



Uncommon specialization in a mutualism between a temperate herbaceous plant guild and an ant: are *Aphaenogaster* ants keystone mutualists?

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Ant-dispersed herbs (myrmecochores) can account for more than one-third of the stems in the temperate deciduous forests of eastern North America. Because many ant species have been observed collecting the seeds, this interaction is often described as a generalized mutualism. Here, we combine fieldwork and meta-analyses to test this assumption. Our meta-analysis demonstrated that *Aphaenogaster* ants (predominantly *A. rudis*) collect approximately $74 \pm 26\%$ (mean \pm SD) of the myrmecochorous seeds in eastern North American forests where any encounters with *Aphaenogaster* were reported, and approximately $61 \pm 37\%$ of the seeds in all the eastern forests where any seed collection has been monitored. This remarkable monopolization of seeds is due to at least two factors: 1) *Aphaenogaster* are significantly more likely to collect the ant-adapted seeds they discover than are ten other ant genera found in these forests and 2) the densities of *Aphaenogaster* and myrmecochorous plants are positively correlated at three nested spatial scales (within 20×20 m patches, among patches within a forest, and among 41 forests in the eastern United States). Although other ants can collect seeds, our analyses demonstrate that *A. rudis* is the primary seed dispersal vector for most of this rich temperate ant-dispersed flora. The low levels of plant partner diversity for myrmecochores demonstrated here rivals that of tropical ant-plants (myrmecophytes) and well exceeds that typically observed in temperate plant–frugivore and plant–pollinator mutualisms and myrmecochory in other biomes.

Ecologists have become increasingly interested in exploring the structure of interaction networks among species in a community. This exploration can link the frequency of interactions to their effect (Vazquez et al. 2005), highlight the incidence of asymmetrical specialization among the participants (Fonseca and Ganade 1996, Bascompte and Jordan 2007, Guimarães et al. 2007), and test the consequences of specialization (Sahli and Connors 2006). The networks for mutualisms, interactions that benefit both participant species, can be both highly structured and speciose (Fonseca and Ganade 1996, Bascompte et al. 2003, Bascompte and Jordan 2007, Blüthgen et al. 2007, Guimarães et al. 2007). However, the consequences of partner diversity are unclear (Stanton 2003, Vazquez et al. 2005, Sahli and Conner 2006). Specialization by one prospective mutualist on only one or a few partner species has been inferred to increase the vulnerability of the mutualist to perturbations in the partner populations (Pauw 2007). Complementarily, the adoption of a 'generalist' approach to mutualism by many exotic species has been invoked to explain their success in a variety of sites and habitats (Richardson et al. 2000). Some communities may also involve a series of highly asymmetric mutualisms,

wherein whole guilds rely on the resources provided by 'keystone mutualists' whose positive effects are disproportionately large relative to their abundance or biomass in the community (Gilbert 1980, Gove et al. 2007).

Plant–animal mutualisms involving some pollinators and/or symbiotic, plant-dwelling ants are widely believed to be more specialized than interactions between plants and seed dispersing frugivores (Wheelwright and Orians 1982, Blüthgen et al. 2007). Our study focuses on mutualistic seed dispersal by ants, an interaction known as myrmecochory. Ants collect myrmecochorous seeds to deliver an attached lipid-rich food reward, called an elaiosome, to their colony (Morales and Heithaus 1998). The elaiosome functions as a dead-insect analogue (Hughes et al. 1994), and, unlike granivory, its manipulation and consumption requires no obvious morphological adaptations by an omnivorous ant. Plant propagules benefit by dispersal from their maternal plant, delivery to particular microsites, and/or escape from rodent seed predators (Heithaus 1981, Giladi 2006). Myrmecochory is not rare; myrmecochorous species are found in >80 plant families, the guild can include 40% of the herbaceous species and 60% of emergent stems in portions of temperate deciduous forests

of the eastern United States (Beattie and Culver 1981, Handel et al. 1981) and densities can be similar in comparable biomes in northern Europe and Japan. Richness can be even greater in drier biomes in South Africa and Australia. The morphological features associated with myrmecochory have evolved at least twenty times in the monocots (Dunn et al. 2007). As befits such diversity, myrmecochorous seeds vary in a number of ways (Giladi 2006), including 1) shape and weight, 2) food reward (caloric and nutritional content of elaiosomes), 3) presentation (e.g. diurnal and monthly phenologies, passively dropped vs ballistically dispersed) and 4) histological origin of the elaiosome. This variation does not distinguish myrmecochory from other mutualisms; that myrmecochory exhibits the same range of specialization, generalization and among-site heterogeneity in animal partners as other plant mutualisms is a reasonable null hypothesis.

Here, we describe the variation in these ant–plant mutualisms within the temperate deciduous forests of North America, complementing recent macro-ecological studies of myrmecochory that focused on the Mediterranean (Rey and Manzaneda 2007) and Australia (Gove et al. 2007). We test the hypothesis that one particular ant, *Aphaenogaster rudis*, is a ‘keystone mutualist’ to myrmecochorous plants throughout the temperate deciduous forests of North America. Such a relationship has been implied by work in several sites in West Virginia (Beattie and Culver 1981), North Carolina (Zelikova et al. 2008) and Georgia, USA (Giladi 2004, Ness 2004). First, we contrast the likelihood of seed collection by *Aphaenogaster* with that by other North American ants. Second, we test the hypothesis that richness and density of myrmecochores in North American temperate deciduous forests are positively correlated with the distribution of *Aphaenogaster* ants within and among forests.

Last, we contrast the partner specialization reported for myrmecochory in North American temperate deciduous forests (the biome occupied by *Aphaenogaster rudis*) with that in other well-studied plant–animal mutualisms including pollination and frugivory in temperate biomes, myrmecochory in sclerophyllous habitats, tropical ant–plants and extrafloral nectary-bearing plants. Intriguingly, no study of myrmecochory we are aware of takes a community-wide perspective, one that would facilitate a description of the network structure of the ant–seed interaction web for the myrmecochorous plant and ant communities at a particular site and permit comparisons with other mutualistic networks (Bascompte and Jordano 2007). Although such comparisons have been made in reference to ant gardens (the formation of which involve seed collection by ants, although perhaps without seed-associated rewards; Youngsteadt et al. 2008) and ants collecting seeds from fleshy, vertebrate-dispersed fruits or frugivore feces (Blüthgen et al. 2007, Guimarães et al. 2007), neither of these are myrmecochorous in the conventional sense. Here, we quantify plant partner diversity (i.e. the richness and relative frequency of prospective plant mutualists) to test the hypothesis that myrmecochory in temperate deciduous forests is more specialized than an analysis of fruit/seed dispersal (Wheelwright and Orians 1982, Blüthgen et al.

2007) or a characterization of temperate biomes might otherwise suggest.

Methods

Likelihood and incidence of seed collection among ant genera

We used published descriptions of ant responses to myrmecochorous seeds (Appendix 1) to contrast *Aphaenogaster* to other North American temperate deciduous forest ant genera. We focus on genera rather than species because 1) some studies only identify genera and 2) several research groups (Beattie et al. 1979, Pudlo et al. 1980, Smallwood 1982, Ness 2004, Giladi 2004, Zelikova et al. 2008) acknowledge the difficulty of distinguishing sub-species within the *Aphaenogaster rudis* complex. All of these studies included in our analysis monitored seeds placed on index cards, and reported whether ants that walked on the cards removed the seeds. The definition of seed ‘discovery’ differed among studies (e.g. merely walking on the card vs antennating the seed). For our analysis, we identified any ant that walked on the card as a seed ‘discoverer’. We asked whether the proportion of discoverers that were also seed collectors differed among ant genera.

We found 1372 records of ant responses to myrmecochorous seeds ($n = 6$ plant species) in five published studies from North American temperate deciduous forests (Appendix 1). We pooled the records for each ant genus and all seeds to contrast total collection rates among ant genera. Because the responses may not be statistically independent (due to multiple collections by individuals from the same colonies, particular seed species only encountered by only a subset of genera, etc), we present the results but do not analyze them statistically.

Because the number of ant–seed observations differs among the aforementioned studies, the results are strongly influenced by the better-replicated studies. These may involve particular combinations of ant and plant species. To better describe the dispersal of a ‘typical seed in a typical forest’ where these interactions have been followed (irrespective of replication), we described the proportion of collected seeds that were dispersed by *Aphaenogaster* and other ant genera in these studies and, where possible, distinguished seed species by site combinations (Table 1).

Ant and plant densities within a forest

To test for correlations between ant and plant communities, we described ant and myrmecochore distributions within a four by four grid in 20×20 m areas in a 150-acre temperate deciduous forest in upstate New York ($43^{\circ}06'N$, $73^{\circ}47'W$) in June 2006. Grid points were separated by 5–7 m (a distance that well exceeds the median foraging range of most seed-dispersing ants in this site). At each grid point, we monitored the ants attracted to 4 g of tuna after 50 min and recorded the presence of *Asarum canadense*, *Erythronium americanum*, *Hepatica acutiloba*, *Sanguinaria canadensis*, *Trillium* spp. and *Uvularia grandiflorum* (the forest’s most common myrmecochores) within

Table 1. The proportions of myrmecochorous seeds collected by ant genera in studies performed in temperate deciduous forests of North America. Plant species by site combinations are used as replicates (in studies where sites can be distinguished based on published information). Sites judged to be unfavorable for ant dispersed plants are indicated by an asterisk. A period (.) indicates no collection reported for the genera. Ness 2004a, 2004b and 2004c refer to *Sanguinaria canadensis* in non-invaded forest interior sites, non-invaded forest edge sites, and sites invaded by the red imported fire ant, *Solenopsis invicta*, respectively. Giladi 2004a, 2004b and 2004c refer to *Hexatylis arifolia* in the 'main', 'ridge' and 'pine' sites, respectively. Beattie et al. 1979a and 1979b refer to studies for *S. canadensis* and *Hepatica acutiloba*, respectively. Heithaus 1986a, 1986b, 1986c, 1986d, 1986e and 1986f refer to *Asarum canadense* in sites WT, VS, BM1, BM2, AC and S6, respectively. Pudlo et al. 1980a, 1980b and 1980c refer to *S. canadensis* in Rocky Run, Cold Knob and Ginger Creek sites, respectively. Gunther and Lanza 1989a, 1989b and 1989c refer to *Trillium erectum*, *T. grandiflorum* and *T. undulatum*, respectively.

Study	<i>Aphaenogaster</i>	<i>Camponotus</i>	<i>Crematogaster</i>	<i>Formica</i>	<i>Lasius</i>	<i>Mymica</i>	<i>Pheidole</i>	<i>Prenolepis</i>	<i>Solenopsis</i>	<i>Stenemmma</i>
Ness 2004a	0.80	.	0.04	0.1
Giladi 2004a	0.81	0.01	0.01	0.01	.	.	0.04	0.13	.	.
Beattie et al. 1979a	0.67	.	.	0.18	0.12	0.02
Beattie et al. 1979b	0.23	.	.	0.57	0	0.2
Culver and Beattie 1978	0.66	.	.	0.01	0.07	0.17	.	.	.	0.01
Heithaus 1986a	1
Heithaus 1986b	1
Heithaus 1986c	0.84	0.11	.	.	0.05
Heithaus 1986d	0.75	0.17	.	0.04	.	0.04
Heithaus 1986e	0.92	.	.	0.08
Heithaus 1986f	0.92	0.08
Ruhren 1996	1
Pudlo et al. 1980a	0.74	0.01	.	0.21	0.02	0.03
Pudlo et al. 1980b	1
Gunther and Lanza 1989a	1
Gunther and Lanza 1989b	1
Gunther and Lanza 1989c	1
Zelikova et al. 2008	0.99	.	.	0.01
Gibson 1993	0.17	.	.	0.63	0.02	0.17
Ness 2004b*	0.67	0.06	0.02	0.24
Ness 2004c*	0.23	0.05	0.01	0.02	0.68	.
Giladi 2004b*	0.72	.	.	0.14	.	.	.	0.13	.	.
Giladi 2004c*	0.6	0.01	.	0.01	.	.	.	0.39	.	.
Pudlo et al. 1980c*	.	0.5	.	.	.	0.25	.	.	.	0.25

1 m (an area $\sim 3 \text{ m}^2$). Because tuna and elaiosomes both include diglycerides, a compound attractive to seed-collecting ants, tuna baits are often used to describe the seed-collecting ant communities (Mitchell et al. 2002, Heithaus and Humes 2003, Ness and Morin 2008). We chose the 50 min sampling duration because it is typically a sufficient time for baits to be discovered by both subordinate opportunists and more dominant ant species (those that typically discover seeds quickly and more slowly, respectively) although not so great in duration that dominators will monopolize all the baits (Ness and Giladi unpubl.). *Aphaenogaster rudis* is a subordinate opportunist (Fellers 1987, Andersen 1997), hence our sampling design offers a conservative description of the density of that ant. Sampling was repeated in 35 grids ('patches') within the forest, each separated by $> 100 \text{ m}$. More detailed descriptions of the study site are available in Ness and Morin (2008).

We used paired one-sided t-tests to test the hypothesis that the ant genera most often attracted to the baits (*Aphaenogaster*, *Myrmica*, *Camponotus* and *Leptothorax*) would be more often found at grid points within 1 m of

a myrmecochore than at grid points without a myrmecochore. We compared the proportion of baits occupied by each ant genus at baiting stations with and without myrmecochores within each $20 \times 20 \text{ m}$ patches, and paired these comparisons by patch. The analysis was limited to 19 patches after we omitted patches where no myrmecochores were found within 1 m of all baiting stations ($n = 12$) and those where all baiting stations included myrmecochores within 1 m ($n = 4$). For some of the ant species, this type of analysis was further restricted to < 19 replicates because not all patches included each ant species.

We used simple linear regressions and the data from the 35 $20 \times 20 \text{ m}$ patches to test the hypotheses that *Aphaenogaster* density and myrmecochore density were positively correlated among patches within a forest, and that myrmecochore density and diversity were not simply correlated with ant activity or ant richness. We estimated *Aphaenogaster* density as the proportion of the 16 baiting stations within each patch that were occupied by that ant, myrmecochore species density as the mean number of myrmecochore species within 1 m of a baiting station in the patch, ant activity as the total number of ant-bait

discoveries per patch, and richness (plant or ant) as the number of genera observed within the patch.

Ant and plant densities among forests

The meta-analysis included studies performed in 41 forests in North America including North Carolina (Mitchell et al. 2002), West Virginia (Beattie and Culver 1981), Ohio (Heithaus and Humes 2003, S. Philpott pers. comm.), New York (Ellison et al. 2007), and Massachusetts and Vermont (Gottelli and Ellison 2002). These studies were chosen because they provide data on 1) the identity of ants attracted to tuna baits and 2) herbaceous plant communities, and we used these data to identify the richness of myrmecochores and percentage of plant species that were myrmecochores within study plots. We treated forests as individual replicates. In studies where multiple forests were followed but only study-wide mean values were given (Mitchell et al. 2002, Heithaus and Humes 2003) we used the mean values for our analyses. When multiple plots were sampled within a single forest (either in space or time; Ellison et al. 2007, S. Philpott pers. comm.), we used the average values among plots for our analyses. We focused our analyses on the proportion of ant-discovered baits that were discovered by *Aphaenogaster* (hereafter $P_{A|D}$). We favor this proportion (rather than the proportion of all baits with *Aphaenogaster*) because it describes the ant communities observed in each study. Ants are ubiquitous, and we attribute the dissimilarity among studies in the proportion of baits that attracted any ants to the idiosyncratic sampling configurations and durations, abiotic conditions, or sampling periods of particular studies. We believe using $P_{A|D}$, rather than the proportion of all baits that attracted *Aphaenogaster*, diminishes the influence of these among-study differences. By necessity, this analysis relied on pitfall traps, rather than baiting data, for one study (Mitchell et al. 2002).

We used a χ^2 statistic to test the hypothesis that the incidence of myrmecochores and *Aphaenogaster* among forests were indistinguishable from random. We used one-sided t-tests to test the hypotheses that forests with myrmecochores would have a greater proportion of inspected baits discovered by *Aphaenogaster* (relative to forests without myrmecochores) and that forests with *Aphaenogaster* would have more myrmecochores (richness and proportion of the herbaceous community) than did forests where that ant was not detected. Proportions were arcsine transformed prior to analysis. Last, we used linear regressions to test the hypotheses that the richness of ant-dispersed plants, and the proportion of the plant community that is ant-dispersed, increases with the proportion of ant-discovered baits that attracted *Aphaenogaster*.

Plant partner diversity among comparable mutualisms

In an effort to contrast myrmecochory with other temperate deciduous plant-animal mutualisms and ant-plant mutualisms in other biomes, we used a crude estimate of the diversity of plant partners. We calculated the Shannon

diversity index (H') for the species of plant partners participating in mutualisms including 1) myrmecochory in the temperate deciduous forests of North America and Japan, 2) myrmecochory in other biomes, including central American rainforests and sclerophyllous vegetation in the Mediterranean, California, and Australia; 3) avian frugivory in temperate deciduous forests of Britain, 4) myrmecophytic ('ant-housing') plants in Central and South America and southeast Asia, 5) extrafloral nectary-bearing plants tended by ants, and 6) pollination in temperate deciduous forests of America (see Table 2 and Supplementary material Appendix 3). We sought to include studies that focused on multiple plant species, and only included studies and sites with at least five interactions with prospective partners (i.e. five individuals interacting with the plant, or at least five recorded visits) reported for a species. Where possible, we calculated the average among sites in instances where species had been studied in more than one location because pooling sites could inflate the perceived partner diversity experienced by plants.

Results

Likelihood and incidence of seed collection among ant genera

Aphaenogaster ants (always *rudis* when identified to species) collected 71% of the seeds they encountered. Average collection rates for the other ten genera were $18 \pm 14\%$ (mean \pm SD; Fig. 1). Three genera (*Crematogaster*, *Myrmecina* and *Prenolepis*) never collected seeds in these studies; collection rates for the seven other 'seed collecting' taxa were $26 \pm 9\%$.

If seed species by site combinations in temperate deciduous forests of North America are used as replicates (Table 1), *Aphaenogaster* collected $61 \pm 37\%$ (mean \pm SD) of the observed seeds. *Aphaenogaster* collected $74 \pm 26\%$ of the seeds in sites where the ant's presence is documented (i.e. where at least one collection by *Aphaenogaster* was observed).

Ant and plant densities within a forest

'Ant activity' (i.e. the sum of all ant species observations at the baits) was not significantly related to myrmecochore density ($F = 2.12$, $DF = 1,33$, $p = 0.16$) or myrmecochore species richness ($F = 0.84$, $DF = 1,33$, $p = 0.36$). Species richness of ants at the 20×20 m patch scale was not significantly related to myrmecochore richness ($F = 0.30$, $DF = 1,33$, $p = 0.59$) or myrmecochore density ($F = 0.09$, $DF = 1,33$, $p = 0.76$).

Aphaenogaster rudis ants were observed $>50\%$ more frequently at baiting stations within 1 m of a myrmecochore (one-sided paired t-test, $t = 2.33$, $DF = 18$, $p = 0.016$), relative to stations without a myrmecochore. *Leptothorax* exhibited a similar pattern, although that ant was found in fewer grids ($t = 1.41$, $DF = 7$, $p = 0.1$). Distributions of *Myrmica* and *Camponotus* supported the null hypothesis of no difference between the microhabitats ($t = 0.047$, $DF = 16$, $p = 0.96$ and $t = 2.88$, $DF = 4$, $p = 0.95$, respectively).

Table 2. Comparisons of partner diversity for different plant–animal mutualisms. Partner diversity was described from the plant’s perspective, and estimated using Shannon’s diversity index (H'). Only studies and sites with at least five reported interactions with prospective partners for a species were included. The average among sites was calculated in instances where species had been studied in more than one location within a study. References are described in the Supplementary material Appendix 3.

Mutualism	Reference	H (avg \pm SD)	Plant species	Location
Myrmecochory in temperate deciduous forests	Beattie et al. (1979)	0.94 \pm 0.05	<i>Hepatica acutiloba</i> , <i>Sanguinaria canadensis</i>	West Virginia, USA
	Culver and Beattie (1978)	1.12	<i>Viola</i> spp.	West Virginia, USA
	Gibson (1993)	1.67	<i>Malanpyrum lineare</i>	Michigan, USA
	Giladi (2004)	0.76 \pm 0.06	<i>Hexastylis arifolio</i>	Georgia, USA (3 sites)
	Zelikova et al. (2008)	0.007	<i>Hexastylis arifolio</i>	Smoky Mountains, USA (sum of 7 sites)
	Heithaus (1986)	0.31 \pm 0.30	<i>Asarum canadense</i>	Georgia, USA (sum of 5 non- <i>Solenopsis</i> invaded forest interiors)
	Ness (2004)	0.70	<i>Sanguinaria canadensis</i>	West Virginia, USA (6 sites)
	Pudlo et al. (1980)	0.60 \pm 0.39	<i>Sanguinaria canadensis</i>	Hokkaido, Japan
	Ohkawara et al. (1996)	0	<i>Erythronium japonicum</i>	Hokkaido, Japan
	Ohkawara et al. (1997)	0.52	<i>Corydalis ambigua</i>	Hokkaido, Japan
	Ohkawara and Higashi (1994)	0	<i>Viola selkirkii</i>	Hokkaido, Japan
	Ohara and Higashi (1987)	0.94	<i>Trillium kamschaticum</i>	Hokkaido, Japan
	Ohara and Higashi (1987)	0.73	<i>Trillium tschonosku</i>	Hokkaido, Japan
Myrmecochory in other biomes	Andersen and Morrison (1998)	1.95	<i>Acacia holosericea</i>	Northern Territories, Australia (‘Natural’ site)
	Gomez and Espadaler (1998)	1.31	<i>Euphorbia characias</i>	Barcelona, Spain
	Bossard (1991)	1.40	<i>Cytisus scoparius</i>	Sierra Nevada, CA
	Mossop (1989)	1.43	<i>Acacia terminalis</i> and <i>Bossiaea obcordata</i>	Infertile and fertile sites, Sydney Australia (4 sites)
	Hughes and Westoby (1990)	1.39	<i>Acacia linifolia</i>	Sydney, Australia
	Hughes and Westoby (1990)	1.99	<i>Bossiaea obcordata</i>	Sydney, Australia
	Hughes and Westoby (1992)	1.41	<i>Acacia terminalis</i> and <i>Dillwynia retorta</i>	Sydney, Australia
	Schatral et al. (1994)	0.49	<i>Hibbertia hypericoides</i>	Western Australia
	Parr et al. (2007)	2.05	<i>Acacia holosericea</i>	Northern Territory, Australia
	LeCorff and Horvitz (1995)	1.9	<i>Calathea micans</i>	La Selva, Costa Rica
Horvitz and Schemske (1986)	1.22	<i>Calathea ovandensis</i>	San Andreas Tuxtla, Mexico	
Myrmecophytes (‘ant-plants’)	Fiala et al. (1999)	0.73 \pm 0.49	20 <i>Macaranga</i> spp.	South East Asia
	Fonseca and Ganade (1996)	0.77 \pm 0.36	10 spp.	Brazil
	Davidson and Fisher (1991)	0.55 \pm 0.36	5 <i>Cecropia</i> spp.	Costa Rica
	Davidson et al. (1989)	0.64 \pm 0.16	7 spp.	
	Frederickson (2005)	0.77 \pm 0.28	<i>Cordia nodosa</i> and <i>Duroia hirsuta</i>	Peru
	Extrafloral nectary attendance by ants	Schemske (1982)	2.23 \pm 0.49	4 <i>Costus</i> spp.
Bluthgen et al. (2004)		1.97 \pm 0.61	19 spp.	Australia
Avian frugivory	Snow and Snow (1988)	1.09 \pm 0.45	30 spp.	Britain
	Sorensen (1981)	0.92 \pm 0.38	10 spp.	Britain
	Baird (1980)	0.92 \pm 0.52	6 spp.	New Jersey, USA
	Malmberg and Willson (1988)	0.90 \pm 0.45	7 spp.	Illinois, USA
Flower visitation by insects	Schemske et al. (1978)	1.27 \pm 1.09	6 spp.	Illinois, USA
	Motten (1986)	1.29 \pm 0.50	13 spp.	North Carolina, USA
	Kato et al. (1990)	2.30 \pm 0.95	50 spp.	Japan
	Barret and Helenurm (1987)	1.54 \pm 1.07	12 spp.	New Brunswick, Canada

All of the patches included *Aphaenogaster rudis*. Myrmecochore species density was correlated with *Aphaenogaster* density (simple linear regression, $F = 10.23$, $DF = 1,33$, $p = 0.003$, $R^2 = 0.24$; Fig. 2) and the proportion of ant-attended baits within each grid that attracted *Aphaenogaster* (simple linear regression, $F = 5.6$, $DF = 1,33$, $p = 0.023$, $R^2 = 0.15$). Myrmecochore species richness was positively correlated with *Aphaenogaster* density ($F = 5.3$, $DF = 1,33$, $p = 0.028$, $R^2 = 0.14$) and the proportion

of ant-attended baits within each grid that attracted *Aphaenogaster* ($F = 3.99$, $DF = 1,33$, $p = 0.054$, $R^2 = 0.11$).

Ant and plant densities among forests

The 41 forests included an average of 3.1 ± 3.9 (\pm SD) ant-dispersed plant species (Fig. 3a), and these species accounted for $13.1\% \pm 12.9$ of the herbaceous plant species

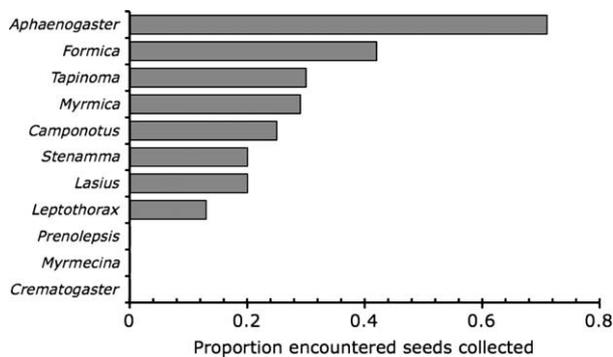


Figure 1. The proportion of encountered seeds collected by ant genera in North American temperate deciduous forests.

(Fig. 3b) within the study plots. The presence of both *Aphaenogaster* and at least one myrmecochore were recorded in 22 out of the 41 of the forests included in our analysis, 11 hosted *Aphaenogaster* but no myrmecochore, six hosted at least one myrmecochore and no *Aphaenogaster* and two had neither. This pattern of presence/absence of myrmecochores and *Aphaenogaster* was indistinguishable from a random distribution ($\chi^2 = 0.37$).

The ‘myrmecochore-occupied’ forests ($n = 27$) had an average of 4.7 ± 4.0 (\pm SD) ant-dispersed plant species in a plot, and these species accounted for $21\% \pm 9$ of the herbaceous plant species; these two measures were positively correlated in those forests (simple linear regression, $F = 15.3$, $DF = 1,18$, $p = 0.0011$, $R^2 = 0.46$). Forests with myrmecochores included a greater proportion of baits that attracted *Aphaenogaster* than did forests without myrmecochores (mean proportions of baits that attracted ants \pm SD = 0.52 ± 0.40 , $n = 27$, and 0.33 ± 0.27 , $n = 14$, respectively; one-sided t of arcsine transformed values = 1.89, $DF = 39$, $p = 0.03$). The *Aphaenogaster*-occupied forests ($n = 33$) included a greater number of myrmecochore species than did forests without *Aphaenogaster* ($n = 8$; mean richness \pm SD = 1.2 ± 1.14 and 3.7 ± 4.3 , respectively; one-sided $t = 1.81$, $DF = 39$, $p = 0.039$). However, as myrmecochores represented similar proportions of the herbaceous plant communities in forest with and without *Aphaenogaster* (mean proportion \pm SD = 0.133 ± 0.133 ($n = 29$) and 0.126 ± 0.115 ($n = 8$), respectively; one-sided $t = 0.13$, $DF = 35$, $p = 0.4$), the association between *Aphaenogaster* and myrmecochore-rich forests could merely reflect an association with forests rich in plant species in general.

Linear regressions focused using the subset of forests that included both myrmecochores and *Aphaenogaster* demonstrated that the proportion of ant-discovered baits with *Aphaenogaster* was positively correlated with myrmecochore species richness ($F = 16.3$, $DF = 1,21$, $p = 0.0007$, $r^2 = 0.46$; Fig. 3a) and the proportion of the herbaceous plant community that was ant-dispersed ($F = 15.2$, $DF = 1,17$, $p = 0.0014$, $r^2 = 0.50$; Fig. 3b) within these forests.

Plant partner diversity among comparable mutualisms

The diversity of plant partners, as characterized by Shannon’s index, varied among the plant–animal mutual-

isms (Table 2). Diversity was lowest for the North American temperate deciduous myrmecochore–ant interactions and tropical ant–plants (mean \pm SD using studies as replicates = 0.64 ± 0.49 and 0.69 ± 0.10 , respectively), intermediate for fruit-bearing plants that attract avian frugivores (0.96 ± 0.09), myrmecochores in other habitats (1.5 ± 0.45), and insect-pollinated flowers (1.6 ± 0.48), and highest for extrafloral-nectary bearing plants that attract ants (2.1 ± 0.18). Although plant partner diversity for temperate myrmecochores and myrmecophytes (and-dispersed and ant-hosting plants, respectively) were comparable, the myrmecochorous flora largely interact with one species complex (*Aphaenogaster*).

Discussion

Our study suggests that the mutualism between temperate deciduous myrmecochores and their ant dispersers is asymmetrical, and that this common North American plant guild functions as de-facto *Aphaenogaster* specialists (at the species complex level, if not species). However, among-forest comparisons provide no evidence that this ubiquitous plant–ant interaction is an obligate one; forests without *A. rudis* can include myrmecochores and vice versa. Below, we discuss why this interaction may be both mutualistic and common, whether describing it as ‘specialized’ is appropriate, and describe the implications of the ant and plant guild’s correlated distributions. We conclude by placing this mutualism in the context of other mutualisms in temperate deciduous habitats and ant–plant interactions in other biomes.

Mutualism requires reciprocal benefit, and there is evidence that the ants and myrmecochorous seeds benefit from their interactions. *Aphaenogaster rudis* colonies provided elaiosomes produce more female alates (Morales and Heithaus 1998). Although there is no evidence that these benefits are unique to *A. rudis*, evidence involving other ants has rarely been sought or found. There are several lines of evidence that seeds benefit from collection by *Aphaenogaster*. *Aphaenogaster rudis* simultaneously disperses seeds rapidly enough to reduce predation risk (Heithaus 1981, Ruhren and Dudash 1996, Ness and Morin 2008), far enough to reduce distance-related and density-dependent costs (Kalisz et al. 1999, Ness et al. 2004), and to sites disproportionately suitable for establishment (*A. rudis* nests are high light sites (Smallwood 1982) with different soil characteristics from the surrounding habitat (Ruhren and Dudash 1996)). These responses satisfy the criteria attributed to an ideal myrmecochore partner (Giladi 2006). The net benefit received by *Aphaenogaster*-dispersed seeds may also be high because the costs incurred by seeds are low. This occurs for several reasons. First, because *A. rudis* foragers are subordinate opportunists (Fellers 1987, Andersen 1997), their foraging territories are ‘permeable’ (sensu Andersen 1988) and overlap with other colonies and other ant species. As a consequence, seeds from any one maternal plant are likely dispersed to multiple nests (Kalisz et al. 1999, Giladi 2004), can be transported a variety of distances, and may be less affected in the event that a particular colony becomes satiated (Heithaus et al. 2005). In effect, *A. rudis*

can provide great benefits without wholly excluding other prospective partners. Second, the solitary foragers typical of an *A. rudis* colony quickly deliver resources to a nest rather than dissecting it on-site, thereby avoiding any damage to the seeds or 'elaiosome-robbing'.

The interaction between *Aphaenogaster* and seeds is the modal dispersal mechanism for these seeds for at least three reasons. First, *A. rudis* is one of the most common and abundant epigenic ants in North American temperate deciduous forests (Culver 1974, Lynch 1981, Gotelli and Ellison 2002). It is inevitable that many prospective food items will be inspected by this ant. Second, the foraging ranges of *Aphaenogaster* colonies often overlap. Based on the even distribution of nests (Giladi 2004), estimates of colony density (>1 colony per m²; Headly 1952, Talbot 1957) and the mean and maximum distances that collected items are discovered from the nest (~50 cm and 1.5 m respectively, Ness et al. 2004), *Aphaenogaster* can provide more than 100% coverage of the forest floor. Based on the high frequency of *Aphaenogaster*-seed interactions, that ant might have a great effect on myrmecochores almost irrespective of their per-interaction effect (Vazquez et al. 2005). Third, because *Aphaenogaster* collects encountered seeds more readily than other ants, the proportions of seeds collected by that ant are even greater than would be predicted by encounter rate alone. The combination of high encounter rates, unusually high collection rates upon encountering seeds, and the benefit conferred to the seeds (above) qualifies *A. rudis* as a keystone mutualist.

It is striking how many myrmecochore-ant interactions include *Aphaenogaster* rather than other more common and widespread omnivores in the Nearctic (e.g. *Lasius*, *Leptothorax* and *Stenamma* spp. in Ward 2000). If the interaction is specialized, how might plants filter prospective partners and target a particular taxon within an ant guild? One series of explanations focuses on the rewards associated with individual seeds. Elaiosomes have chemical cues that induce collection behaviors by *A. rudis* (Marshall et al. 1979). However, similar responses can be elicited from a variety of ants (Skidmore and Heithaus 1988). Compositionally, the elaiosome requires no obvious morphological adaptations by a foragers to manipulate or nestmates to consume. Nonetheless, certain diaspores may be sufficiently small to be unrewarding for some ants to collect or sufficiently large that collection can be a challenge. If these distinctions are informed by the size of the ants (Beattie et al. 1979, Christian 2001) or their jaws, an intermediate-sized ant such as *A. rudis* may be capable and willing to collect diaspores unavailable or unattractive to some portions of the omnivorous ant community.

At a finer resolution of partner choice, we favor an explanation whereby plants may target a particular disperser by modifying the spatio-temporal distribution of seeds (Giladi 2006). Insofar as plants 1) offer few seeds at any one time (e.g. 'drip' rather than 'pour' seeds (Ohara and Higashi 1987), or produce few large seeds rather than many small seeds, as do *Sanguinaria*, *Trillium* and *Erythronium* spp.) and/or 2) distribute their seeds in space such that highly competitive ants cannot discover/dominate all the seeds (as can occur when myrmecochory and ballistic dispersal are coupled, as in many *Viola* spp., Beattie et al. 1979), they increase the likelihood of seed discovery and collection by

subordinate ants such as *A. rudis*. Intriguingly, the perceived relative importance of subordinate ants such as *A. rudis* could be inflated insofar as ecologists monitor seeds for overly short time periods or offer/monitor fewer seeds than would naturally be produced by prospective plant mutualists. This potential bias can be overcome by conducting longer term experiments as well as manipulating both the spatial and temporal distribution of seeds while monitoring ant-specific seed discovery and removal rates for various focal plant species. Such studies could dramatically change our sense of the functioning of this common and widespread plant-animal mutualism.

Whether the de facto specialization of myrmecochores on *Aphaenogaster* is evolved or incidental, the absence or rarity of that ant taxon is correlated with decreases in population density of myrmecochorous species (Giladi 2004, Pudlo et al. 1980), and the richness and proportional representation of myrmecochores within the larger plant community (this study). The correlated distributions of myrmecochores and *Aphaenogaster* at several nested spatial scales (within 20 × 20 m plots, within forests, and among forests; Gove et al. 2007) are consistent with that predicted for a widespread, pervasive mutualism. Although this correlation could merely indicate that myrmecochores and *Aphaenogaster* both favor rich mesic forests, variation in myrmecochore density is not correlated with gross measures of 'ant' activity or diversity (this study; Mitchell et al. 2002) or with the rate of seed collection by 'ants' (Jules and Rathke 1999, Ruhren and Handel 2003), measures that might also be expected to be higher in rich mesic forests. This lack of a correlation has been interpreted as evidence that the success (or lack thereof) of myrmecochores is not limited by access to 'ants'. However, our demonstration of the strong correlations between myrmecochore densities and the ant taxon unique in North American temperate forests for collecting a majority (>50%) of the seeds they encountered hints that the identity of the seed-collecting ant may be critical to the plant. That inference is supported by the benefits described above, as well as by the positive correlations between the proportion of ant-collected seeds that are collected by *A. rudis* and myrmecochore densities (Ness and Morin 2008, Zelikova et al. 2008), seed survival rates (Ness and Morin 2008) and population structure consistent with successful dispersal (Pudlo et al. 1980). We believe these links between *Aphaenogaster* and myrmecochorous herbs are largely causal rather than correlative. If they are solely correlative, *Aphaenogaster* is nonetheless a remarkably reliable dispersal agent for most myrmecochores.

Within forests, we hypothesize that the myrmecochorous plant guild tracks *Aphaenogaster* more strongly than vice versa. Forests with high myrmecochore richness invariably included *Aphaenogaster* at a majority of ant-discovered baits, whereas *Aphaenogaster* can be common in forests where richness was low or the plants are absent (Fig. 3a). This asymmetry may arise from the nature of the interaction. While the benefit for *Aphaenogaster* results from the augmentation of resources (Morales and Heithaus 1998, Bono and Heithaus 2002) that are also found in the regular diet of the ant (Fischer et al. 2008), the services provided by *Aphaenogaster* to the plant directly affect the success of a crucial demographic transition in the plant life cycle. In

addition, from the plant's perspective, the interaction concludes once the seed is literally pulled into the ant colony's sphere of influence. In contrast, the interaction increases or reconfigures the production of the propagules (winged alates) of an *A. rudis* colony (Morales and Heithaus 1998); these progeny leave the nest and any plants or seeds accumulated near the maternal colony to independently colonize other suitable habitats.

At larger spatial and temporal scales, the interaction may become increasingly sensitive to external drivers. Because many myrmecochores are capable of vegetative reproduction and/or can live >70 years (e.g. some *Trillium* spp.), the modern-day distributions of such long-lived plant species can be shaped by anthropogenic extirpations and/or interactions with past partners that may differ from those presently available. Soil characteristics also play a critical role in determining the plant distributions within and among forests. These are two sources of the residual variation apparent in Fig. 2 and 3. Last, the dispersal services that ants can provide are limited in spatial scope. Although seed-dispersing ants reconfigure plant populations (Pudlo et al. 1980, Kalisz et al. 1999), no ant carries a seed from one forest to another. Rather, the presence of *Aphaenogaster* may increase the likelihood that relict populations, or the rare propagules that arrive in forests by other vectors (e.g. deer in Vellend et al. 2003), flourish.

The low levels of partner diversity, from the plant's perspective, documented in North American temperate deciduous myrmecochore-ant interactions are comparable to that observed for tropical ant-plants (Table 2), albeit with the critical distinction that myrmecochore flora largely interacts with *Aphaenogaster*. Remarkably, the *A. rudis* complex collected the most seeds in all but one (Gibson 1993) of the studies performed in the temperate deciduous forests of North America. There is also evidence that

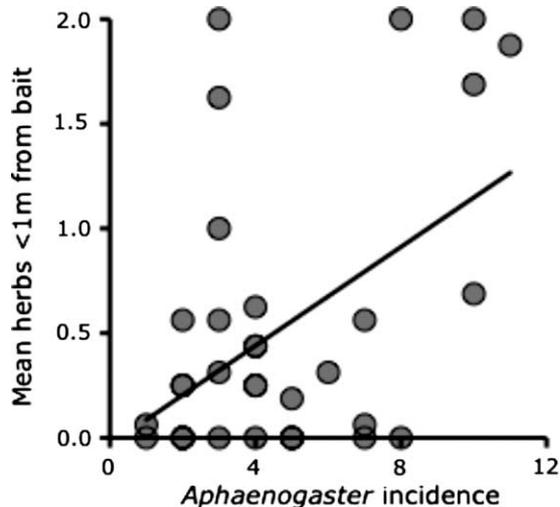


Figure 2. The incidence of *Aphaenogaster rudis* and the density of ant-dispersed plant species (myrmecochores) among 35 400-m² baiting grids in a forest in New York, USA. *Aphaenogaster* incidence was estimated by the number of 16 available ant baits in each grid that were occupied after 50 min. Each grid was separated by >100 m. Myrmecochore density was estimated as the by the mean incidence of *Trillium*, *Uvularia*, *Sanguinaria*, *Asarum* and *Hepatica* spp. within 1 m of a bait in each grid.

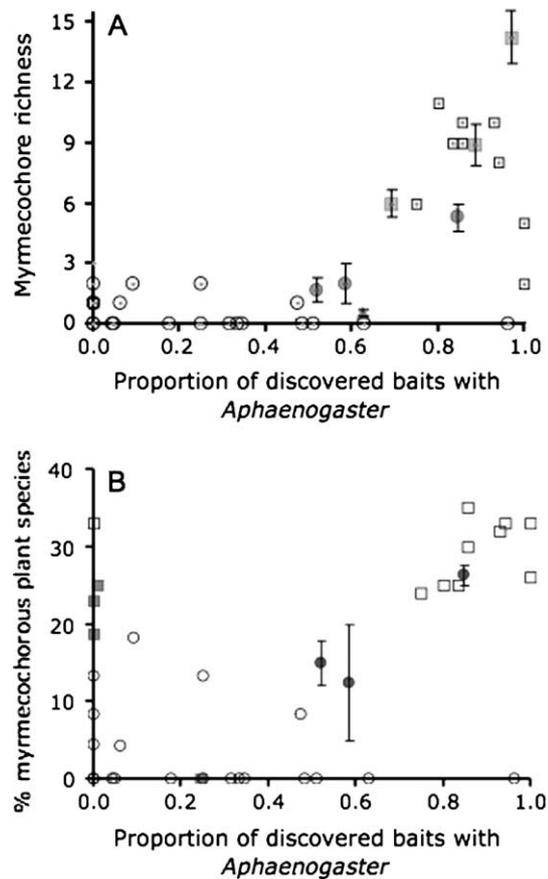


Figure 3. The distribution of ant dispersed plants and the proportion of ant baits visited by *Aphaenogaster* ants in North American temperate deciduous forests. (A) the richness of ant dispersed plants within study plots, (B) the percentage of the herbaceous plant community that was is ant dispersed. Empty squares indicate forests in Beattie and Culver (1981), empty circles forests from Gotelli and Ellison (2002), filled circles indicate forests in Heithaus and Humes (2003), a filled triangle indicates the forest in Ellison (2007), and filled squares indicate forests in Mitchell et al. (2002) in (A) and forests sampled by S. Philipott in (B).

myrmecochores in Japan, where *A. rudis* is absent, interact with few partners (i.e. low partner diversity, largely limited to *Myrmica* and *Aphaenogaster* spp.), although the seed dispersal phenomenon has been documented in fewer sites. This specialization by temperate deciduous myrmecochores is markedly greater than that reported for frugivory by temperate deciduous birds, pollination by temperate deciduous insects, ants attracted to extrafloral nectaries, or myrmecochory in other biomes (Rey and Manzaneda 2007, but see Gove et al. 2007). Recognizing the caveats that 1) these interactions are studied with different methodologies, 2) the criteria for distinguishing visitors and parasites from true mutualists differs, and 3) that we have focused on partner diversity from the plant perspective (rather than characterizing the degree of reciprocal specialization by the plant and animal communities), these comparisons underscore that partner diversity differs among mutualistic interactions and that the temperate-tropical distinction can be misleading for

some mutualisms. Insofar as low partner diversity in the presence of many prospective partners is a hallmark of specialization, temperate myrmecochores may be unexpectedly similar to tropical ant-plants.

We conclude that myrmecochory in temperate deciduous habitats exhibits a degree of low partner diversity hitherto limited to obligate plant–animal mutualisms in the tropics, and that a single ant species complex is the primary seed disperser for the myrmecochore guild in North America. This pattern lacks evidence of the coevolution so apparent in tropical ant-plants, although future phylogenetic work on the *A. rudis* species complex could change this view. Further, a highly interactive and abundant mutualist may be capable of coevolving with entire networks (Guimarães et al. 2007). Understanding the influence of *A. rudis* complex may be a critical step in the conservation of North American myrmecochores. More specifically, studies performed at the ‘margins’ of that interaction where plants are incapable of interacting with *A. rudis* (e.g. due to forest edges, biological invasions, range limits or dissimilar responses to climate change) could help identify vulnerabilities associated with the reliance of temperate deciduous myrmecochores on that ant.

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Supplementary material (available online as Appendix O17430 at <www.oikos.ekol.lu.se/appendix>). Appendix 3.

Appendix 1. Responses of North American temperate deciduous ant species to seeds adapted for ant dispersal (myrmecochory). 'Seeds encountered' refers to observations of ants walking on cards with seeds. 'Seeds collected' refers to the subset of those encountered seeds that were removed from the cards (i.e. dispersed).

Genus	Species	Seed species	Seeds encountered	Collected	Proportion	Reference
<i>Aphaenogaster</i>	<i>rudis</i>	<i>Hepatica acutiloba</i>	8	7	0.88	Beattie et al. 1979
	<i>rudis</i>	<i>Sanguinaria canadensis</i>	174	128	0.74	Culver and Beattie 1978
	<i>rudis</i>	<i>Sanguinaria canadensis</i>	34	29	0.85	Ness and Morin 2008
	spp. (<i>fulva</i> , <i>rudis</i> and <i>texana</i>)	<i>Viola</i> spp.	116	71	0.61	Culver and Beattie 1978
	POOLED		332	235	0.71	
<i>Camponotus</i>	sp.	<i>Sanguinaria canadensis</i>	16	4	0.25	Ness and Morin 2008
	<i>pennsylvanicus</i>	<i>Sanguinaria canadensis</i>	12	3	0.25	Culver and Beattie 1978
	POOLED		28	7	0.25	
<i>Crematogaster</i>	sp.	<i>Sanguinaria canadensis</i>	0	1	0	Ness and Morin 2008
<i>Formica</i>	<i>integra</i>	<i>Viola</i> spp.	40	4	0.10	Culver and Beattie 1978
	<i>subsericea</i>	<i>Hepatica acutiloba</i>	17	17	1.00	Beattie et al. 1979
	<i>subsericea</i>	<i>Viola</i> spp.	5	1	0.20	Culver and Beattie 1978
	<i>subsericea</i>	<i>Sanguinaria canadensis</i>	47	24	0.51	Pudlo et al. 1980
	POOLED		109	46	0.42	
<i>Lasius</i>	sp.	<i>Sanguinaria canadensis</i>	4	1	0.25	Ness and Morin 2008
	<i>alienus</i>	<i>Hepatica acutiloba</i>	8	0	0	Beattie et al. 1979
	<i>alienus</i>	<i>Viola</i> spp.	27	8	0.30	Culver and Beattie 1978
	<i>alienus</i>	<i>Sanguinaria canadensis</i>	15	2	0.13	Pudlo et al. 1980
	POOLED		54	11	0.20	
<i>Leptothorax</i>	sp.	<i>Sanguinaria canadensis</i>	12	1	0.08	Ness and Morin 2008
	<i>curvispinosus</i> + <i>longispinosu</i>	<i>Viola</i> spp.	4	1	0.25	Culver and Beattie 1978
	POOLED		16	2	0.13	
<i>Myrmecina</i>	<i>americana</i>	<i>Viola</i> spp.	6	0	0	Culver and Beattie 1978
<i>Myrmica</i>	sp.	<i>Sanguinaria canadensis</i>	87	41	0.47	Ness and Morin 2008
	<i>emeryana</i>	<i>Sanguinaria canadensis</i>	3	1	0.33	Culver and Beattie 1978
	<i>punctiventris</i>	<i>Hepatica acutiloba</i>	6	6	1	Beattie et al. 1979
	<i>punctiventris</i>	<i>Viola</i> spp.	40	18	0.45	Culver and Beattie 1978
	<i>punctiventris</i>	<i>Trillium erectum</i>	271	71	0.26	Guntherand Lanza 1989
	<i>punctiventris</i>	<i>Trillium grandiflorum</i>	127	29	0.23	Guntherand Lanza 1989
	<i>punctiventris</i>	<i>Trillium undulatum</i>	222	53	0.24	Guntherand Lanza 1989
	<i>punctiventris</i>	<i>Sanguinaria canadensis</i>	12	3	0.25	Pudlo et al. 1980
	POOLED		768	222	0.29	
<i>Prenolepis</i>	<i>imparis</i>	<i>Sanguinaria canadensis</i>	39	0	0	Pudlo et al. 1980
<i>Stenamma</i>	<i>schmitti</i>	<i>Viola</i> spp.	6	1	0.17	Culver and Beattie 1978
	sp.	<i>Sanguinaria canadensis</i>	1	0	0	Ness and Morin 2008
	sp.	<i>Sanguinaria canadensis</i>	3	1	0.33	Pudlo et al. 1980
	POOLED		10	2	0.20	
<i>Tapinoma</i>	<i>sessile</i>	<i>Viola</i> spp.	10	3	0.3	Culver and Beattie 1978

Appendix 2. Descriptions of the herbaceous plant community and the proportion of ant-discovered baits that were discovered by *Aphaenogaster* ant for sites in North American temperate deciduous forests. Mean values (and standard errors if possible) are provided for studies where multiple forests were followed and only study-wide mean values were given. Where multiple plots were sampled within a single forest (either in space or time), average values among plots are provided.

Study	Site	Ant discovered baits w/ <i>Aphaenogaster</i>	Myrmecochores richness	SE	% plant spp.	SE
Gottelli and Ellison 2002	arc	0.63	0		0	
Gottelli and Ellison 2002	bh	0.00	0		0	
Gottelli and Ellison 2002	cb	0.33	0		0	
Gottelli and Ellison 2002	ckb	0.51	0		0	
Gottelli and Ellison 2002	haw	0.96	0		0	
Gottelli and Ellison 2002	Hbc	0.32	0		0	
Gottelli and Ellison 2002	ob	0.25	0		0	
Gottelli and Ellison 2002	qP	0.00	0		0	
Gottelli and Ellison 2002	rP	0.04	0		0	
Gottelli and Ellison 2002	skp	0.48	0		0	
Gottelli and Ellison 2002	spr	0.18	0		0	
Gottelli and Ellison 2002	sw	0.35	0		0	
Gottelli and Ellison 2002	win	0.05	0		0	
Gottelli and Ellison 2002	pea	0.06	1		4.3	
Gottelli and Ellison 2002	car	0.00	1		4.5	
Gottelli and Ellison 2002	chi	0.00	1		8.3	
Gottelli and Ellison 2002	tpb	0.47	1		8.3	
Heithaus and Humes 2003	high disturbance (avg)	0.58	2	1	12.5	7.5
Gottelli and Ellison 2002	mol	0.25	2		13.3	
Gottelli and Ellison 2002	moo	0.00	2		13.3	
Heithaus and Humes 2003	moderate disturbance (avg)	0.52	1.7	0.61	15	2.94
Gottelli and Ellison 2002	col	0.09	2		18.2	
Beattie and Culver 1981	4	0.75	6		24	
Beattie and Culver 1981	3	0.83	9		25	
Beattie and Culver 1981	8	0.80	11		25	
Beattie and Culver 1981	5	1.00	5		26	
Heithaus and Humes 2003	low disturbance (avg)	0.85	5.3	0.67	26.3	1.2
Beattie and Culver 1981	6	0.86	9		30	
Beattie and Culver 1981	2	0.93	10		32	
Beattie and Culver 1981	9	0.00	1		33	
Beattie and Culver 1981	10	1.00	2		33	
S. Philpott unpubl.	Hill Forest, OH	0.00	3		23	
S. Philpott unpubl.	Mulberry Forest, OH	0.24	0		0	
S. Philpott unpubl.	Jermain Forest, OH	0.01	1		25	
S. Philpott unpubl.	Stranahan Arboretum, OH	0.00	3		18.8	
Ellison 2007	Black Rock Forest (avg plot)	0.63	0.56	0.17		
Beattie and Culver 1981	7	0.94	8		33	
Beattie and Culver 1981	1	0.86	10		35	
Mitchell et al. 2002	small plot-high disturbance (avg)	0.69	6	0.69		
Mitchell et al. 2002	large plot-high disturbance (avg)	0.89	8.9	1.04		
Mitchell et al. 2002	large plot-low disturbance (avg)	0.97	14.25	1.31		