Differential dispersal of *Chamaesyce maculata* seeds by two ant species in Japan

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Abstract

Seed dispersal by ants (myrmecochory) is a widely distributed plant-animal interaction in many ecosystems, and it has been regarded as a generalized (multiple species) interaction in which specialization on specific ant partners is uncommon. In this paper, we demonstrate species-specific seed dispersal of spotted spurge (*Chamaesyce maculata*) by ants in Japan. *Chamaesyce maculata* produces seeds from summer to autumn in Japan. The seeds produced in autumn are carried by two ant species, *Tetramorium tsushimae* and *Pheidole noda*. We performed laboratory experiments to investigate the fate of *C. maculata* seeds in the nests of *T. tsushimae* and *P. noda*. *Pheidole noda* consumed the seeds in the nest and rarely carried seeds out of the nest, while *T. tsushimae* consumed only the seed coat, and subsequently carried the seeds out of the nest. Removal of the seed coat by *T. tsushimae* may increase seed survival by reducing their susceptibility to infection by fungi. We also observed ant responses to filter paper soaked with an aqueous extract of the seed coat. *Pheidole noda* did not react to the filter paper, but *T. tsushimae* carried the filter paper into their nest. Analysis by high-pressure liquid chromatography (HPLC) revealed that the aqueous extract contained at least four sugars and one unknown substance. Myrmecochory has been regarded as a generalized interaction with specialization for specific ant partners uncommon. However, our study suggests there is a species-specific interaction in seed dispersal by ants in autumn-flowering individuals of *C. maculata* in Japan.

Key words: myrmecochory; *Pheidole noda*; *Tetramorium tsushimae*; seed coat; specialization

Introduction

Some species interact with only one or a few species, while others interact with many species. The process of divergence toward specialization or generalization has long been a central issue in evolutionary and ecological research (Manzaneda and Rey 2009). Specialization in antagonistic interactions is likely to occur via coevolutionary arms races, which are positive feedback mechanisms in which organisms develop adaptations and counter-adaptations against each other (Dawkins and Krebs 1979; Thompson 2005). Specialization in mutualistic associations also occurs. For example, interactions between plants and pollinating seed parasites, such as yuccas and yucca moths (Pellmyr and Leebens-Mack 1999), figs and fig wasps (Janzen 1979), and Phyllantheae plants and *Epicephala* moths (Kato et al. 2003) have evolved as highly specialized and pair-wise mutualisms. However, in general, many mutualisms are facultative and involve multiple species interacting opportunistically with one another.

Ant-plant interactions are common examples of the multiple-species mutualisms. The basic ant-plant mutualisms have been categorized into four types, based on the types of services that ants provide to plants (Beattie 1985): (1) protection from herbivores, (2) provision of essential nutrients, (3) pollination, and (4) seed dispersal. In return, plants provide rewards such as nest sites or food. Associations involving protection and seed dispersal by ants are found in a diverse range of habitats including arid, tropical and temperate regions (Beattie 1985; Koptur 1992; Lengyel et al 2010). In many ecosystems, these interactions involve multiple interacting species (Bentley 1976; Beattie 1985; Koptur 1992; Katayama and Suzuki 2005).

Many plant species depend on ants for their seed dispersal (myrmecochory). Myrmecochory has been reported in over 11,000 plant species in 334 genera (Lengyel et al 2010). The geographical and taxonomic distributions of myrmecochory suggest that it has independently evolved many times, possibly as a response to different selection pressures operating under different ecological circumstances (Westoby et al. 1991; Dunn et al. 2007).

Myrmecochorous plants produce seeds with a nutrient-rich structure called an elaiosome. The elaiosome acts as an attractant which may elicit seed collection by ants (Marshall et al. 1979; Lanza et al. 1992). Typically, ants carry the seeds into their nest, remove the elaiosome, and feed it to their larvae (Handel and Beattie 1990). After removing the elaiosome, the ants carry the intact seeds to waste disposal areas, where the seeds may germinate. The benefits of myrmecochory to plants have been described by several authors (e.g., Auld 1986; Ohkawara et al. 1996; Whitney 2002). Ant nests where seeds are carried are thought to be preferred with safe sites for the seeds by reducing the risk of mortality due to fire (Auld 1986) or predators (Ohkawara et al. 1996). Soils near the ant nests often have high nutrient contents and thereby increase seedling survival and growth (Horvitz 1981; Beattie and Culver 1983; Beattie 1985). Finally, the seeds escape from competition with the parent plant and other competitors (Bond et al. 1991). Although average seed dispersal distances by ants are typically just a few meters (Wilson 1993), in some case, longer distance dispersal can be achieved by ants (Whitney 2002).

Although it is generally believed that specialized associations in ant-mediated seed dispersal are rare, there is increasing evidence of more specialized relationships in ant-seed interactions (Zelikova et al. 2008; Manzaneda and Rey 2009). Several studies reported plant elaiosomes that attract specific guilds of ants (Marshall et al. 1979; Lanza et al. 1992; Giladi 2006). However, most previous studies only examined seed removal rate, and studies evaluating actual seed fate after ant dispersal are lacking. In this study, we performed three laboratory experiments and one chemical analysis to demonstrate species-specific seed dispersal by ants in the spotted spurge, *Chamaesyce maculata* in Japan, and to evaluate fate of seeds carried into ant nests. *Chamaesyce maculata* (Euphorbiaceae) is a prostrate annual weed, and produces small seeds (about 0.8 mm and 0.15 mg fresh) from summer to autumn in western Japan (Suzuki and Teranishi 2005; Suzuki and Ohnishi 2006). It has two different modes of seed dispersal depending on season (Ohnishi et al. 2008). The seeds produced in summer are dispersed farther

from the parents by automatic mechanical seed dispersal (autochory). However, no autochory occurs in the seeds produced in autumn, and some seeds are instead dispersed by ants. Although 18 species of ants have been recorded visiting *C. maculata* plants at 50 sites in western Japan, only two, *Tetramorium tsushimae* and *Pheidole noda*, have been observed carrying the seeds of *C. maculata* into their nests (Ohnishi et al. 2008). In this study, we investigated the fate of *C. maculata* seeds in nests of *T. tsushimae* and *P. noda*. Our results showed that only *T. tsushimae* carried seeds out of their nest after removing the seed coat. Second, we demonstrated that removal of the seed coat is potentially beneficial for the seeds by decreasing the infection rate by fungi. Third, we observed ant behavior in response to an aqueous extract of the seed coat, which suggested that the seed coat was important in inducing *T. tsushimae* to carry the seeds into their nest. Finally, we carried out a chemical analysis which revealed that the aqueous extract contained at least four sugars and one unknown substance.

Materials and methods

Study species

Chamaesyce maculata is distributed in North America and East Asia. In Japan, the plants grow in open areas such as waste grounds, footpaths, and rock crevices from early June to November. *Chamaesyce maculata* does not produce typical elaiosomes on its seeds (Ohnishi and Suzuki 2011). However, we found that its seeds were covered with a thin coat. We randomly collected over 2,000 seeds of *C. maculata* produced in autumn from several mature plants on the campuses of Saga University (city of Saga, 33°14′N, 130°18′E) in western Japan for use in the following experiments.

Workers of *T. tsushimae* and *P. noda* are small omnivorous ants about 2 mm in body length. They colonize open lands such as grasslands, wastelands and raised areas between farm fields. Colonies of *T. tsushimae* and *P. noda* (>10,000 workers and >20 queens each) were

collected in the city of Saga, and reared separately in large plastic cages (27 × 17 × 17 cm). Ants in each cage were fed 10% sucrose solution and 10 mealworm larvae (*Tenebrio molitor*) once a day. Sucrose solution was poured in a test tube (diameter 1.2 cm, length 12 cm) plugged at the top with cotton wool, to be freely used by ants.

Experiment 1: Fate of seeds in ant nests

To evaluate the fate of seeds in the nests of *T. tsushimae* and *P. noda*, we carried out a laboratory experiment. We made five artificial nests of *T. tsushimae* and *P. noda*, respectively (Appendix Fig. S1). Five hundred workers, 100 larvae, and one queen were put in a plastic cage (13.5 × 6.2 × 3.5 cm). The bottom of each cage was covered with plaster, 2.5 cm deep, to maintain a suitable humidity, and the top of each cage was covered with a red cellophane film to maintain darkness for ants while allowing for observation of ant behaviors in the nest.

Ants were starved for four days prior to the beginning of the experiment, and we connected one nest to a plaster stage (2.5 cm deep) in a plastic cup (diameter 10 cm, depth 4.5 cm) by a vinyl chloride tube (6 mm in inner diameter and 10 cm long). The inside of the plastic cup was coated with talc powder to prevent ants from escaping. Hereafter, the plaster stage in the plastic cup is referred to as the arena. We placed 100 *C. maculata* seeds on the arena and counted the number of seeds carried into each nest within 12 hours. After 12 hours, we removed the remaining seeds from the arena and then we counted number of seeds that were carried back to the arena from the nest once a day. Then we checked the seed status (damaged or undamaged, and with or without seed coat), and calculated the proportion of damaged seeds to the total seeds brought back to the arena. After three days, we opened the ant nest, and checked the status of seeds in the nest. Based on the number of undamaged seeds remaining, we estimated the number of consumed seeds. The trials were replicated one time for each of the *T. tsushimae* and *P. noda* nests.

Experiment 2: Benefit for seeds by removing seed coat

To evaluate the benefits of seed coat removal for *C. maculata*, we measured the fungal infection rates of seeds with and without seed coats. We collected 40 seeds from five nests of *T. tsushimae* after the previous experiment. Then, we placed them on wet plaster in a plastic cup (diameter 10 cm, depth 4.5 cm, i.e., 40 seeds per cup) at 25°C and 0L24D photoperiod conditions. All of the seeds that had been placed in the ant nest for three days were undamaged, but the seed coats had been removed by *T. tsushimae*. After three days, we recorded the proportion of seeds that were infected by fungi. As a control, we put 43 seeds with seed coats on wet plaster in another plastic cup under same condition mentioned above, and recorded the proportion of seeds infected by fungi.

Experiment 3: Ant behavior responses to seed coat extract

To evaluate effects of the seed coat on ants seed dispersal, we carried out a laboratory experiment using similar experimental settings as previously described (Appendix Fig. S1). We made 20 and 18 artificial nests (with 500 workers, 100 larvae, and one queen) of *T. tsushimae* and *P. noda*, respectively. Twenty seeds were placed in a 1.5-mL tube with 50 µL distilled water. After five min, we dripped 5 µL of aqueous extract onto a piece of filter paper (2 × 2 mm). Twenty such pieces of filter paper were placed in an arena connected to an ant nest (500 workers, 100 larvae, and one queen), and we recorded the number of pieces of paper remaining in the arena for 90 min at 10-minute intervals. We conducted this treatment using half of ant nests (i.e., replications of *T. tsushimae* and *P. noda* were 10 and 9, respectively). We conducted a control treatment using the remaining nests. Twenty pieces of filter paper treated with 5 µL distilled water were placed in the arena, and we recorded the number of pieces of filter paper remaining in the arena for 90 min at 10-minute intervals.

Sugar analysis

We analyzed sugar contents in the seed coat. Two hundred seeds were placed in a 1.5-mL tube along with 1 mL distilled water. After five min, the water extract was transferred to another 1.5-mL tube, and was freeze-dried for 24 hours. The sample was dissolved in 10 µl of Milli-Q-Water, and sugar contents in the sample were analyzed by high-pressure liquid chromatography (HPLC), using a Wakosil $5NH_2$-MS packed column (4.6 x 150 mm; Wako Pure Chemical, Osaka, Japan) and 78% acetonitrile mobile phase at room temperature. Peak size for the various sugars present in the sample was calculated directly using a refractive index detector (RID; Shimadzu Corp., Kyoto, Japan). The sample was optimized using two sugar standards containing five or six sugars, with each sugar at a concentration of 5 µg μL^{-1} (standard 1: fructose, glucose, sucrose, maltose, and melezitose, standard 2: xylose, galactose, melibiose, mannitol, lactose, and raffinose). The composition of each sample was determined by comparison of retention times with those from a standard sample measured on the same day. We replicated this analysis five times.

Statistical analysis

The proportions of seeds carried into the nest, carried out of the nest, consumed and damaged by ants were compared between ant species using Mann Whitney *U*-tests. The sum of proportions of seeds consumed and damaged by ants was also compared between ant species using Mann Whitney *U*-tests. The proportion of seeds infected by fungi was compared between ant species using Chi-square tests. One-way repeated measures ANOVA was used to analyze the ants' carrying rate of filter paper with and without seed coat extract. All statistical procedures were conducted using JMP ver. 7 (SAS Institute Inc.).

Results

Fate of seeds in ant nests

Over the course of 12 hours, workers of both ant species carried most seeds into their nests. The number of seeds carried into the nest did not differ between ant species (Z = 0.645, P = 0.517, Fig. 1a). However, the ant's behavior after 12 hours differed. Workers of *T. tsushimae* brought 68% of the seeds that were carried into the nest back to the arena within three days (Fig. 1b). Workers of *P. noda* rarely carried seeds (11%) out of their nest (Z = -2.514, P = 0.012, Fig. 1b). Inside the nests, *T. tsushimae* consumed 32% of the seeds remaining in the nest, while *P. noda* consumed most seeds (96%) in the nest (Z = 2.514, P = 0.012, Fig. 1c). Out of the nests of *T. tsushimae* and *P. noda*, 14% and 46% seeds were damaged, respectively (Z = -2.087, P = 0.034, Fig. 1d, Fig. 2a). In total, 22% and 90% of seeds were damaged or consumed by *T. tsushimae* and *P. noda*, respectively. The total proportion of seeds damaged or consumed by *T. tsushimae* was significantly smaller than the proportion damaged or consumed by *P. noda* (Z = 2.514, P = 0.012). Before the experiment, thin seed coats were present on all seeds (Fig. 2b). However, the seed coats of the seeds carried out of *T. tsushimae* nests and of those remaining in the nests were removed (Fig. 2cd).

Benefit for seeds by removing seed coat

Over 95% (39/43) of seeds with intact seed coats were infested by fungi after three days, while less than 3% (1/40) of seeds without seed coats were infected by fungi (Fig. 3). The infection rate of seeds with intact seed coats was significantly greater than for those without seed coats (χ^2 = 64.56, P < 0.001). *Tetramorium* ants frequently removed the seed coat from the seed, and this seed coat-removal behavior was repeatedly observed in each trial in experiment 1. Therefore, we consider that our experiment can be reproducible.

Ant responses to seed coat extract

The extract of the seed coat induced *T. tsushimae* to carry treated filter papers into their nest (Fig. 4a). *Tetramorium tsushimae* workers carried more pieces of filter paper with the extract than those that had been treated only with distilled water (one-way repeated measures ANOVA; treatment: $F_{1,18}$ = 5.438, P = 0.032; time: $F_{8,11}$ = 2,503; P = 0.080, treatment × time: $F_{8,11}$ = 2.944; P = 0.050). *Pheidole noda* workers carried some pieces of the filter paper during the experiment: however the seed coat-extract did not induce *P. noda* ants to carry the paper into their nest (treatment: $F_{1,16}$ = 1.381, P = 0.257, time: $F_{9,8}$ = 2.175, P = 0.144, treatment × time: $F_{9,8}$ = 0.498, P = 0.840, Fig. 4b).

Sugar analysis

Four sugars (fructose, glucose, sucrose, and maltose) were detected in the seed coat (Appendix Fig. S2). Amounts of each sugar per seed are as follows: fructose: 46.9 ± 6.6 ng, glucose: 65.3 ± 17.9 ng, sucrose: 10.1 ± 4.8 ng, maltose: 18.3 ± 3.6 ng. In addition to these sugars, one peak was detected ahead of the peak of fructose although the retention time did not overlap with any of those of 11 reference sugars. The amount of the unknown substance was calculated using fructose as a reference; it was 106.1 ± 14.4 ng per seed.

Discussion

Our study demonstrates that *P. noda* rarely carried *C. maculata* seeds out of their nest but instead consumed the seeds inside their nest. This ant species is likely to behave as a seed predator. This seed-predatory ant species did not react to the substances in the seed coat. *Tetramorium tsushimae* often carried *C. maculata* seeds out of their nests after removing the seed coat. These results are consistent with field observation (Ohnishi et al. 2008) that both ant species carried seeds into their nests, but that only *T. tsushimae* frequently carried the seeds out

of their nest. Ohnishi and Suzuki (2011) considered *C. maculata* to be a species that lacks elaiosomes. However, our results suggest that the seed coat acts as an elaiosome to attract workers of *T. tsushimae*.

Although it has been recognized that elaiosome size and chemistry are important in determining seed removal rates by ants (Hughes and Westoby 1992; Lanza et al. 1992; Gorb and Gorb 1995; Mark and Olesen 1996; Pizo and Oliveira 2001; Peters et al. 2003), most previous studies investigate the responses of either a single ant species or of the overall ant assemblage to variation in elaiosome traits. Therefore, the possible effects of these traits on partner choice have not been adequately evaluated. Our study experimentally demonstrated that substances present on the seed coat specifically elicited *T. tsushimae* to carry the filter paper into their nest, but did not attract *P. noda*. These results suggest species-specific seed dispersal by ants of *C. maculata* in Japan.

Ohnishi and Suzuki (2009) carried out a laboratory experiment to investigate germination rates of *C. maculata* seeds before and after they had been carried by *T. tsushimae*. They demonstrated that seeds carried out of the ant nest did not lose the ability to germinate. In contrast, the germination rate slightly but significantly increased in the seeds that remained in the nests of *T. tsushimae* (germination rate after one month: control seeds: 46%: 92/200); seeds remaining in *T. tsushimae* nests: 60%: 105/175). Increased germination rate is likely caused by removal of the seed coat by *T. tsushimae*. Our study demonstrated that over 95% of seeds with intact seed coats were infested by fungi, while seeds from which the seed coat had been removed by *T. tsushimae* were rarely attacked by fungi. The seed coat contains several kinds of sugars, and it is possible that the high concentration of available carbons might increase the risk of microbial infection. *Tetramorium tsushimae* removes the seed coat, and consequently may increase survival rate of the seeds. Seed coat may generate an ecological trade-off (i.e., it acts as an ant-attractant, but has costs associated with fungal infection), although there are other

benefits of seed removal by ants, such as reducing the risk of mortality by predators (Ohkawara et al. 1996)

The present results suggest that *P. noda* behaves as a seed predator. However, we suspect that *P. noda* contributes to seed dispersal of *C. maculata* in the same manner as harvester ants do for other plants (Y. Ohnishi, unpublished data). Non-myrmecochorous seeds without special rewards for ants are also collected by harvester (granivorous) ants (Pizo and Oliveira 1998; Retana et al. 2004). They consume some of these seeds but others are abandoned in their nest. The leftover seeds may contribute to the seed bank. In addition, *P. noda* lose *C. maculata* seeds while carrying them from plants to the nest (Kobayashi 2009; Y. Ohnishi, unpublished data). Ohnishi and Suzuki (2011) stated that *P. noda* was a good disperser. To reinforce this hypothesis, additional experiments are needed to evaluate the effects of *P. noda* on seed dispersal in *C. maculata*.

Myrmecochory has been regarded as a generalized (multiple-species) interaction, in which specialization is rare. However, specialization may be more common than expected in myrmecochorous interactions (Giladi 2006; Zelikova et al. 2008; Manzaneda and Rey 2009; Youngsteadt et al. 2009). Seeds of the neotropical ant-garden epiphyte *Peperomia macrostachya* are removed by only three ant species (Youngsteadt et al. 2009). The myrmecochorous herb *Helleborus foetidus* is ecologically specialized to different local ant dispersers across a wide geographic range (Manzaneda and Rey 2009). Seeds of *Trillium* spp. and *Hexastylis arifolia* in Great Smoky Mountains National Park (USA) are removed by one dominant ant species *Aphaenogaster rudis* at every site although the seed removal rate decreases with elevation (Zelikova et al 2008). Because seed dispersal is extremely important for plant fitness, plant traits that affect seed dispersal are likely to be under strong selection pressure (Nathan and Muller-Landau 2000; Garrido et al. 2002). If an ideal ant partner rapidly disperses seeds to favorable habitats and is available in every habitat, or if plants require directional dispersal

toward a specific habitat, specialization to specific ant species is likely to occur. On the other hand, if ant faunal composition temporally and frequently fluctuates, or if plants cannot, over evolutionary time, anticipate the local ant fauna, the plant traits would evolve to permit association with multiple ant partners.

The present study provides one example of species-specific interactions in seed dispersal by ants. *Tetramorium tsushimae* is a dominant ant species in western Japan, and the ant positively affects seed survival and dispersal of *C. maculata*. It colonizes open lands, which are desirable habitats of *C. maculata*. The small size of *T. tsushimae* workers (about 2 mm) is matched to the size of *C. maculata* seeds. They do not feed on the seeds of *C. maculata*, probably because the seeds contain some substances that *T. tsushimae* cannot detoxify. These characteristics of *T. tsushimae* are favorable for *C. maculata*. In addition, *T. tsushimae* uses other myrmecochorous plants (Y. Ohnishi and N. Katayama, personal obs.), and therefore, this system is not a specialist-specialist interaction but a specialist-generalist interaction. Recent studies revealed that asymmetrical (specialist-generalist) interaction might be biologically advantageous in plant-animal mutualisms due to their robustness against local extinction of partners (Bascompte et al. 2003; Guimarães et al. 2006). Our results do not suggest possible explanations for why *C. maculata* depends on a specific ant partner, or how this system is specialized. However, this study presents a system in which to explore the process of divergence toward specialization or generalization in seed dispersal by ants, providing the first step for evaluating the above questions.

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References

Auld TD (1986) Population dynamics of the shrub *Acacia suaveolens* (Sm.) Willd.: dispersal and the dynamics of the soil seed-bank. Aust J Ecol 11:235–254

Bascompte J, Jordano P, Melián CJ, Olesen JM (2003) The nested assembly of plant–animal mutualistic networks. Proc Natl Acad Sci USA 100:9383–9387

Beattie AJ (1985) The evolutionary ecology of ant-plant mutualisms. Cambridge University Press, New York

Beattie AJ, Culver DC (1983) The nest chemistry of two seed-dispersing ant species. Oecologia 56:99–103

Bentley BL (1976) Plants bearing extrafloral nectaries and the associated ant community: interhabitat differences in the reduction of herbivore damage. Ecology 57:815-820

Bond WJ, Yeaton R, Stock WD (1991) Myrmecochory in Cape Fynbos. In: Huxley CR, Cutler DF (eds) Ant–plant interactions. Oxford Science publications, Oxford, pp 448–462

Dawkins R, Krebs JR (1979) Arms races between and within species. Proc R Soc Lond B 205:489-511

Dunn RR, Gove AD, Barraclough TG, Givnish TJ, Majer JD (2007) Convergent evolution of an ant–plant mutualism across plant families, continents, and time. Evol Ecol Res 9:1349–1362

Garrido JL, Rey PJ, Cerdá X, Herrera CM (2002) Geographical variation in diaspore traits of an ant-dispersed plant (*Helleborus foetidus*): are ant community composition and diaspore traits correlated? J Ecol 90:446-455

Giladi I (2006) Choosing benefits or partners: a review of the evidence for the evolution of myrmecochory. Oikos 112:481–492

Gorb SN, Gorb EV (1995) Removal rates of seeds of five myrmecochorous plants by the ant *Formica polyctena* (Hymenoptera, Formicidae). Oikos 73:367–374

Guimarães Jr PR, Rico-Gray V, dos Reis SF, Thompson JN (2006) Asymmetries in specialization in ant–plant mutualistic networks. Proc R Soc B 273:2041–2047

Handel SN, Beattie AJ (1990) Seed dispersal by ants. Sci Amer 263:58–64

Horvitz CC (1981) Analysis of how ant behaviors affect germination in a tropical myrmecochore *Calathea microcephala* (P. & E.) Koernicke (Marantaceae): Microsite selection and aril removal by neotropical ants, *Odontomachus*, *Pachycondyla*, and *Solenopsis* (Formicidae). Oecologia 51:47–52

Hughes L, Westoby M (1992) Effect of diaspore characteristics on removal of seeds adapted for dispersal by ants. Ecology 73:1300-1312

Janzen DH (1979) How to be a fig. Ann Rev Ecol Syst 10:13-51

Katayama N, Suzuki N (2005) The importance of the encounter rate between ants and herbivores and of ant aggressiveness against herbivores in herbivore exclusion by ants on *Vicia angustifolia* L. (Leguminosae) with extrafloral nectaries. Appl Entomol Zool 40:69-76

Kato M, Takimura A, Kawakita A (2003) An obligate pollination mutualism and reciprocal diversification in the tree genus *Glochidion* (Euphorbiaceae). Proc Natl Acad Sci USA 100:5264-5267

Kobayashi Y (2009) The significance of seed dispersal by ants in the prostrate annual, *Chamaesyce maculata* (L.) Small (Euphorbiaceae) and benefits of carrying the seeds for ants. PhD thesis, Kagoshima University, Kagoshima (in Japanese with English summary)

Koptur S (1992) Extrafloral nectary-mediated interactions between insects and plants. In: Bernays E (ed) Insect–plant interaction, vol IV. CRC Press, Boca Raton, pp 81-129

Lanza J, Schmitt MA, Awad AB (1992) Comparative chemistry of elaiosomes of three species of *Trillium*. J Chem Ecol 18:209-221

Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR (2010) Convergent evolution of seed

dispersal by ants, and phylogeny and biogeography in flowering plants: A global survey. Persp Plant Ecol Evol Syst 12:43-55

Manzaneda AJ, Rey PJ (2009) Assessing ecological specialization of an ant–seed dispersal mutualism through a wide geographic range. Ecology 90:3009–3022

Mark S, Olesen JM (1996) Importance of elaiosome size to removal of ant-dispersed seeds. Oecologia 107:95-101

Marshall DL, Beattie AJ, Bollenbacher WE (1979) Evidence for diglycerides as attractants in an ant-seed interaction. J Chem Ecol 5:335-344

Nathan R, Muller-Landau HC (2000) Spatial patterns of seed dispersal, their determinants and consequences for recruitment. Trends Ecol Evol 15:278-285.

Ohkawara K, Higashi S, Ohara M (1996) Effects of ants, ground beetles and the seed-fall patterns on myrmecochory of *Erythronium japonicum* Decne (Liliaceae). Oecologia 106:500–506

Ohnishi KY, Suzuki N (2009) Preferential removal of non-injured seeds by an omnivorous ant, *Tetramorium tsushimae* Emery, in the seed dispersal of *Chamaesyce maculata* (L.) small. Ecol Res 24:1155-1160

Ohnishi KY, Suzuki, N (2011) Seed predator deterrence by seed-carrying ants in a dyszoochorous plant, *Chamaesyce maculata* L. Small (Euphorbiaceae). Popul Ecol 53:441-447

Ohnishi Y, Suzuki N, Katayama N, Teranishi S (2008) Seasonally different modes of seed dispersal in the prostrate annual, *Chamaesyce maculata* (L.) Small (Euphorbiaceae), with multiple overlapping generations. Ecol Res 23:299-305

Pellmyr O, Leebens-Mack J (1999) Forty million years of mutualism: Evidence for Eocene origin of the yucca-yucca moth association. Proc Natl Acad Sci USA 96:9178–9183

Peters M, Oberrath R, Bohning-Gaese K (2003) Seed dispersal by ants: are seed preferences

influenced by foraging strategies or historical constraints? Flora 198: 413-420

Pizo MA, Oliveira PS (1998) Interaction between ants and seeds of a nonmyrmecochorous neotropical tree, *Cabralea canjerana* (Meliaceae), in the Atlantic forest of southeast Brazil. Am J Bot 85:669–674

Pizo MA, Oliveira PS (2001) Size and lipid content of nonmyrmecochorous diaspores: effects on the interaction with litter-foraging ants in the Atlantic rain forest of Brazil. Plant Ecol 157:37-52

Retana J, Picó X, Rodrigo A (2004) Dual role of harvesting ants as seed predators and dispersers of a non-myrmecochorous Mediterranean perennial herb. Oikos 105:377–385

Suzuki N, Ohnishi Y (2006) Significance of the simultaneous growth of vegetative and reproductive organs in the prostrate annual *Chamaesyce maculata* (L.) Small (Euphorbiaceae). Ecol Res 21:91-99

Suzuki N, Teranishi S (2005) Phenology and life cycle of the annual, *Chamaesyce maculata* (L.) Small (Euphorbiaceae), with multiple overlapping generations in Japan. Ecol Res 20: 425-432

Thompson JN (2005) The geographic mosaic of coevolution. University Chicago Press, Chicago

Westoby M, French K, Hughes L, Rice B, Rodgerson L (1991) Why do more plant species use ants for dispersal on infertile compared with fertile soils. Aust J Ecol 16:445-455

Whitney KD (2002) Dispersal for distance? *Acacia ligulata* seeds and meat ants Iridomyrmex viridiaeneus. Austral Ecol 27:589–595

Wilson MF (1993) Dispersal mode, seed shadows, and colonization patterns. Vegetatio 107/108:261–280

Youngsteadt E, Baca JA, Osborne J, Schal C (2009) Species-specific seed dispersal in an obligate ant-plant mutualism. PLoS One 4:1-11

1 Zelikova TJ, Dunn RR, Sanders NJ (2008) Variation in seed dispersal along an elevational
2 gradient in Great Smoky Mountains National Park. Acta Oecologica 34:155-162
3

Figure legends

Fig. 1 Proportions of seeds (a) carried into the nest, (b) carried out of the nest, (c) consumed by ants in the nest, and (d) damaged outside the nest of *Tetramorium tsushimae* and *Pheidole noda* ants, respectively. Error bars indicate SE.

Fig. 2 Pictures of (a) damaged seed, (b) seed with seed coat, (c) seed carried out of a nest of *Tetramorium tsushimae*, and (d) non-damaged seed remaining in a nest of *Tetramorium tsushimae*.

Fig. 3 (a) Picture of seed infected by fungi, and (b) difference in fungal infection of seeds before and after seed coat removal by *Tetramorium tsushimae.* Solid and open columns in (b) indicate the proportion of infected and non-infected seeds, respectively.

Fig. 4 The number of filter papers with distilled water (open circle) or the extract of *Chamaesyce maculata* seed coats (solid circle) remaining in the arena after 90 min. (a) *Tetramorium tsushimae* and (b) *Pheidole noda*. Error bars indicate SE.

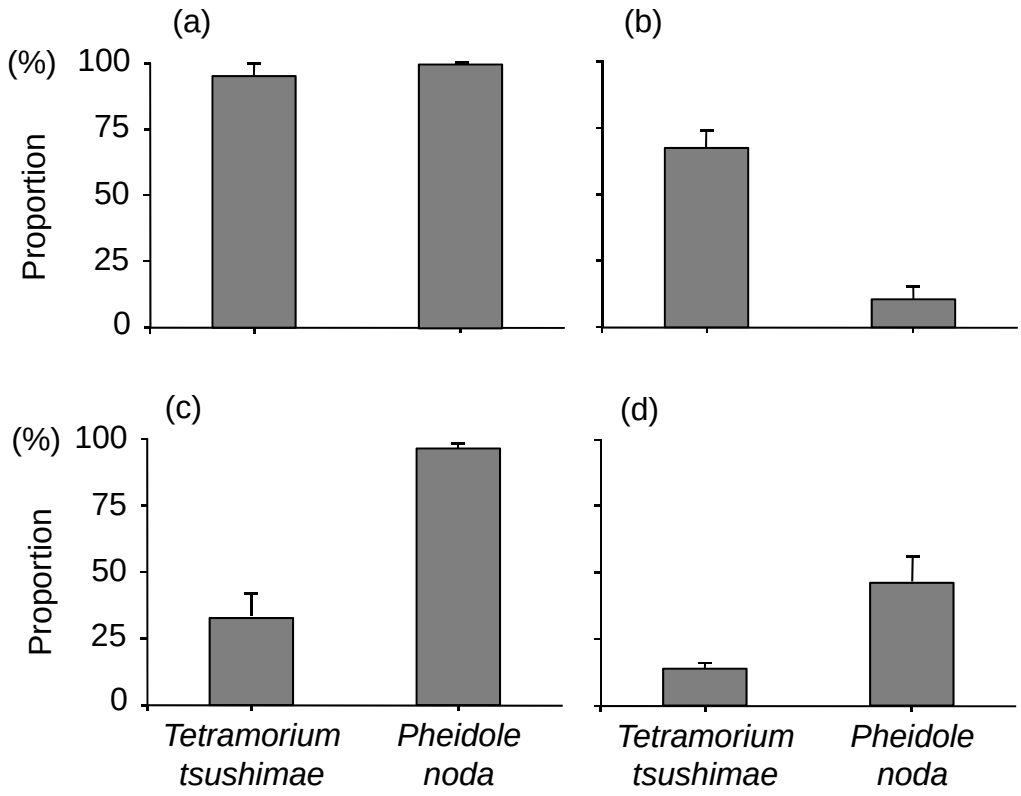
(a)
(b)
(c)
(d)
(%)
100
75
50
25
0
Proportion
Tetramorium tsushimae
Pheidole noda

Fig. 1


(a) Damaged seed
(b) Seed with seed coat
(c) Seed carried out of ant nest
(d) Non-damaged seed in ant nest

Fig. 2

(a)

(b)

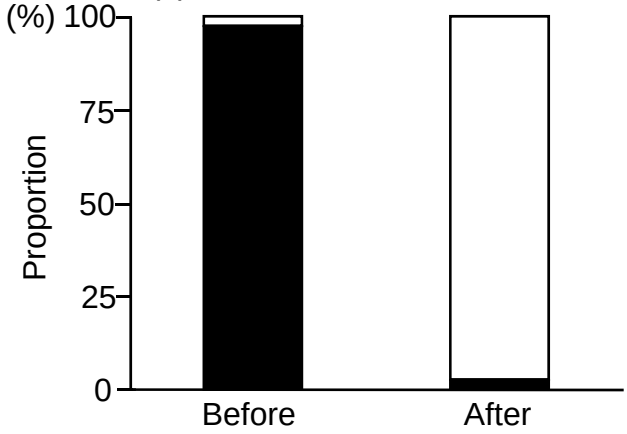

Fig. 3

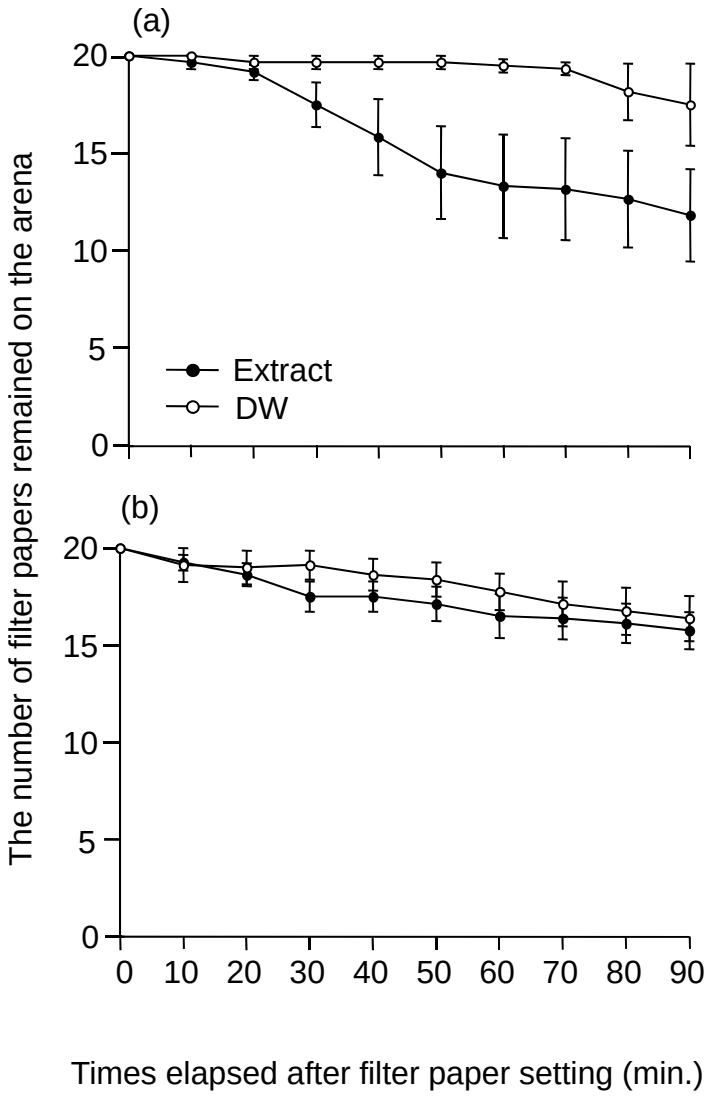
(a)
20
15
10
5
0
Extract
DW
(b)
20
15
10
5
0
0
10
20
30
40
50
60
70
80
90
The number of filter papers remained on the arena
Times elapsed after filter paper setting (min.)

Fig. 4

SUPPLEMENTARY MATERIALS

Differential dispersal of *Chamaesyce maculate* seeds by two ant species in Japan

Yoshihiro K. Ohnishi, Noboru Katayama, and Nobuhiko Suzuki

Appendix: Fig. S1. Illustration of experimental setting.

Appendix: Fig. S2. Chromatographs of water extract of spotted spurge's seeds.

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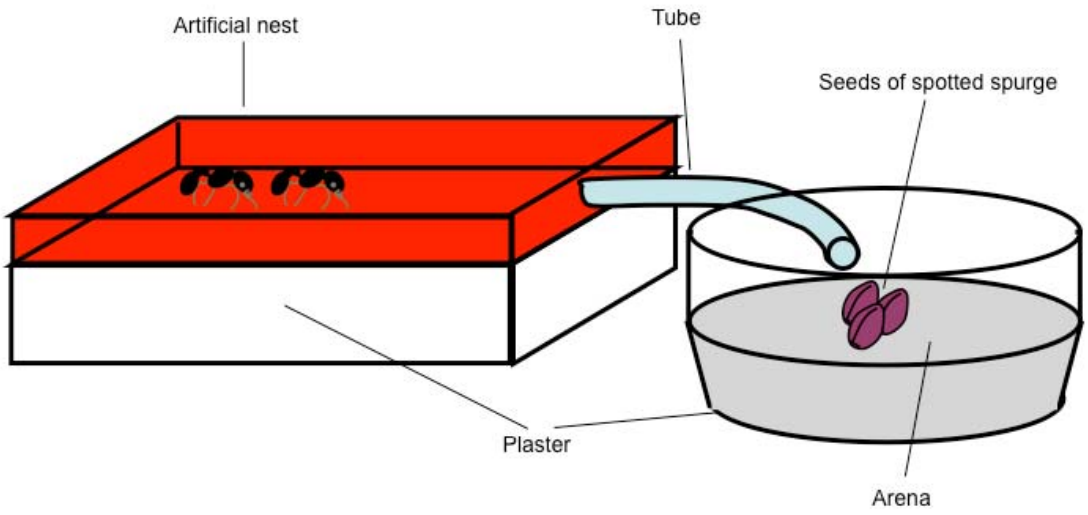

Fig. S1 Illustration of experimental setting. An artificial ant nest consisting of 500 workers, 100 larvae, and one queen is housed in a plastic cage. The nest is connected to a plaster stage (arena) in a plastic cup by a vinyl chloride tube. One hundred seeds are placed in the arena. The inside of the plastic cup is coated with talc powder to prevent ants from escaping.

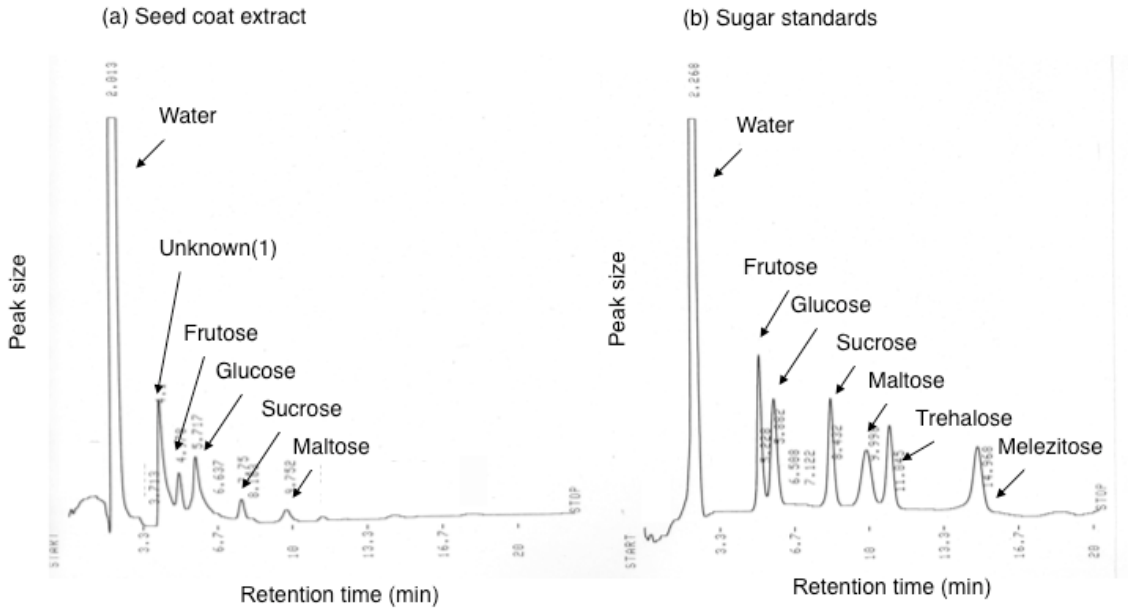

Fig. S2 Chromatographs of (a) water extract of *Chamaesyce maculata* seeds and (b) water standard containing fructose, glucose, sucrose, maltose, trehalose and melezitose. Four sugars (fructose, glucose, sucrose, and maltose) were identified in the seed coat by comparison of retention times with those from a standard sample. One peak was detected ahead of the peak of fructose although the retention time did not overlap with those of any reference sugars.