Seed handling behaviours of native and invasive seed-dispersing ants differentially influence seedling emergence in an introduced plant

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Abstract. 1. Myrmecochory, or ant-mediated seed dispersal, is an important ecological interaction in which ants benefit by gaining nutrition from lipid-rich elaiosomes attached to seeds and plants benefit from having their seeds dispersed away from parent plants. Most research on the benefits of myrmecochory focuses on primary dispersal, in which ants move seeds to nests, or secondary dispersal, in which ants deposit intact seeds in middens after consuming elaiosomes. Less is known about how ants handle seeds inside nests and if handling influences plant fitness.

2. The seed handling behaviours of a native 'keystone disperser', *Aphaenogaster rudis* s.l., and an invasive seed-disperser, *Myrmica rubra* L., on an introduced herb, *Chelidonium majus* L., were compared. We conducted a greenhouse experiment to test if handling by ants, manual removal of elaiosomes, or no handling (controls) influenced seedling emergence. Colony-level differences in handling behaviours and plant responses were also examined.

3. *Aphaenogaster rudis* retained seeds inside nests longer than *M. rubra*, but there was no difference in the amount of elaiosome removed by the two species. There was no difference in the proportion of seedlings that emerged among treatments, but seedlings emerged earlier when handled by *A. rudis*. Additionally, more seedlings emerged and seedlings emerged earlier the longer seeds were retained inside ant nests.

4. This study suggests that handling by ants may be a benefit of myrmecochory. This is probably not due to elaiosome removal; rather favourable nest conditions may enhance emergence. Also, functional differences in ant species may result in different outcomes for plant partners.

Key words. *Aphaenogaster rudis*, biological invasion, invasive ant, mutualism, myrmecochory, *Myrmica rubra*, seed dispersal, seedling emergence.

Introduction

Myrmecochory, or ant-mediated seed dispersal, is a widespread and important ecological interaction. Over 11 000 plant species are myrmecochorous, and this interaction plays an integral role in structuring plant communities (Bond & Slingsby, 1984; Christian, 2001; Giladi, 2006; Lengyel *et al.*, 2010). Myrmecochory is described as a mutualism, in which ants gain nutrition from consuming lipid-rich elaiosomes on seeds and plants benefit from being dispersed away from parent plants (Bronstein *et al.*, 2006; Giladi, 2006). Ants carry seeds to

their nests (primary dispersal), remove and feed elaiosomes to larvae inside nests (handling), and then deposit intact seeds in middens inside or outside of nests (secondary dispersal) (Giladi, 2006; Servigne & Detrain, 2010; Canner *et al.*, 2012). Most research has focused on the benefits of primary and secondary dispersal to plants, but less is known about how seed handling by ants inside nests benefits plants (Servigne & Detrain, 2010).

Understanding the evolutionary forces that shape mutualisms requires knowledge about the benefits gained by interacting species and if the benefits outweigh associated costs (Bronstein *et al.*, 2006). Ant partners benefit from myrmecochory by gaining nutrition. However, diets of seed dispersers are varied, and the degree to which elaiosomes contribute to colony

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growth and fitness remain largely unknown (Bronstein *et al.*, 2006, but see Morales & Heithaus, 1998; Bono & Heithaus, 2002; Fokuhl *et al.*, 2012; Turner & Frederickson, 2013). There are multiple hypotheses as to how plants benefit from myrmecochory, and these benefits are more well documented (Giladi, 2006). Plants, for example, can benefit from being dispersed away from parent or sibling plants, thus escaping competition or inbreeding depression (i.e. dispersal distance) (Anderson, 1988; Bond & Stock, 1989; Kalisz *et al.*, 1999). Seeds can end up in favourable microsites for germination and establishment (i.e. directed dispersal) (Beattie & Culver, 1983; Hanzawa *et al.*, 1988). Seeds may also escape from predation from granivores by being moved to nests or buried (i.e. predation escape) (Culver & Beattie, 1978; Heithaus, 1981). There is evidence for all of these benefits, and these benefits are not always mutually exclusive (Giladi, 2006). The handling of seeds by ants inside nests could also influence plant fitness (Culver & Beattie, 1980; Lobstein & Rockwood, 1993; Boyd, 2001), but mechanisms for how this may occur are not well understood or documented (Giladi, 2006; Servigne & Detrain, 2010).

There are multiple hypotheses as to how handling seeds may influence plant fitness. For example, removal of the elaiosome could scarify the seed coat and promote germination by enabling the increased uptake of nutrients and water (Culver & Beattie, 1980). The presence of an elaiosome may also chemically inhibit germination (Lobstein & Rockwood, 1993). Favourable conditions inside nests may promote germination (Horvitz, 1981). Handling by ants can also interact with other benefits to enhance fitness. For example, removal of elaiosomes can decrease the probability of granivores finding seeds (Heithaus, 1981; Slingsby & Bond, 1985; Boyd, 2001; Christian & Stanton, 2004). Elaiosome removal can also interact with nest conditions to influence germination (Culver & Beattie, 1978; Horvitz, 1981). There are mixed results as to the effects of seed handing and specifically elaiosome removal on plant performance with documentations of decreases, no changes, and increases in seedling germination and emergence (Culver & Beattie, 1978, 1980; Horvitz & Beattie, 1980; Horvitz, 1981; Lobstein & Rockwood, 1993; Christian & Stanton, 2004; Ohkawara, 2005; Imbert, 2006; Garrido *et al.*, 2009). These mixed results probably reflect species-specific differences in how plants benefit from handling and in how ants handle seeds.

Ant partner identity can influence the outcome of myrmecochory. Differences among ant species in body size, foraging behaviour, or nest characteristics, for example, may determine the rate at which seeds are picked up, how far they are moved, how they are processed in the nest, and where they are deposited (Hughes & Westoby, 1992; Gorb & Gorb, 2003; Ness *et al.*, 2004; Servigne & Detrain, 2010). Myrmecochory has been traditionally described as a diffuse mutualism, in which multiple species of ants disperse multiple species of plants. However, recent studies suggest that this interaction is more specialised with certain species or guilds of ants acting as high-quality or 'keystone dispersers' (Gove *et al.*, 2007; Manzaneda & Rey, 2009; Ness *et al.*, 2009). Introduced ants provide good cases to uncover the importance of ant identity

in structuring myrmecochorous communities. Generally, introduced ants such as *Linepithema humile* (Mayr) have negatively affected plant communities by replacing native ants that are high-quality dispersers and by acting as low-quality dispersers that fail to disperse seeds, deposit seeds in sub-optimal locations, or act as granivores (Christian, 2001; Rodriguez-Cabal *et al.*, 2009).

Myrmica rubra L. (Myrmicinae) is native to Europe and has been introduced into North America. It occurs in a variety of habitats, including deciduous forests, where it overlaps with a keystone disperser *Aphaenogaster rudis* s.l. (Myrmicinae), and many species of myrmecohorous plants in its introduced range (K. Prior, pers. obs.). In contrast to other well-studied introduced ants that rarely disperse seeds in their native range (Rodriguez-Cabal *et al.*, 2009), *M. rubra* may act as a high-quality disperser given that it disperses seeds in its native range (Gorb *et al.*, 2000; Servigne & Detrain, 2010). Differences between *M. rubra* and native ants such as *A. rudis* in seed dispersal behaviours or colony characteristics, however, could alter the outcome of myrmecochorous interactions. We examine how the seed handling behaviours of *M. rubra* and *A. rudis* influence the emergence of a fast-growing, European perennial, *Chelidonium majus* L. (Papaveraceae). This plant has also been introduced into North America into habitats where both ant species occur (K. Prior, pers. obs.). *Myrmica rubra* disperses *C. majus* in its native range; thus, we may expect that *M. rubra* is a more effective disperser of *C. majus* than *A. rudis*. However, given that species specificity is often quite low in myrmecochory, and that many ant species will pick up and move many myrmecochorous plant species (Giladi, 2006; Lengyel *et al.*, 2010), it would not be surprising if *A. rudis* also effectively disperses *C. majus*. Thus, while we do expect that these two seed-dispersing ants will likely differ in the degree to which they provide benefits to *C. majus*, we have no *a priori* prediction about which species may provide the greatest benefit.

Here, we examine if and how seed handling by ants benefits plants. First, we ask if handing by ants influences seedling emergence, and also compare how handling by two ant species influence emergence. Second, we compare the seed handling behaviours of two ant species to uncover how differences in behaviours affect the benefits of handling. This study provides insight into the benefits of handling, an understudied portion of the dispersal process, and uncovers the potential for ant species identity to influence the outcome of myrmecochory. This study also contributes to our understanding of how introduced ants impact myrmecochorous communities.

Materials and methods

Study species

Aphaenogaster rudis is a numerically dominant ant in woodlands of eastern North America (Ness *et al.*, 2009); including deciduous forests in southern Ontario, where we conducted this study. The taxonomy of this species is under consideration and it is currently referred to as belonging to the *A.rudis*-group (Lubertazzi, 2012). *Aphaenogaster rudis*

workers are medium-sized ants (∼4 mm in length) that are general scavengers. Small invertebrates probably comprise a significant portion of their diet (Lubertazzi, 2012), but they are also keystone dispersers of seeds of myrmecochorous plants (Ness *et al.*, 2009). Their colonies are monogynous with approximately 266–613 workers (Lubertazzi, 2012). Rotten fallen wood seems to be their preferred nesting site, but they also nest under rocks, in leaf litter, and in soil (Lubertazzi, 2012).

Myrmica rubra was introduced from Europe into the eastern United States in the early 20th century and now occurs in various locations throughout North America, including southern Ontario (Groden, *et al.*, 2005). *Myrmica rubra* is considered 'invasive' as it occurs at high densities and can be a nuisance to people owing to its tendency to sting (Holway *et al.*, 2002; Garnas, 2005; Groden *et al.*, 2005). Little is known, however, about the ecological effects of this species on native communities (except see Garnas, 2005). *Myrmica rubra* occurs in a variety of habitats in its native and introduced ranges, including deciduous forests (Groden, *et al.*, 2005). The distribution and invasion status of *M. rubra* in southern Ontario has not been well documented; however, we have observed this species in forest patches that include *A. rudis*, but we do not know if *M. rubra* is displacing *A. rudis* (K. Prior, pers. obs.). We often observe patches of forest that have high abundances of *A. rudis* or *M. rubra* with the other species present, and some patches where *A. rudis* or *M. rubra* occur exclusively. *Myrmica rubra* might prefer moist soil and often occurs in high abundances in riparian-forested areas (Groden *et al.*, 2005), but we have found *M. rubra* in more upland forest patches with relatively dry soil and *A. rudis* in patches near streams with relatively moist soil. *Myrmica rubra* often nests in rotten fallen wood (Groden *et al.*, 2005). Colonies are polygynous and polydomous with queen and worker numbers being highly variable (queens range from 1 to 194 and workers from 297 to > 10 000 in a colony) (Elmes, 1973; Groden *et al.*, 2005). *Myrmica rubra* are medium-sized ants (∼4 mm in length) that are primarily insectivorous, but also tend hemipterans and consume elaiosomes (Gorb *et al*., 2000).

Chelidonium majus is a weedy, short-lived perennial or biennial herb native to Eurasia that has been widely introduced into North America (Mack, 2003). It grows at the edge of forests or in disturbed forests (Kang & Primack, 1991). *Myrmica rubra* disperses *C. majus* in its native range (Gorb *et al.*, 2000), and we have observed it in areas where both *A. rudis* and *M. rubra* occur in its introduced range in North America (K. Prior, pers. obs.). Its seeds are small (∼2 mm in length) and dark brown with a white, fleshy elaiosome that comprises about 30% of the mass of the diaspore (elaiosome plus seed) (Servigne & Detrain, 2008). Plants flower from June to September and once fruits dehisce, seeds fall to the ground and are collected by ants (Oberrath & Bohning-Gaese, 2002). Seeds do not undergo dormancy and germinate in the fall, with a leafy rosette also emerging in the fall. Aerial shoots with leaves and flowers are produced from May to June of the following year (Kang & Primack, 1991). We chose this species to examine the effects of handling by ants because, unlike

other species of myrmecochorous plants, this plant germinates without requiring a period of dormancy.

Collection and maintenance of seeds and ant colonies

We collected *Chelidonium majus* seeds from June to September when seed pods were naturally dehiscing from plants at the University of Toronto's research station, Koffler Scientific Reserve (KSR) at Jokers Hill in King City, Ontario (44°02′N, 79°32′W). Seeds were stored at −23 °C in vials until use in behavioural trials (∼7 months) (see *Seed handling behaviour trials*). For the seedling emergence experiment, seeds were collected late in the season and stored at 4 °C with moist filter paper for approximately 2 weeks and were removed on the day that they were provided to ant colonies (see *Seedling emergence experiment*). Previous studies have shown that ants have no preference for fresh or previously frozen seeds (Servigne & Detrain, 2008).

We collected *A. rudis* colonies from KSR and *M. rubra* colonies from multiple sites around the Greater Toronto area (43°35′N, 79°33′N; 43°40′N, 79°22′W; 43°50′N, 79°11′W). We collected *A. rudis* colonies from artificial nest boxes (see Lubertazzi, 2012 for details) that were placed on the forest floor between March and July. We also collected both species by breaking open rotten logs on the forest floor. All colonies were collected in August and September 2012. We collected all possible workers and brood for *A. rudis* colonies and only retained colonies in which we found queens. Given the large size of *M. rubra* colonies, we collected as many brood and queens as we could and approximately 300–500 workers from each colony.

We standardised 20 colonies of each species to contain 200 workers, up to 50 brood, and a single queen. In an attempt to have similar numbers of brood care workers and foragers in each colony, approximately 100 ants were collected that were tending brood inside 'nests' (see below for a description of 'nests') and 100 workers were collected that were foraging outside of the nests. All colonies were housed in plastic containers (17 × 15 × 12 cm) that had sides covered with Fluon [Insect-a-Slip (Bioquip, Rancho Dominguez, California)]. A single test tube, filled one-third with distilled water, plugged with cotton, and covered with aluminum foil was placed in each plastic container to serve as a 'nest'. We fed colonies approximately 0.2 g of standardised diet modified from the Bhatkar–Whitcomb diet approximately three times a week (Dussutour & Simpson, 2008). Colonies were kept in an environmental chamber set to 23 °C and a LD 12:12 h cycle at the University of Toronto.

Seed handling behaviour trials

We fed 20 seeds of *C. majus* to eight colonies of *A. rudis* and nine colonies of *M. rubra* (only 17 standardised colonies were used for these trials). Colonies were starved for 4 days prior to the start of the trials, but fed as described above throughout the trials. Seeds were presented to ants in 10-cm Petri dishes ('depots') placed in the plastic containers. In each colony, ants

removed all the seeds from the depots within 24 h. Seeds that were deposited outside of the nests were collected from the bottom of the plastic containers. These seeds were assumed to have been handled by ants as both of these species have been observed bringing seeds into artificial nests (K. Prior, pers. obs.). Seeds were counted and collected until all seeds had been found (11 days). Handled seeds were examined under a dissecting microscope and the amount of elaiosome remaining was scored as: completely intact, partially removed (i.e. some tissue remaining on seed), or completely removed.

Seedling emergence experiment

Five hundred *C. majus* seeds were used in each of four treatments to examine how handling by ants affected seedling emergence (see Appendix S1). Seeds were not handled (controls), elaiosomes were manually removed with a razor blade, or seeds were handled by 20 *A. rudis* colonies or 20 *M. rubra* colonies. For the ant handling treatments, we fed 30 seeds each to 20 colonies each of *A. rudis* and *M. rubra* as described above (see *Seed handling behaviour trials*). These colonies were the same colonies that were used in the handling behaviour trials, but this experiment was conducted 4 months prior to the behavioural trials. Ants removed 98% of seeds from depots within 24 h and all of the seeds within 48 h. Thus, all of the seeds were handled by ants. We collected handled seeds once they were removed from the nests starting 24 h (day 1) after they had been fed to colonies and for 5 days afterwards. After 6 days, we removed any remaining seeds from the nests. We limited the number of days that seeds were allowed to be handled by ants, so that we could plant all the seeds within the timeframe of a week. The first 25 seeds collected from each colony were planted (see below).

We planted each seed in commercial potting soil [Premium Nature Mix (Premier Tech Home and Garden, Rivière-du-loup, Quebec, Canada)] in a single cell (3 cm^2) in a planting tray (28 × 53 cm). Trays were kept in a greenhouse set to 25 °C under natural lighting at the University of Toronto. Two planting trays were placed adjacent to each other in the greenhouse and considered a replicate (block). Trays were arranged along multiple tables and blocks accounted for variation in greenhouse conditions. There were 20 trays in total with 10 replicates of each treatment. We randomly assigned a treatment to half of a tray. Fifty seeds from each treatment were planted in a tray with 500 seeds planted from each treatment in total. We planted seeds from the ant handling treatments on the day that they were collected and seeds from the control and manual removal treatments on day 1.

We assigned seeds in the control and manual removal treatments haphazardly to cells. Seeds from different ant colonies were randomly assigned to cells across replicates with the same random arrangement for each replicate. Seeds collected on different days were also assigned to cells across replicates such that all seeds collected on a certain day were not clumped within a replicate. We planted half of the replicates in the first week and the other half in a second week. Trays were watered every other day or as needed and loosely covered

with clear plastic to help retain moisture. Seedlings started to emerge approximately 21 days after seeds were planted. We recorded seedling emergence once per week until we stopped observing emergence (approximately 70 days). We estimated time until emergence from the first day of the week in which seeds were planted to account for measuring emergence on a weekly basis. Because there could be a fixed time between primary dispersal and emergence, we also calculated the number of days elapsed between primary dispersal (the day seeds were fed to ants) and seedling emergence.

Statistical analyses

We conducted survival analyses to compare the time (number of days) for *A. rudis* and *M. rubra* colonies to remove seeds from nests for the behavioural trials (see *Seed handling behaviour trials*) and for the trials conducted for the seedling emergence experiment (see *Seedling emergence experiment*). In the first set of trials, we recorded the total number of days it took for colonies to remove all seeds from nests; therefore, our model did not include censoring. In the experiment trials, some seeds were collected from within nests. Thus, we included censoring that assumes that all seeds would eventually be removed from nests. Censoring in survival analysis accounts for not fully recording the time to an event occurring (e.g., seed removal from nest). In both analyses, we incorporated time-specific hazard distributions (weibull) given that removal rates were not constant over time (Crawley, 2007). A generalised linear mixed model (GLMM) was conducted with a binomial error distribution and logit link function to compare the proportion of handled seeds (i.e. removed from nests) with their elaiosomes completely removed over time. Ant species was a fixed factor in the model and time was a random factor nested within each colony. The full model was compared to a simpler model that included only species as a fixed factor. Model comparisons and model significance were tested with χ^2 tests (Crawley, 2007).

For the emergence experiment, a generalised linear model (GLM) was conducted to assess the effects of week planted and treatment on the proportion of emerged seedlings. A binomial error distribution and a logit link function were used, given there was no evidence of overdispersion. Significance was tested with a χ^2 test (Crawley, 2007). We compared differences in time until seedling emergence among treatments with a survival analysis using a weibull distribution and including censoring to assume that seedlings could emerge at a future date. Week planted and plant tray (block) were included in the full model. Model comparisons and significance was tested with a χ^2 tests test. Differences among treatments were assessed by comparing *P*-values using sequential Bonferroni (Rice, 1989; Crawley, 2007).

We examined if colony-level variation in handling times influenced seedling emergence by conducting a GLM on the timing and proportion of emerged seedlings and the maximum number of days it took for seeds to be removed from the nests of ant colonies. We included species as a factor and used a binomial distribution and logit link function. Additionally,

we conducted a survival analysis with a weibull distribution and censoring to see if time in the nest influenced time until seedling emergence with colony, species, and plant tray (block) as factors. All statistical tests were conducted in R version 2.15.1 (R core team, Vienna, Austria). The lmer function in the lme4 package was used for GLMM (Bates *et al.*, 2012). The survival package and survreg were used for the survival analysis (Therneau, 2013).

Results

Aphaenogaster rudis retained seeds inside nests for longer than *M. rubra* both in the behavioural trials (survival: $\chi^2 = 17.66$, d.f. $= 1$, $P < 0.0001$; Fig. 1) and in the trials for the emergence experiment (survival: $\chi^2 = 47.21$, d.f. $= 1$, $P < 0.0001$). There was no difference in the proportion of seeds with elaiosomes completely removed from seeds between species (GLMM: Wald $z = -0.35$, d.f. $= 1$, $P = 0.727$; Fig. 2), and time did not significantly improve the fit of the model (SD $= 0.74$).

There was no effect of treatment (GLM: $\chi^2 = 5.02$, d.f. $= 3$, $P = 0.1706$; Fig. 3a) or week planted ($P > 0.05$) on the proportion of emerged seedlings. There was, however, an effect of treatment on time until emergence with week planted removed from the final model, but plant tray retained (survival: $\chi^2 = 27.59$, d.f. $= 12$, $P = 0.0063$; Fig. 3b). When seeds were handled by *A. rudis*, seedlings emerged faster (mean $\pm$ SE $= 43 \pm 0.75$ days) compared with seeds handled by *M. rubra* (46 ± 0.77 days; $P = 0.0028$), with their elaiosomes manually removed (45 ± 0.96 days; $P = 0.0140$), and controls (46 ± 0.86 days; $P = 0.0288$). Treatment and plant tray similarly affected time until emergence when emergence time was estimated from primary dispersal (survival: $\chi^2 = 25.85$, d.f. $= 12$, $P = 0.0110$), indicating that this result is not simply as a result of a fixed time between primary dispersal and seedling emergence.

There was a positive relationship between the proportion of emerged seedlings from seeds handled by colonies and the maximum number of days seeds were retained inside nests (GLM: $\chi^2 = 7.23$, d.f. $= 1$, $P < 0.0072$; Fig. 4a). The proportion of seedlings that emerged from handled seeds ranged from 0.48 ± 0.04 for seeds that were handled for 1 day to 0.63 ± 0.02 for seeds that were handled for 6 days (Fig. 4a). There was also a negative relationship between time until seedling emergence and the number of days that seeds were retained inside nests (survival: $\chi^2 = 13.42$, d.f. $= 1$, $P = 0.0002$; Fig. 4b); species and colony were not included in the final model. Seedlings took 48 ± 1.17 days to emerge if they were handled for 1 day, but only 36 ± 1.87 days to emerge when handled for 6 days (Fig. 4b). There were similar effects when time until emergence was calculated from primary dispersal (survival: $\chi^2 = 10.43$, d.f. $= 1$, $P = 0.0012$).

Discussion

Handling by ants or manual removal of elaiosomes did not increase the proportion of emerged seedlings; however, seeds that were handled by *A. rudis* emerged slightly earlier
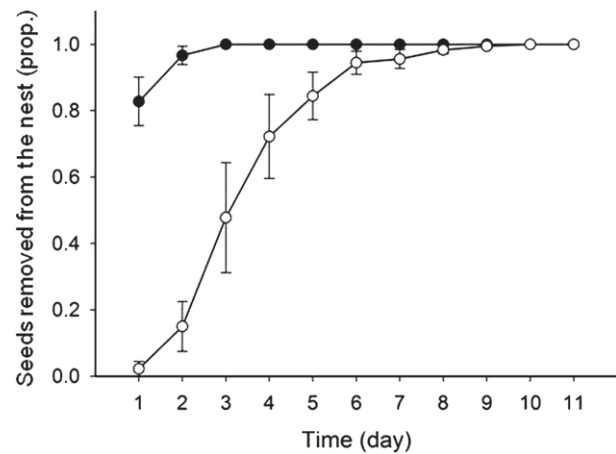

Fig. 1. The cumulative proportion of seeds of *Chelidonium majus* that *Aphaenogaster rudis* (white) and *Myrmica rubra* (black) removed from their nests over time (days) (see *Seed handling behaviour trials*). Each point is the average ($+/-$ SE) of eight and nine colonies of each species, respectively.

than seeds that were handled by *M. rubra*, control seeds, and seeds that had elaiosomes manually removed. Early emergence may be beneficial to plants if individuals gain a competitive advantage over later-emerging seedlings. Previous studies of herbaceous plants have found that early-germinating individuals are likely to be vigorous and become dominant in populations, facilitating early flowering and large seed crops (Naylor, 1972; Ross & Harper, 1972). Emergence was only 3 days earlier when seeds were handled by *A. rudis*. We do not know if early emergence by this number of days would significantly contribute to plant fitness especially in the context of phenological variation in dehiscence times in more natural conditions and relative to benefits gained from primary and secondary dispersal. Other studies have also documented early germination and emergence of handled seeds (Culver & Beattie, 1980; Horvitz, 1981). Thus, handling by ants may provide a benefit to plants by facilitating early emergence, but we do not know the extent to which early emergence would contribute to plant fitness.

Both of these species secondarily disperse seeds outside their nests in waste piles or middens (Servigne & Detrain, 2010; Canner *et al.*, 2012). *Myrmica rubra* deposited the majority of seeds outside nests 1 day after seeds were fed to colonies and all seeds were removed after 3 days. *Aphaenogaster rudis* kept seeds inside nests for up to 11 days and there was more variation among colonies in the time that seeds were retained inside nests. Furthermore, the longer a colony kept seeds in the nest, the more seedlings emerged and the earlier they did so. Conditions that seeds experience inside ant nests could be a benefit of handling. Micro-environmental factors provide important conditions for germination and establishment (Hartgerink & Bazzaz, 1984) and perhaps the moist and humid conditions inside nests prime seeds for germination and emergence. Our artificial nests did not completely mimic natural nest conditions. However, these artificial nests and natural nests in cavities of rotting wood
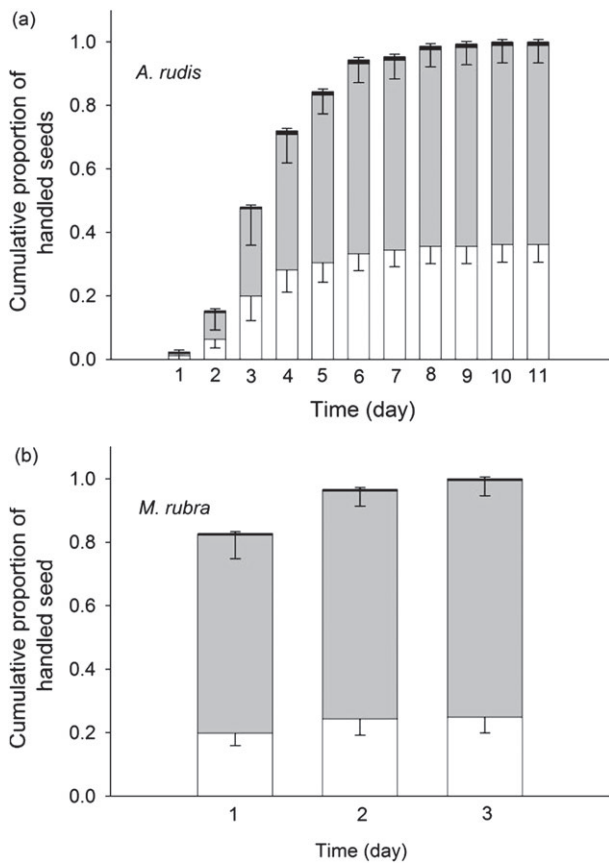
Fig. 2. The cumulative proportion of seeds of *Chelidonium majus* that were discarded from nests over time (days) with elaiosomes completely removed (white), partially removed (grey), or not removed (black) by (a) *Aphaenogaster rudis* or (b) *Myrmica rubra*. Bars represent the average (− or + SE for clarity) of eight and nine colonies of each species, respectively.

Fig. 3. Box plots of (a) the proportion of emerged seedlings and (b) the number of days until seedlings emerged for *Chelidonium majus* seeds that were handled by *Aphaenogaster rudis*, *Myrmica rubra*, that had elaiosomes manually removed, or that were not manipulated (controls). The top and bottom lines of the boxes represent the 75th and 25th percentiles, the middle line represents the median, and the circles represent outliers. Significant differences compared with *A. rudis* denoted as (**).

are similar in that they both probably provide moist and humid environments for ant colonies and seeds relative to the surrounding soil. In fact, in our experiment seeds probably experienced favourable soil conditions in the greenhouse compared with what they may experience in nature. Thus, our results could be conservative with respect to the effect that time in the nest has on seedling emergence.

Elaiosome removal has been proposed to be a benefit of seed handling by ants. However, we found no difference in the amount of elaiosome removed when seeds were handled by either species of ant, and the amount of elaiosome removed was not influenced by the length of time that seeds remained inside nests. Additionally, we found no effect of manual removal on the proportion of emerged seedlings. Taken together, these results suggest that elaiosome removal does not benefit *C. majus* seedlings. Elaiosomes have different tissue origins and placements which could influence how removal affects seedling success (Gorb & Gorb, 2003). For example, elaiosome removal could decrease the germination success of seeds in which elaiosomes develop from internal cells (e.g. Imbert, 2006). Germination success often increases or does not change
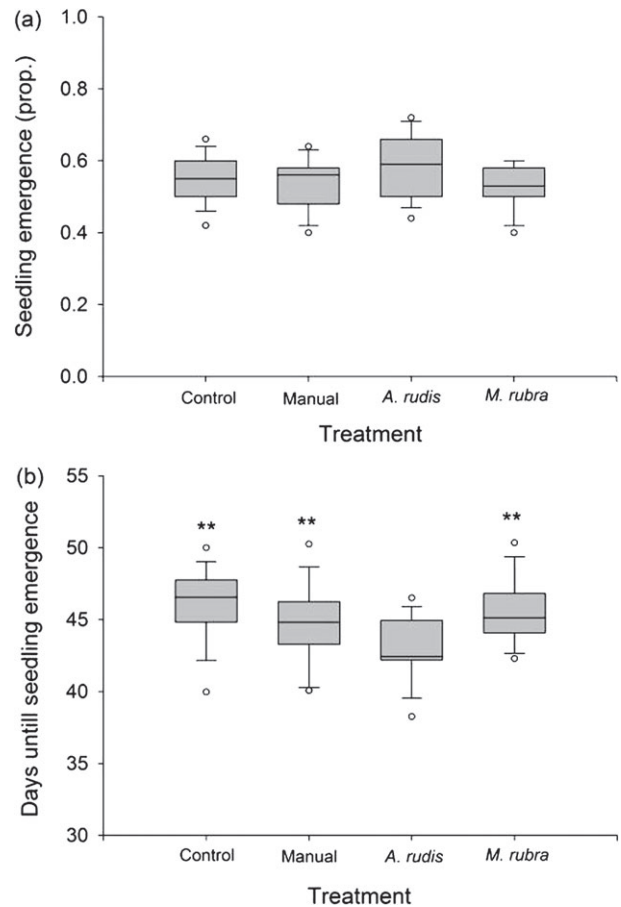
when elaiosomes are removed from seeds in which elaiosomes develop from external cells, as in *C. majus* (Lisci *et al.*, 1996; Gorb & Gorb, 2003). Seeds in the family Euphorbiaceae have elaiosomes that cover the micropyle region of the seed or the area responsible for water intake and elaiosome removal in these species often facilitates germination (Gorb & Gorb, 2003; Leal *et al.*, 2007). Some previous studies have found that elaiosome removal increases germination in some contexts but not others. For example, Horvitz (1981) found that elaiosome removal only increased germination for seeds that experienced dry conditions. Elaiosome removal can also increase fitness by reducing predation (e.g. Kwit *et al.*, 2012), which was not a factor in our experiment. Further exploration of elaiosome removal of *C. majus* seeds by ants under natural abiotic and biotic conditions could reveal that elaiosome removal provides benefits, but our results suggest that perhaps not differently for seeds handled by these two species of ants.
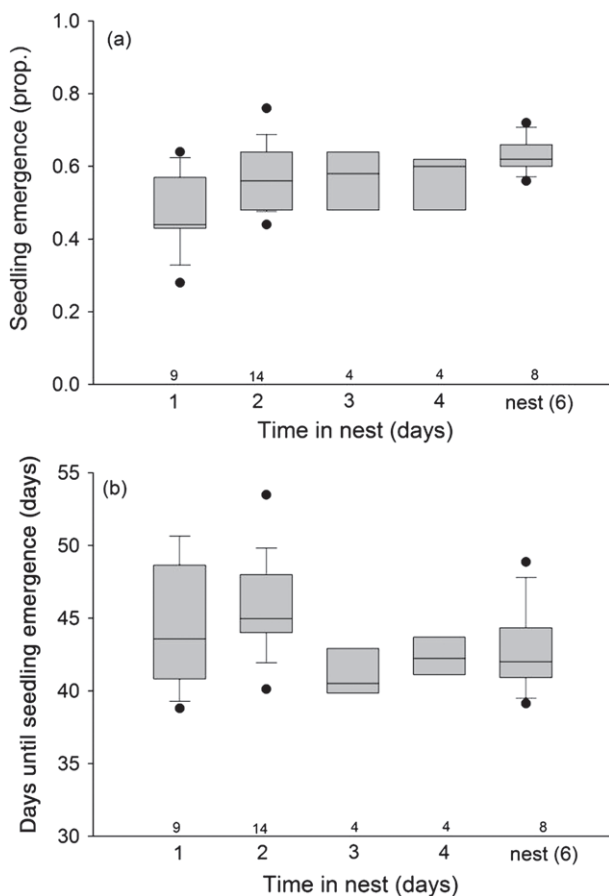
Fig. 4. Box plots of the (a) proportion of emerged seedlings and (b) the time to emergence from seeds that were handled by 20 *Aphaenogaster rudis* and 20 *Myrmica rubra* colonies that took 1–5 days to remove all seeds from nests or with seeds still remaining in nests (day 6). The top and bottom lines of the boxes represent the 75th and 25th percentiles, the middle line represents the median, and the circles represent outliers. Numbers below the boxes represent colonies. Data are not presented for the one *A. rudis* colony that took 5 days to remove seeds from nests (proportion emerged = 0.56; time until emergence = 41 days).

Studies on the selective advantages of myrmecochory suggest that plant fitness depends on the identity and behaviour of ant partners (Culver & Beattie, 1980; Giladi, 2006; Servigne & Detrain, 2008, 2010). We documented differences in seed handling behaviours between *A. rudis* and *M. rubra*. Servigne and Detrain (2010) also found that *M. rubra* removed seeds at a faster rate from their nests than *Lasius niger*. However, they did not measure the effects of handling by different species on plant performance. They suggested that quick removal from nests may be a result of *M. rubra* being largely insectivorous and having hygienic tendencies. Insect prey is also part of the diet of *A. rudis*, but there may be differences in the contribution of this food source to the overall diet of these species. Workers of both species feed elaiosomes to their larvae and larval nutritional requirements probably influences seed handling behaviours (Bono & Heithaus, 2002; Fokuhl *et al.*,

2012). Differences in handling times between these species could be as a result of species-specific differences in seed processing times. These ant species also probably differ in their foraging behaviours, and they do differ in colony size and structure and in nest structure. Examining how differences in all of these features affect plant fitness is essential to fully uncover how ant identity influences plant fitness.

Handling behaviours that we observed in the lab may not accurately reflect how ants handle seeds in more natural conditions. For example, we would expect greater variation in seed-processing times in the field given that colonies may vary in size, age, and in the amount and type of food they have already acquired. Although there may be greater variation in handling times in the field, our results probably reflect real and ecologically significant differences in handling behaviours between species. We found large and constant differences in handling times between species. Also, our results support previous studies that assessed removal rates from nests or secondary dispersal. For example, Servigne and Detrain (2010) found that *M. rubra* removed the majority of seeds of *C. majus* from artificial nests within 6 h of picking up seeds, whereas Canner *et al.* (2012) found 6.8% of seeds remained in nests 7 days after seeds were fed to *A. rudis* colonies in the field.

Myrmecochory in this ecosystem is unevenly diffuse (Ness *et al.*, 2009). Thus, we may expect that an introduced ant could have particularly large effects on plant populations and communities. Conversely, given that ants interact with many plant species and have varied diets, introduced plant partners may have relatively smaller effects on ant populations and communities. We found that both species of ants readily picked up *C. majus* seeds. Elaiosomes are thought to be the result of convergent evolution favouring seed traits that facilitate dispersal by a guild of ants that are subordinate, omnivorous foragers (Giladi, 2006); a guild that both of these species belong to (Gorb *et al*., 2000; Lubertazzi, 2012). So it is not unexpected that both of these species readily dispersed *C. majus*. This suggests that both native and invasive ants could contribute to the spread of *C. majus*; an expected response for introduced plants involved in diffuse mutualisms (Richardson *et al.*, 2000). In contrast, we did find that *A. rudis* and *M. rubra* may not function similarly in the benefits that they provide to *C. majus*. Handling by *A. rudis* may provide greater benefits to *C. majus* than handling by *M. rubra*. Thus, differences in the behaviour and colony characteristics of ant species have the potential to influence plant fitness and the evolutionary trajectories of plant traits mediated by these mutualisms.

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Supporting Information

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Appendix S1. Diagram of the seedling emergence experiment.

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