

Mutualism fails when climate response differs between interacting species

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Abstract

Successful species interactions require that both partners share a similar cue. For many species, spring warming acts as a shared signal to synchronize mutualist behaviors. Spring flowering plants and the ants that disperse their seeds respond to warming temperatures so that ants forage when plants drop seeds. However, where warm-adapted ants replace cold-adapted ants, changes in this timing might leave early seeds stranded without a disperser. We investigate plant seed dispersal south and north of a distinct boundary between warm- and cold-adapted ants to determine if changes in the ant species influence local plant dispersal. The warm-adapted ants forage much later than the cold-adapted ants, and so we first assess natural populations of early and late blooming plants. We then transplant these plants south and north of the ant boundary to test whether distinct ant climate requirements disrupt the ant–plant mutualism. Whereas the early blooming plant's inability to synchronize with the warm-adapted ant leaves its populations clumped and patchy and its seedlings clustered around the parents in natural populations, when transplanted into the range of the cold-adapted ant, effective seed dispersal recovers. In contrast, the mutualism persists for the later blooming plant regardless of location because it sets seed later in spring when both warm- and cold-adapted ant species forage, resulting in effective seed dispersal. These results indicate that the climate response of species interactions, not just the species themselves, is integral in understanding ecological responses to a changing climate. Data linking phenological synchrony and dispersal are rare, and these results suggest a viable mechanism by which a species' range is limited more by biotic than abiotic interactions – despite the general assumption that biotic influences are buried within larger climate drivers. These results show that biotic partner can be as fundamental a niche requirement as abiotic resources.

Keywords: *Anemone americana*, *Aphaenogaster*, *Asarum arifolium*, climate warming, mutualism, myrmecochory, phenological synchrony, species distributions, species interactions

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Introduction

Timing is everything when species interact. Phenology is the study of seasonal life-history events, such as spring animal emergence and plant seed set. Shared phenological cues, such as rising spring temperatures, ensure that pollinators emerge when flowers open, herbivores feed when foliage flushes, and dispersers forage when propagules ripen (Burns, 2002; van Asch & Visser, 2007; Bartomeus *et al.*, 2011). Phenological synchrony requires that cooperating organisms initiate interactions at the same time, which requires that the seasonal behaviors of each participant share similar cues. Changes in those cues, such as warming, can disrupt mutualistic interactions if the partners respond individually (Cleland *et al.*, 2007), or if one partner is replaced by a warm-adapted species with different phenology (Urban *et al.*, 2012). As such, a great unknown in the ecological sciences is the future of

species interactions in a changing climate (Davis *et al.*, 1998; Agrawal *et al.*, 2007; Araujo & Luoto, 2007; Urban *et al.*, 2012).

Many early blooming wildflowers depend on early spring *Aphaenogaster* ants for seed dispersal (Ness *et al.*, 2009). Warm-adapted, low-elevation *Aphaenogaster rudis* ants have moved up the southern edge of the Appalachian Mountains and replaced cold-adapted, high-elevation *A. picea* ants in many locations (Warren & Chick, 2013) – the upward shift corresponding to a regional warming in minimum temperatures. The distribution boundary between *A. rudis* and *A. picea* is consistent with *A. picea*'s ability to tolerate minimum temperatures ca. 2 °C lower than *A. rudis* (Warren & Chick, 2013), a difference reflected in the broad-scale distributions of the species as *A. picea* is a distinctively northerly, high-elevation species (Umphrey, 1996; Warren *et al.*, 2011b). These thermal tolerances not only influence the ant distributions but also influence the ant behaviors. *Aphaenogaster rudis* requires 6 °C higher spring temperatures than *A. picea* to break winter

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diapause and begin foraging. As a result, *A. rudis* begins foraging about 4 weeks later than *A. picea* – too late to overlap with seed set by early blooming woodland herbs (Warren *et al.*, 2011a). We build on these findings by examining the consequences of species-specific *Aphaenogaster* foraging phenology on local dispersal of early and late blooming plants. Our study is novel because it tests how failed dispersal synchrony affects local plant population and distributions.

We ask whether dispersal synchrony fails for early (*Anemone americana*) or late (*Asarum arifolium*) blooming plants north and south of the *A. rudis*/*A. picea* boundary in north Georgia, USA. Given that seedlings cluster around parent plants when ant-mediated seed dispersal fails due to natural (e.g., high soil moisture, Warren *et al.*, 2010) or experimental (e.g., ant enclosures, Zelikova *et al.*, 2011) disruption, we expect greater plant aggregation in early (*A. americana*) than late seed set (*A. arifolium*) plant populations if *A. rudis* synchrony fails at the southern sites. Given that observations of natural populations cannot fully decouple abiotic and biotic influences on plant distributions (Warren & Bradford, 2011), adult *A. americana* and *A. arifolium* plants translocated north of the *A. rudis*/*A. picea* boundary allow us to test whether the ant-dispersal synchrony affects seedling aggregation. At the northern site where *A. picea* forages early enough for effective dispersal of both species, we expect lesser seedling aggregation around transplanted adult plants, whereas we expect greater seedling aggregation around early blooming *A. americana* (but not late blooming *A. arifolium*) adults at the southern site where *A. rudis* forages too late for effective dispersal.

Materials and methods

Plant species

Anemone americana (DC.) H. Hara (Ranunculaceae) occurs from northern Georgia to Nova Scotia, west to Alabama and Montana. It is also widespread in Asia and Europe. Endangered disjunct populations of *A. americana* occur south of its range edge in north Florida (USDA, NRCS, 2012) in refugia that harbor glacial relicts (Delcourt, 2002). Even in the southern edge of the contiguous range, *A. americana* populations are far more regionally patchy than 200 km north where the plant essentially is contiguous (USDA, NRCS, 2012). *Asarum arifolium* Michx. (Aristolochiaceae) is limited to the southeastern USA from Florida to Virginia, North Carolina to the Mississippi River, and its regional distributions are much less patchy than those of *A. americana*. Both species are small (10–15 cm tall), long-lived (50–100 years) woodland herbs most common in mature, mesic deciduous forests of eastern North America. Neither species produces clonal offspring or seed banks. *Anemone americana* produces ca. 28 small seeds (ca. 0.8 mg,

dry weight) and blooms regularly; *A. arifolium* produces ca. 10 large seeds (ca. 3 mg) and blooms intermittently. Although *A. americana* produces relatively small seeds that provide little nutritional benefit for ants that collect them, the seeds contain the same chemical cues as more attractive seeds that prompt dispersal by seed-dispersing ants (Pfeiffer *et al.*, 2009), particularly members of the *Aphaenogaster rudis* complex, which includes both *A. rudis* and *A. picea*.

Anemone americana (formerly *Hepatica nobilis*) reaches its equatorial limit in the southeastern USA where it exists in patchy, waning populations (Warren, 2010; Warren & Bradford, 2011). By comparison, *Asarum arifolium* (formerly *Hexastylis arifolia*) is a southeastern USA endemic, and its populations and individuals are more widespread (Warren, 2007, 2008; Warren & Bradford, 2011). These distribution patterns suggest the southeastern USA habitat is more suitable for *A. arifolium*, but local- and regional-scale transplant experiments indicate that *A. americana* survives, reproduces, and recruits better in the southeastern habitats than *A. arifolium* (Warren, 2010; Warren & Bradford, 2011). The success of *A. americana* transplants suggests that it is not directly limited by abiotic conditions in the southeastern USA, highlighting that biotic interactions might explain its apparent demise there.

Study sites, experimental design, and plant measures

Natural *A. americana* and *A. arifolium* populations were surveyed at Whitehall Forest (WHF) in Clarke County, Georgia, USA (33°53'N, 83°21'W; 150–240 m elevation, 122 cm mean annual precipitation, 17 °C mean annual temperature) in 2006 as part of a long-term, demographic research project (Warren *et al.*, 2007), with abundance sampled in 480 m² grids divided into 4 m² cells. We also collected 144 reproductive-sized adults of each plant species at WHF in 2006, and half of each species were translocated at WHF (approximately 50 km south of the *A. rudis*/*A. picea* boundary) and the other half approximately 50 km north of the *A. rudis*/*A. picea* boundary at Coweeta Hydrologic Laboratory (CWT) in Macon County, North Carolina, USA (35°03'N, 83°25'W; 750–1025 m elevation, 183 cm mean annual precipitation, 13 °C mean annual temperature). At each location, we established six experimental grids (5 × 7 m, Fig. S1) at similar slope degrees (mean = 17.5°), three across north- and three across south-facing slopes ($n = 12$ total grids). One individual of each species was placed at random in 12, 1 m² cells within each grid, leaving 1 m² spaces between each cell. This resulted in 12 experimental grids (six at WHF and six at CWT) with 144 occupied cells (12 cells × 12 grids), with each containing an individual of each species. The WHF and CWT sites were located in relatively mature (ca. 80 years) oak-hickory forests (see Warren, 2010; Warren *et al.*, 2011a; Warren & Bradford, 2011 for additional details).

In June 2011, we surveyed the transplant grids for offspring from surviving transplants which subsequently produced seeds for 5 years (i.e. after spring 2006 translocation). Offspring were classified as seedlings (distinct dicotyledons for both species), juvenile (leaf area: *A. americana* <9 cm²; *A. arifolium* <3 cm²), and adult (leaf area: *A. americana* >9 cm²;

A. arifolium >3 cm²) (Warren, 2007). None of the offspring were of reproductive size (based on leaf area: *A. americana* >30 cm²; *A. arifolium* >25 cm²) (see Warren, 2007). We investigated offspring aggregation by distance and polar angle from putative parents. Offspring were associated with the nearest transplant within 1.5 m. This distance was appropriate as mean local ant-seed transport at WHF is ca. 50 cm (Giladi, 2004; Zelikova *et al.*, 2011). Given these short dispersal distances, the potential for nontransplant seed contamination was minimal: all grid sites were located at least 500 m away from extant populations of the study species, except for two grids at WHF which occurred ca. 10 m away from *A. arifolium* plants.

Ant species and measurements

The key seed-dispersing ants in eastern North American woodlands occur in the *Aphaenogaster* genus (Beattie & Hughes, 2002; Giladi, 2006; Ness *et al.*, 2009). *Aphaenogaster* spp. are exceedingly abundant in eastern deciduous forests (Lubertazzi, 2012), and they account for ca. 75% of removed seeds (Ness *et al.*, 2009; Warren *et al.*, 2010). They also are more 'effective' dispersers than other scavenging ants as they usually disperse the seeds farthest (typically up to 1 m) and damage seeds the least (Giladi, 2004; Ness *et al.*, 2009; Zelikova *et al.*, 2011). Most seed-dispersing *Aphaenogaster* spp. belong to the 'rudis complex,' sibling species which form a cryptic and often difficult to distinguish group (Creighton, 1950; Umphrey, 1996; Ness *et al.*, 2009). *Aphaenogaster rudis* and *A. picea* are almost indistinguishable species, and they often are identified by geographic location rather than morphology, or get lumped together as part of the 'rudis complex.' Indeed, the only notable ecological difference between the two species appears to be greater cold tolerance by *A. picea* (Warren *et al.*, 2011a, b). We take advantage of the difference in cold tolerance, which results in later spring foraging by *A. rudis* than *A. picea*, to examine the consequence of dispersal asynchrony on plant dispersal. To examine *Aphaenogaster* spp. foraging phenology in conjunction with the transplant dispersal patterns, we placed four tuna bait stations at the corners of each 35 m² grid ($n = 48$ total). The bait stations were monitored for 90 min weekly March–June 2010 (Warren *et al.*, 2011a) and monthly March–June 2011. The abundance and identity of all ants visiting the tuna baits were recorded. *Aphaenogaster picea* and *A. rudis* individuals were differentiated by morphological traits and geographic range. Voucher specimens were deposited in Warren's collection at SUNY Buffalo State. We note that tuna bait stations are excellent for monitoring foraging phenology in scavenging ants, but likely overestimate the diversity of seed-dispersing ants (Sanders & Gordon, 2000).

Data analysis

We tested for overdispersion in the abundance of *A. americana* and *A. arifolium* individuals in the natural populations measured in grid cells using the 'gcc' package (Scrucca, 2004) for the 'R' statistical program (R Development Core Team, 2013).

Overdispersion in Poisson distributed data indicates whether the individuals are clustered or dispersed (Cox & Lewis, 1966), and we determined overdispersion in the data by comparing observed variance to theoretical variation based on the Chi-square distribution. We used generalized linear mixed models (GLMMs) to analyze the distance and angle of offspring from transplants. The GLMM structure is well suited for unbalanced data collected in a hierarchical manner. We evaluated offspring distance and angle assuming Poisson error distributions with species identity (*A. arifolium*, *A. americana*) and location (WHF, CWT) as fixed effects and transplant grid as a random effect. Because of overdispersion ($\Phi > 10$) in both models, we used lognormal Poisson models with individual-level random effects. The mixed models were fit using the Laplace approximation in the 'lme4' package for the 'R' statistical program (R Development Core Team, 2013). For GLMM analysis, the angle from transplant to seedling was converted from the 0–360° compass azimuth to a 0–180° linear scale so that values 0–90° were offspring angled upslope from transplants and values 90–180° were angled downslope, using the absolute value of 180° (azimuth-180°). The distance (cm, r) and angle (degrees, θ) between adult transplants and putative offspring were converted to Cartesian coordinates, x [$r \cos(\theta)$] and y [$r \sin(\theta)$] for aggregation analysis and graphing. Aggregation also was analyzed using Ripley's K function and nearest-neighbor analysis in the spatstat package (R Development Core Team, 2013). We considered coefficients with $P < 0.05$ significant and coefficients with $P < 0.10$ marginally significant (sensu Hurlbert & Lombardi, 2009).

Results

We looked at plant clustering in the co-occurring natural *A. americana* (919 individuals) and *A. arifolium* populations (1180 individuals) surveyed in 2006. *Anemone americana* plants were more often absent (78% empty) from individual cells than *A. arifolium* (23% empty) and, when present, *A. americana* occurred at densities (max. = 40 plants m⁻²) (Fig. 1a) more than double that of *A. arifolium* (max. = 17 plants m⁻²) (Fig. 1b). Overall plant density (mean plants m⁻²) was somewhat similar between *A. americana* (2.55) and *A. arifolium* (3.28), but *A. americana* SD m⁻² (7.22) was much higher than *A. arifolium* (3.88), suggesting overdispersed count data. Clumped occurrence data create overdispersion in Poisson distributed count data, and greater overdispersion is consistent with data containing more points containing extreme low and high counts compared to the mean (Cox & Lewis, 1966). As both plants are ant dispersed, the data were expectedly overdispersed, but *A. americana* overdispersion ($\phi = 40.81$, $\chi^2 = 7305$, $P < 0.001$) was much greater than for *A. arifolium* ($\phi = 9.19$, $\chi^2 = 1647$, $P < 0.001$). The spatial patterning is consistent with greater natural aggregation in the early-seeding *A. americana* compared to the later-seeding *A. arifolium* populations.

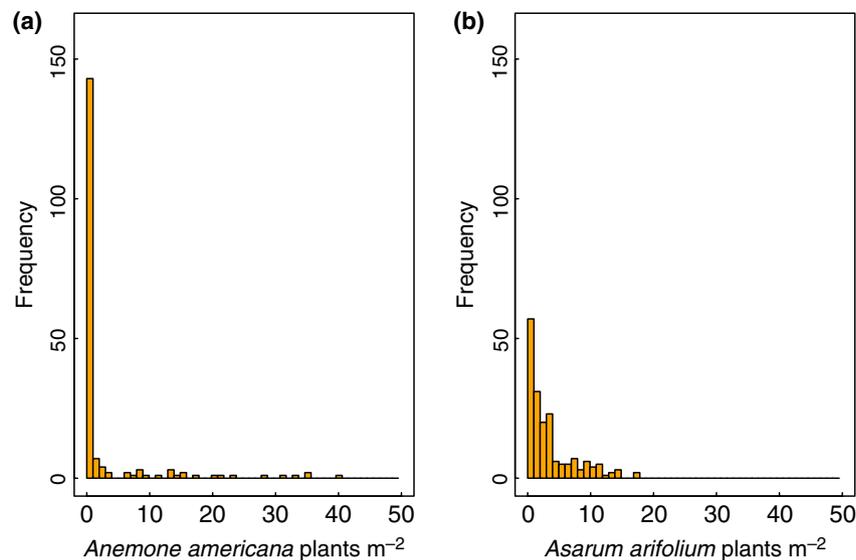


Fig. 1 Histograms showing abundance per m^2 in natural populations of the early-seeding (*Anemone americana*) and late-seeding (*Asarum arifolium*) species. Greater aggregation in the early-seeding species is consistent with dispersal failure.

The adult experimental transplants (*A. americana*: 110 survivors from translocation in 2006; *A. arifolium*: 95 survivors) produced 116 offspring during the 5-year span between transplanting and offspring surveys. Of these, 22.4% of the offspring were seedlings (*A. americana*: 21.6%; *A. arifolium*: 0.8%), 47.5% juveniles (*A. americana*: 21.6%; *A. arifolium*: 25.9%), and 30.1% adults (*A. americana*: 27.5%; *A. arifolium*: 2.6%). A species \times location interaction term (coeff. = -0.576 , SE = 0.340 , $z = -1.697$, $P = 0.09$) indicated that propagules travel similar distances for both species and sites except *A. americana* at WHF. Specifically, *A. americana* offspring were dispersed $2 \times$ further away from the adult plants at CWT (46.7 ± 26.3 cm) than at WHF (23.6 ± 17.2 cm), whereas *A. arifolium* offspring travelled similar distances (mean \pm SD) from the transplants at both WHF (42.0 ± 23.9 cm) and CWT (41.3 ± 21.4 cm).

All of the experimental grids were located in sloped habitat so that propagule movement might occur even without active ant dispersal, but such passive dispersal would be expected to only be downslope from parental plants. The mean (\pm SD) angle of offspring dispersal generally was equal or upslope from transplants at CWT for *A. americana* ($91.0 \pm 50.7^\circ$) and *A. arifolium* ($71.1 \pm 33.6^\circ$), and at WHF for *A. arifolium* ($80.8 \pm 45.0^\circ$), but a significant species \times location interaction term (coeff. = 1.511 , SE = 0.802 , $z = 1.884$, $P = 0.06$) indicated that *A. americana* offspring at WHF generally were significantly more downslope from adults ($126.5 \pm 28.3^\circ$) (Fig. 2).

We analyzed the number of offspring in the radius of adult transplants (i.e., offspring clustering) using

Ripley's K function to further explore aggregation in the offspring distributions. The analyses indicated that all of the offspring were more clumped than expected for a random distribution, but *A. americana* at WHF appeared far more clumped (Fig. S2a) than *A. arifolium* at WHF or either species at CWT (Fig. S2b–d). Similarly, nearest-neighbor analysis indicated that *A. americana* offspring at WHF occurred approx. $2 \times$ as close to other *A. americana* offspring than for either plant species at CWT (Fig. 3). These analyses collectively show that the offspring from *A. americana* at WHF (the southern location) occurred relatively closer to the parent and mostly downslope (Fig. 2a), whereas the *A. americana* offspring at CWT, and *A. arifolium* at both locations, occurred further from parent plants and in all directions (Fig. 2b–d).

Eleven ant species and 3066 individuals were observed at the foraging stations across spring 2010 and 2011. At both sites, the tuna bait stations were visited most by *Prenolepis imparis* ants (66%), which are small ants that aggressively recruit members to food resources but often ignore myrmecochorous seeds or consume the elaiosomes without dispersing the seeds (Giladi, 2004; Ness *et al.*, 2009). The key seed dispersers, *Aphaenogaster* spp., were 9% of the ants visiting the stations and this low abundance is a product of their typical single vs. 'mob' foraging strategy. *Aphaenogaster rudis* was observed at the southern location (WHF) and *A. picea* at the northern location CWT. In both years, *A. rudis* ants were not observed at WHF until late April–early May, whereas *A. picea* foraged at CWT beginning in mid-March (Fig. 4). *Aphaenogaster picea* began foraging at CWT when

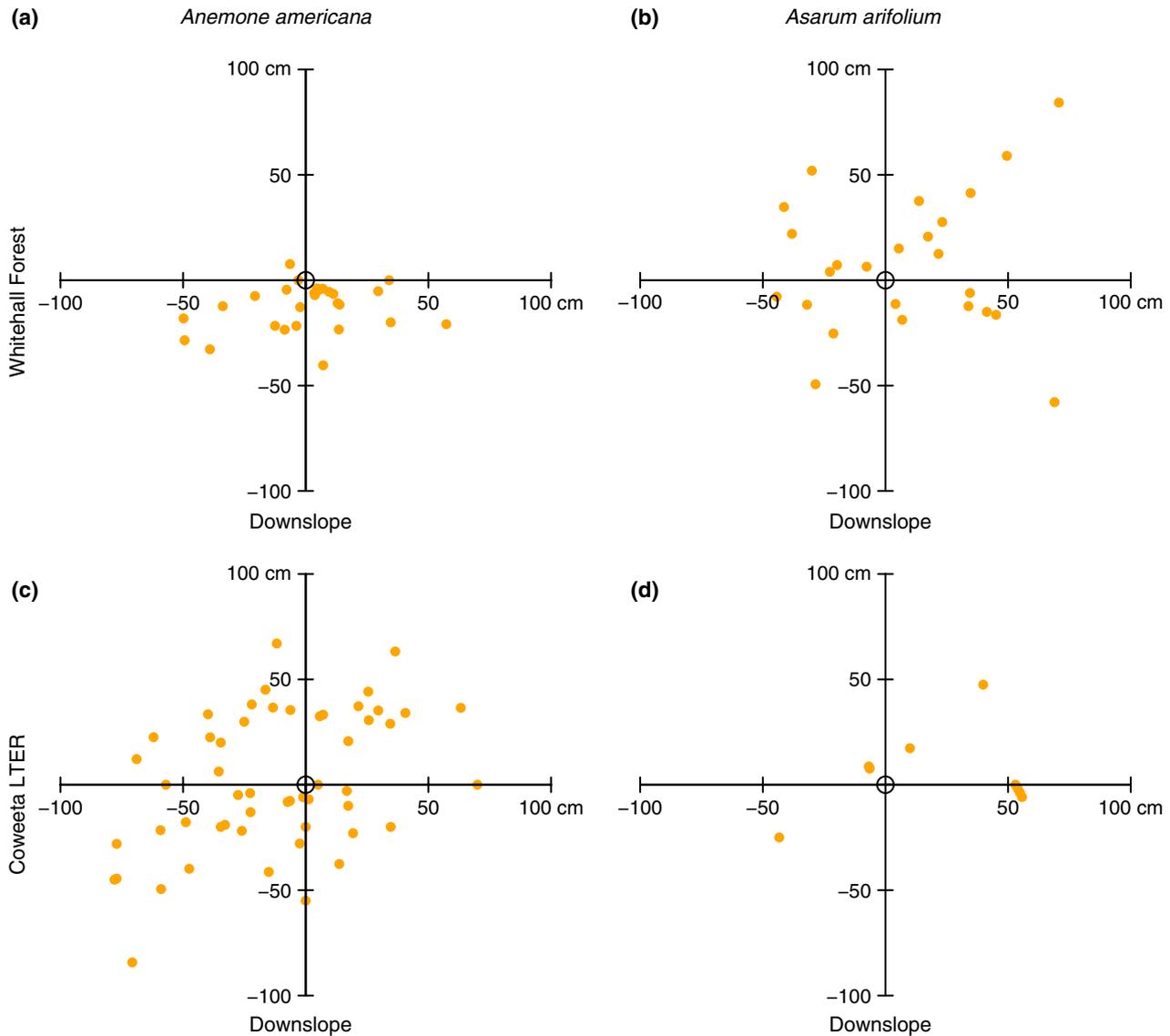


Fig. 2 The spatial distribution of *Anemone americana* and *Asarum arifolium* offspring (solid circles) 1 m² around parental transplants (empty circle at 0). Shown are all offspring and transplants from each species and site centered to a common point on each graphic. Note that negative locations on the *y*-axis indicate offspring found downslope from parental plants. The greater aggregation and downslope-only patterning of *A. americana* offspring at WHF (a) suggests only passive (gravity) dispersal whereas in all other cases active (ant) dispersal moved seeds greater distances and upslope against gravity (b–d).

minimum daily temperature was above 4 °C, but *A. rudis* began foraging at WHF only when this minimum was above 10 °C. Notably, *Aphaenogaster* ants were not active in early spring at WHF when *A. americana* dropped seeds, but they were active when *A. americana* dropped seeds at CWT. *Aphaenogaster* ants were active in late spring at both sites when *A. arifolium* dropped seeds (Fig. 4). We confirmed that *A. rudis* removes *A. americana* seeds if available later in the spring when *A. rudis* is active, by offering them in seed baits in June 2011.

Discussion

Habitat suitability cannot explain why *A. arifolium* populations thrive and *A. americana* populations falter in forests of the southeastern USA (Warren, 2008, 2010; Warren & Bradford, 2011). Instead, local *A. americana* clumping and patchiness is best explained by the plant's failure to synchronize seed release with spring foraging by the keystone seed-dispersing ant, *A. rudis*. The dispersal mutualism recovers, however, if we move *A. americana* 100 km north – crossing an

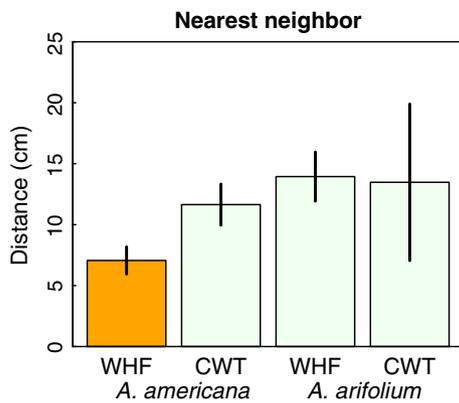


Fig. 3 Mean nearest-neighbor distance ($\pm 95\%$ CI) for *Anemone americana* and *Asarum arifolium* at Whitehall Forest (Georgia, USA) and Coweeta LTER (North Carolina, USA). The nearest-neighbor distance was calculated as the distance between each offspring and the closest occurring sibling offspring.

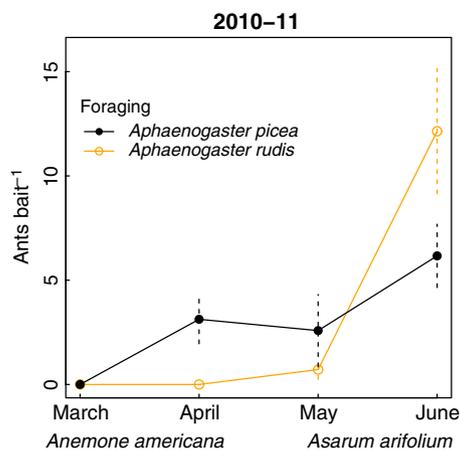


Fig. 4 *Aphaenogaster* ants per bait station ($n = 24$ per location, mean $\pm 95\%$ CI) during spring 2010 and 2011 at the southern location (closed circles: Whitehall Forest: WHF, Georgia, USA) and the northern location (open circles: Coweeta Long Term Ecological Research site: CWT, North Carolina, USA). *Aphaenogaster rudis* were observed foraging at WHF and *Aphaenogaster picea* were observed at CWT. Shown is the seed set window for *Anemone americana* and *Asarum arifolium* transplants (across both sites) for comparison with ant foraging. Some 2010 data were taken from Warren *et al.* (2011a). *Aphaenogaster rudis* only foraged in synchrony with the later-seeding *A. arifolium* plants whereas *A. picea* foraged in synchrony with both plant species.

A. rudis/A. picea distribution boundary – where *A. picea* begins spring foraging early enough to pick up *A. americana* seeds. Our data are novel because they extend observations of a failed interaction (i.e., seed dispersal) to consequences at the population level (i.e., seedling clumping). Reducing the negative consequences of increased density dependence (e.g., Janzen, 1970;

Connell, 1971) caused by the accumulation of seeds around parent plants is considered a major benefit of ant-mediated seed dispersal. Dispersed plant populations have lower intraspecific competition and pathogen accumulation, and improved gene flow (Janzen, 1970; Handel & Beattie, 1990; Gorb & Gorb, 2003; Giladi, 2006; Zhou *et al.*, 2007; Ness & Morin, 2008). Accordingly, the climate response of species interactions, and not just the component species themselves, is integral for understanding ecological responses in a changing climate.

Most plant species must interact with other species for effective seed dispersal (Hanzawa *et al.*, 1988; Cain *et al.*, 1998; Vander Wall, 2001; Burns, 2002; Herrera, 2002). Ant-mediated seed dispersal is a worldwide phenomenon involving >11 000 plant species (Lengyel *et al.*, 2009, 2010). Plants benefit from ant dispersal through reduced seed predation (e.g., Heithaus, 1981; Ness & Bressmer, 2005; Kwit *et al.*, 2012), placement in fertile microhabitat (Culver & Beattie, 1983; Wagner *et al.*, 2004), and lessened intraspecific competition (Handel & Beattie, 1990; Gorb & Gorb, 2003; Giladi, 2006; Ness & Morin, 2008). A clear sign of failed dispersal is the accumulation of offspring around parents, producing clumped patches (Giladi, 2004; Warren *et al.*, 2010; Zelikova *et al.*, 2011). Our data on plants and ants suggest then that the patchy *A. americana* populations at our southern site reflect declining population viability due to deleterious conditions caused by dispersal failure. Both species likely contracted into patchy, isolated patches during the height of cotton farming in the southeastern USA (1920s), but whereas *A. arifolium* has recolonized recovering secondary forests, *A. americana* remains isolated. Habitat limitation does not seem to explain the patchiness because *A. americana* exhibits greater phenotypic flexibility and survival than *A. arifolium* when transplanted into novel local and regional habitats (Warren, 2008, 2010; Warren & Bradford, 2011; Warren & Lake, 2013). Whereas dispersal generally is associated with long-distance colonization, the simple act of dispersing seeds away from parents is a critical demographic stage integral for population survival and so might explain *A. americana's* continuing patchy distribution (Kalisz *et al.*, 1999; Chlumsky *et al.*, 2013; Jones *et al.*, 2012; Montesinos *et al.*, 2006). That is, because local plant populations can fail when local dispersal fails (Brown *et al.*, 1996), the patchy *A. americana* distributions might reflect fragmentation by previous land-use and subsequent dispersal failure, localized extirpation due to dispersal failure or, most likely, both scenarios working at once. In any case, however, the underlying mechanism for patchiness is failed dispersal due to a lack of phenological synchrony with the local seed disperser.

Plant–ant seed dispersal mutualisms have long been considered diffuse interactions with multiple plant and ant species; however, recent work (Gove *et al.*, 2007; Ness *et al.*, 2009; Warren *et al.*, 2010) indicates that the mutualism is much more specialized – at least in some systems. In North American forests, 2–4 *Aphaenogaster* spp. are the dominant and most effective seed dispersers, even though other ant species occasionally pick up seeds (Ness *et al.*, 2009; Warren *et al.*, 2010). We focus on *Aphaenogaster* spp. at each of our study sites, leaving the possibility that a non-*Aphaenogaster* spp. might pick up early spring seeds. However, no active dispersal occurs from experimental transplants in the absence of *A. rudis* foraging at WHF, indicating no seed-dispersing species replaces *Aphaenogaster*, even though our tuna bait observations indicate that other ant species are active when *A. americana* drops seeds. Whereas North American seed-dispersing *Aphaenogaster* species often are lumped together as the *A. rudis* complex, Warren *et al.* (2011b) demonstrate that the member species likely have individual climate requirements. Additional work shows that *A. picea* tolerates colder temperatures and forages earlier in spring than *A. rudis* (Warren *et al.*, 2011a; Warren & Chick, 2013). Consistent with these findings, the distribution boundary between the species shifted upward in elevation with three decades of regional warming (Warren & Chick, 2013). *Aphaenogaster picea*'s more northerly distribution likely dropped into our southern study area during the last glacial maximum and returned northward toward its present range with interglacial warming (Fig. S3). If *A. picea*'s distribution is indeed moving northward, then the early-seeding *A. americana* populations at our southern location are presumably progressing toward extirpation.

Ant-dispersed plants typically migrate at a maximum 1–2 m yr⁻¹, with intermittent long-distance dispersal events (Gomez & Espadaler, 2013). We find with active dispersal *A. americana* and *A. arifolium* seedlings move a mean distance of 43 cm from parental plants (Fig. 2). Similar mean distances (49 and 47 cm) were reported for *A. arifolium* seeds offered to *A. rudis* at WHF by Giladi (2004) and Zelikova *et al.* (2011), respectively. Zelikova *et al.*'s (2011) experimental exclusion of ants reduced mean seed dispersal to 8 cm, whereas we found that without *Aphaenogaster* foraging, *A. americana* seeds dispersed 24 cm (mean) from parents. The difference likely is because our study grids were located on relatively steep slopes (mean: 17.5°), exacerbating passive dispersal through gravity and overland water flow, compared to the relatively level plots used by Zelikova *et al.* (2011) [Warren, pers. obs.]. In contrast to the plants, *Aphaenogaster* ants can migrate 50–100 m yr⁻¹ through aerial mating flights (Hölldobler & Wilson,

1990). The discrepancy in ant and plant mobility suggests that *A. picea* may have outpaced *A. americana* when migrating northward. Such disjunctive patterns might be expected at the trailing edge of a mutualism as species ranges shift northward (Thomas *et al.*, 2006). Species extirpation in peripheral range-edge populations usually is associated with unsuitable abiotic conditions (Lawton, 1993), but we instead demonstrate that extirpation may be caused by an unsuitable biotic environment (i.e., loss of an effective disperser). The possibility that biotic requirements might override abiotic requirements in species responses to climate change is a phenomenon not often considered (Rodríguez-Perez & Traveset, 2012; but see Moeller *et al.*, 2012). For example, we demonstrate that for early spring plant dispersal, the climate conditions that limit ant-mediated seed dispersal may be more important than those directly limiting the plants.

Given that dispersal allows species to track shifting suitable habitat, reduce aggregation, and maintain gene flow, effective dispersal is an integral component of population viability (MacDonald & Johnson, 2001). Plants depend on effective dispersal for population viability, and this is true both for long-range and local dispersal events (Brown *et al.*, 1996). The importance of local dispersal in maintaining populations suggests that the availability of *Aphaenogaster* to ant-dispersed woodland herbs should be considered an integral niche requirement. Whereas classic niche theory considers the presence of biotic interactions (e.g. competition) as limiting (Hutchinson, 1957), biotic interactions might instead be promoting (e.g. mutualism) and hence the loss of a 'biotic resource' therefore could limit populations (Bronstein, 1989; Stanton-Geddes & Anderson, 2011; Pelini *et al.*, 2009). Indeed, recent treatments of the niche consider some biotic interactions as essential requirements in Hutchinson's *n*-dimensional hypervolume (Pulliam, 2000; Bruno *et al.*, 2003), and hence a part of the fundamental niche as opposed to only part of the realized niche, as in the traditional fundamental/realized dichotomy. Interestingly, *Aphaenogaster* niche requirements spatially and temporally limit the distributions of the plants they disperse, highlighting that mutualisms have the potential to contract (Bronstein, 1989; Warren *et al.*, 2010) as well as expand (Bruno *et al.*, 2003) the niche – further challenging continued application of the fundamental/realized niche dichotomy. Indeed, as with pollinators and host plants (e.g., Bronstein, 1989; Pelini *et al.*, 2009), our results suggest that the presence and/or abundance of effective dispersers, and phenological synchrony, are as important as light, water and temperature in shaping a plant's niche.

We demonstrate a viable mechanism by which a species' range is more limited by biotic than abiotic conditions, an important finding considering that biotic interactions often are considered noise in species distributions (see Soberon & Nakamura, 2009). Considering that dispersal failure causes detrimental fitness consequences for individual plants at local scales, and that dispersal failure inhibits population persistence at broader scales, effective dispersers should be considered fundamental niche requirements. In this case, dispersal fails not because a viable mutualist partner is not available, but because phenological timing between partners is asynchronous.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Grid layout for transplant study. Gray cells contain translocated *Anemone americana* and *Asarum arifolium* plants.

Figure S2. (1a) Ripley's K function showing the expected number of offspring within the radius (r) of adult transplants for *Anemone americana* and *Asarum arifolium* at the southern location (Whitehall Forest: WHF, Georgia, US) and the northern location (Coweeta Long Term Ecological Research site: CWT, North Carolina, US). The second-order Ripley's K function describes how spatial interactions change with space.

Figure S3. Geographic suitable habitat projections for *Aphaenogaster rudis* (A & B) and *Aphaenogaster picea* (C & D) during the last glacial maximum, 21 000 years before present (A & C) and in current climate conditions (B & D).