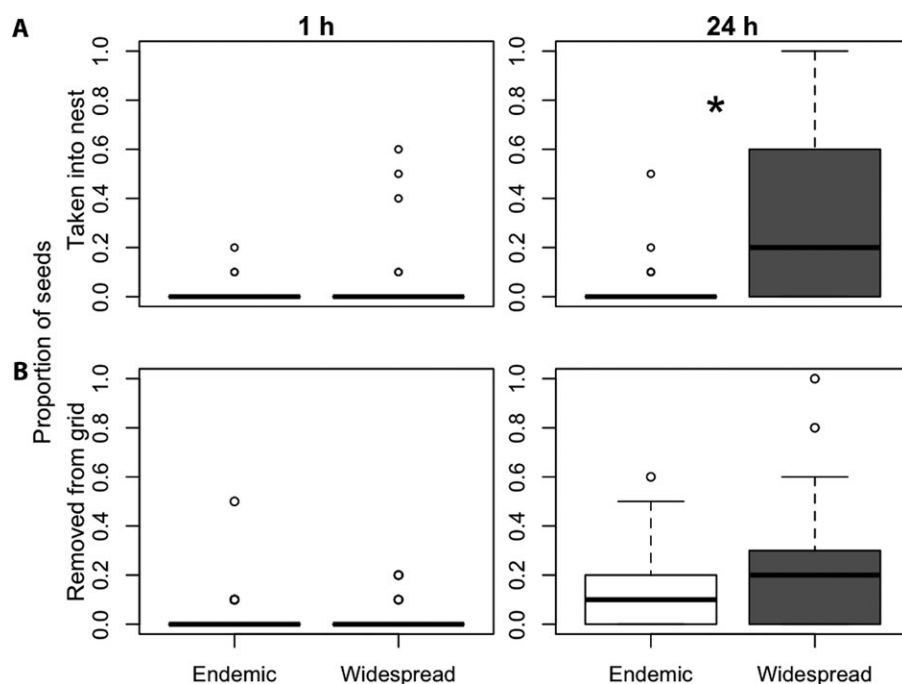

FIGURE 4. Box plots illustrating median, interquartile range, and outliers for (A) proportions of seeds taken directly into ant nests by workers in 1 h and 24 h, and (B) proportions of seeds taken off the seed grid but not into nests in 1 h and 24 h. Asterisks denote significant effects of species identity. A significantly greater proportion of widespread seeds was taken into nests after 24 h. Proportions of seeds taken directly into nests in 1 h did not differ significantly for endemic and widespread species. Proportions of seeds taken off the grid but not into nests did not differ significantly for endemic and widespread species after 1 h or 24 h.

primarily with *A. rudis* and had one additional observed interaction with *Camponotus americanus* Mayr. The endemic *T. lancifolium* interacted with both *A. rudis* and *A. picea,* and four other native species that are non-keystone dispersers. The endemic *T. discolor* was observed to interact with the invasive *B. chinensis.*

DISCUSSION

Our study yielded results that indicate lower quantitative and qualitative seed dispersal effectiveness in some respects for endemic *Trillium* species in comparison to their sympatric, widespread congeners. For three endemic-widespread species pairs across the southern Appalachian region, endemic species (*T. decumbens*, *T. lancifolium*, and *T. discolor*) had significantly fewer seeds dispersed by ants than did their widespread congeners (*T. cuneatum* and *T. catesbaei*), indicating the initial stage of the quantitative portion of SDE is lower for the endemic species. The results of our lab-based choice experiments provides a plausible explanation for our field results. After 1 h of exposure to seeds in the laboratory, *Aphaenogaster picea* behavior was significantly related to seed identity, with widespread seed species experiencing higher levels of discovery, handling, and dispersal by ants into nests than endemic species. After 24 h of exposure, *A. picea* workers dispersed significantly higher proportions of widespread seed species into ant nests. If *Aphaenogaster* species display a consistent preference for the seeds of widespread trilliums over those of their sympatric, endemic congeners in the field, then fewer seeds of the endemic species would be

TABLE 1. Ant specimens collected during field observations and associated *Trillium* species. *N* = number of specimens collected for each ant–plant species pair. The *A. rudis*–*T. cuneatum* pair was represented by the highest number of specimens collected (*N* = 24), followed by *A. rudis*-*T. lancifolium* (*N* = 5).

Ant species	Plant species	Site	*N*
Aphaenogaster rudis	*T. catesbaei*	Whitewater Falls (North Carolina)	3
A. rudis	*T. cuneatum*	Old Mine, Pocket Branch, Cave, Dallas, Butler (Georgia)	24
A. rudis	*T. decumbens*	Old Mine, Pocket Branch, Cave (Georgia)	3
A. rudis	*T. lancifolium*	Dallas (Georgia)	5
A. picea	*T. catesbaei*	Whitewater Falls (North Carolina)	4
A. picea	*T. cuneatum*	Old Mine (Georgia)	1
A. picea	*T. lancifolium*	Dallas (Georgia)	1
Camponotus americanus	*T. decumbens*	Cave (Georgia)	1
C. chromaiodes	*T. cuneatum*	Pocket Branch (Georgia)	1
C. pennsylvanicus	*T. lancifolium*	Dallas (Georgia)	1
Crematogaster ashmeadi	*T. decumbens*	Old Mine (Georgia)	1
C. pilosa	*T. lancifolium*	Dallas (Georgia)	1
C. pinicola	*T. lancifolium*	Butler (Georgia)	1
Formica pallidefulva	*T. cuneatum*	Old Mine (Georgia)	1
Temnothorax torrei	*T. lancifolium*	Butler (Georgia)	1
Nylanderia faisonensis	*T. cuneatum*	Old Mine (Georgia)	1
Brachyponera chinensis	*T. cuneatum*	Boat Ramp (Georgia)	3
B. chinensis	*T. discolor*	Jocasse Gorges (South Carolina)	1

dispersed. In this case, preference for seeds of widespread species by a keystone group of ants (*Aphaenogaster*) could also contribute to lower qualitative SDE for endemic species, with fewer of the latter seeds being dispersed at all, or more seeds being available for lower-quality non-*Aphaenogaster* ant species for dispersal.

This hypothesis is consistent with what we noted through qualitative comparisons of observed ant–trillium interactions in the field, where widespread trilliums appeared to interact more often with members of *Aphaenogaster* than endemic trilliums. Although primarily dispersed by members of *Aphaenogaster*, widespread species had a few connections with other non-keystone dispersing ants. Roughly half of the interactions of endemic species were with non-*Aphaenogaster* species, potentially indicating that endemic trilliums are more often dispersed by less-efficient dispersers than their sympatric, widespread congeners. Widespread species also had a more total interactions than did endemic species, providing further support for the hypothesis that endemic species experience lower qualitative SDE stemming from the frequency and composition of interactions with members of the disperser community. When coupled with the quantitative disadvantages that typify SDE of endemic trilliums in our study, this qualitative aspect of SDE may further accentuate the overall SDE experienced by endemic species. However, the scope of the conclusions drawn from ant–trillium interactions is limited, given the constraints of our sampling design. The number of specimens collected was low compared to the total number of observed interactions in the field, due to our reluctance to interfere with otherwise unmanipulated observations of seed dispersal. Additionally, the higher number of interactions recorded for *Trillium cuneatum* may be due to its inclusion in two species pairs.

We observed seed dispersal for this species at six sites, whereas the others were observed at three sites (*T. decumbens* and *T. lancifolium*) or two sites (*T. catesbaei* and *T. discolor*). Finally, our comparisons of trillium–ant interactions should be considered in light of the geographic separation of the three species pairs, which may explain some of the observed differences. Ideally, interactions with disperser communities would have been compared only within sympatric species pairs; however, due to the low number of specimens collected, some of these comparisons would not have been meaningful. Thus, although comparisons across the entire study system yielded interesting and potentially important insights, some of the results may be an artefact of overlapping distributions of particular ant species and *Trillium* species as reflected by the choice of study sites.

It is possible that significantly more seeds of the widespread *Trillium* species were dispersed in the field than those of their endemic congeners due to some non-random distribution of individual widespread trilliums and *Aphaenogaster* nests. Attractive myrmecochore seeds will ideally be collected by workers of the nearest nests and be dispersed to those nests until the colony is satiated; keystone dispersers generally remove >75% of available seeds (Zelikova et al., 2008; Ness et al., 2009; Warren et al., 2010). In this case, dispersal distances and numbers of seeds dispersed will be dictated in part by the spatial distribution of nests in relation to the spatial distribution of plants. It is unlikely that the locations of plants will be correlated with the locations of ant nests due to past ant–seed dispersal events because *Aphaengoaster* colonies move nests in the spring and often again in the summer (Lubertazzi, 2011). It is possible that *Aphaenogaster* colonies and these particular widespread *Trillium* species have correlated microhabitat preferences, such as lower soil moisture (Warren et al., 2010), that make their proximity more likely, thus explaining higher SDE for these species. Because we did not record locations of *Aphaenogaster* nests nor any microhabitat characteristics, we are unable to test this hypothesis. However, many of our results do not support this explanation. First, dispersal distances for widespread and endemic species in the field were not significantly different, suggesting that there was not a consistent trend for widespread trilliums to be located closer to *Aphaenogaster* nests. Second, the results of our laboratory experiments, which showed *A. picea* preferred the seeds of widespread species in the absence of differing proximities to ant nests and microhabitat characteristics, make the above explanation unlikely.

Aspects of diaspores (seeds, elaiosomes, or their combination) may explain our findings of lower SDE for endemic trilliums. Because ant–seed foraging is stimulated by the presence of oleic acid, which elicits a carrying response in ants, differences in quantities of oleic acid present in elaiosomes can affect disperser preference (Boulay et al., 2006; Turner and Frederickson, 2013), and thus dispersal success (Boulay et al., 2007). Total lipid content (Boulay et al., 2006) is a representative measure of elaiosome nutritional quality and may also play a role in shaping ant preference; seed dispersing ants can detect and preferentially disperse diaspores with greater nutritional rewards (Leal et al., 2014). Physical characteristics, such as elaiosome-seed mass, length, and width ratios, have also been shown to explain preference in seed-dispersing ants (Mark and Olesen, 1996), and generally these metrics are positively correlated with seed removal rates and dispersal distances (Davidson and Morton, 1981; Oostermeijer, 1989; Ruhren and Dudash, 1996; Gorb and Gorb, 2003; Peters et al., 2003; Bas et al., 2009; Warren et al., 2014). Gunther and Lanza (1989) specifically noted this for

members of *Trillium*. Additionally, some ant-dispersed plants display cheating behaviors by producing large quantities of cheap signaling compounds and provide little in the way of nutritional reward to ants (Pfeiffer et al., 2010; Turner and Frederickson, 2013). It is possible that the widespread species investigated in this study are characterized by physical and/or nutritional profiles that are more attractive, that they display cheating behavior more often than endemic species, or that a combination of these characteristics leads to the observed preference in *A. picea*. Exploring the chemical and physical variability in seed traits for co-occurring, related rare and common myrmecochores will contribute to our understanding of this mutualism in the context of plant rarity and may establish a mechanism explaining disperser preference for certain myrmecochore species. In turn, physical and chemical seed attributes may be linked to lower SDE for myrmecochores that produce diaspores and elaiosomes that are less attractive to ant partners.

Other studies have found that rare species are characterized by poorer measures of ecological and demographic success than widespread relatives. For example, studies have correlated plant rarity with poor competitive ability (Rabinowitz and Rapp, 1981; Prober and Austin, 1993; Baskin et al., 1997; Lynch et al., 1999; Walck et al., 1999), poor ability to survive disturbances before reaching reproductive maturity (Enright et al., 1996), and strong effects of habitat loss and fragmentation on seed-set and seed viability through impacts of pollinator availability (Cunningham, 2000) and pollen limitation (Brown and Kephart, 1999). The finding that endemic *Trillium* species may experience lower SDE than common, geographically widespread relatives adds another dimension to the literature comparing ecological attributes of rare and common and specifically endemic and widespread, plant species.

The seed life-history stage has the potential to contribute to patterns of endemism. Establishment limitation, which may be tied to aspects of seed dispersal, has been documented in some endemic plants (Dinsdale et al., 2000). In addition, endemic plants in the family Fabaceae have been noted to be more susceptible to predispersal seed predation than widespread, sympatric congeners, as well as being characterized by lower seed production (Combs et al., 2013). Similar findings elsewhere (Pirie et al., 2000) suggest that endemic plant species may be both intrinsically and extrinsically seed-limited, a conclusion potentially corroborated by our results. We found that some endemic trilliums exhibit lower fecundity by producing fewer seeds than their common congeners, a result that may or may not be consistent with lower intrinsic seed-limitation and which is in need of further testing, especially in light of the life-history strategies of narrow endemics. We also note the exception of Species Pair 3 to this conclusion, which may be credited to *T. catesbaei* being a declinate-flowered, pedicellate species and *T. discolor* being sessile (Ohara and Utech, 1986, 1988). Our results also provide a potential basis for extrinsic seed-limitation in endemic *Trillium* species as a consequence of poorer quantitative and/or qualitative aspects of the ant–seed dispersal mutualism, resulting in lower SDE. The latter result is, to our knowledge, novel in the context of rare–common plant comparisons.

Although we did not address whether post-seed dispersal demography differed for endemic and widespread trilliums, our results on seed dispersal patterns warrant further work in this area. In our study system, endemic trilliums had fewer seeds dispersed relative to sympatric widespread congeners, thereby having fewer seeds' elaiosomes removed—a seed treatment that has been shown to positively impact seed germination (Horvitz and Beattie, 1980;

Peternelli et al., 2003; Cuautle et al., 2005; Salazar-Rojas et al., 2012; Prior et al., 2014). Whether endemic species with low quantitative and qualitative aspects of SDE relative to other plant species experience lower emigration and colonization of new sites at local scales (e.g., meters) or larger regional scales (e.g., kilometers) is far from clear. In our study system, lower SDE stemming from seed dispersal by ants may be overcome by other long-distance forms of seed dispersal, such as dispersal by yellow jackets (Jules, 1996; Zettler et al., 2001; Bale et al., 2003) or white-tailed deer (Vellend et al., 2003; Myers et al., 2004). Regardless, successful dispersal of sufficient seeds to safe sites is of crucial importance to the persistence of metapopulations (Washitani et al., 1997), and therefore should be considered in conservation efforts for endemic plant species.

AUTHOR CONTRIBUTIONS

C.N.M. and C.K. conceived and designed the observations and experiments. C.N.M. performed the observations and experiments. C.N.M. analyzed the data. C.N.M. and C.K. wrote the manuscript.

DATA ACCESSIBILITY

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.778hc71 (Miller and Kwit, 2018).

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