



Title	Differential dispersal of <i>Chamaesyce maculata</i> seeds by two ant species in Japan
Author(s)	Ohnishi, Yoshihiro K.; Katayama, Noboru; Suzuki, Nobuhiko
Citation	Plant Ecology, 214(7), 907-915 https://doi.org/10.1007/s11258-013-0217-7
Issue Date	2013-07
Doc URL	http://hdl.handle.net/2115/56457
Rights	The final publication is available at link.springer.com .
Type	article (author version)
File Information	Ohnishi et al 2013.pdf



[Instructions for use](#)

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

3

4 Yoshihiro K. Ohnishi^{1,4}, Noboru Katayama^{2,3}, and Nobuhiko Suzuki^{1,4}

5

6 ¹*Department of Applied Biological Sciences, Faculty of Agriculture, Saga University;*

7 *Honjo 1, Saga 840-8502, JAPAN*

8 ²*Center for Ecological Research, Kyoto University, 509-3, 2-chome, Hiranocho, Otsu, Shiga*

9 *520-2113, JAPAN*

10 ³*Present address: Field Science Center for Northern Biosphere, Hokkaido University,*

11 *Toikanbetsu 131, Horonobe-cho, Hokkaido 098-2943, JAPAN*

12 ⁴*The late doctors*

13

14 *Final word count: 4984 (Abstract to References)*

15

16 ***Corresponding author:*** Noboru Katayama

17 *Field Science Center for Northern Biosphere*

18 *Hokkaido University*

19 *Toikanbetsu 131*

20 *Horonobe-cho, Hokkaido 098-2943, Japan*

21 E-mail: noborukata1913@gmail.com

22 Phone: +81-1632-6-5211

23 Fax: +81-1632-6-5003

24

25 Author Contributions: YKO originally formulated the idea, and NK and NS developed methodology. YKO and NK
26 performed the experiments, and analyzed the data. NK wrote the manuscript.

27

1 **Abstract**

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

20

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

1 **Introduction**

2 Some species interact with only one or a few species, while others interact with many species.
3 The process of divergence toward specialization or generalization has long been a central issue
4 in evolutionary and ecological research (Manzaneda and Rey 2009). Specialization in
5 antagonistic interactions is likely to occur via coevolutionary arms races, which are positive
6 feedback mechanisms in which organisms develop adaptations and counter-adaptations against
7 each other (Dawkins and Krebs 1979; Thompson 2005). Specialization in mutualistic
8 associations also occurs. For example, interactions between plants and pollinating seed parasites,
9 such as yuccas and yucca moths (Pellmyr and Leebens-Mack 1999), figs and fig wasps (Janzen
10 1979), and Phyllanthaceae plants and *Epicephala* moths (Kato et al. 2003) have evolved as highly
11 specialized and pair-wise mutualisms. However, in general, many mutualisms are facultative
12 and involve multiple species interacting opportunistically with one another.

13 Ant-plant interactions are common examples of the multiple-species mutualisms. The
14 basic ant-plant mutualisms have been categorized into four types, based on the types of services
15 that ants provide to plants (Beattie 1985): (1) protection from herbivores, (2) provision of
16 essential nutrients, (3) pollination, and (4) seed dispersal. In return, plants provide rewards such
17 as nest sites or food. Associations involving protection and seed dispersal by ants are found in a
18 diverse range of habitats including arid, tropical and temperate regions (Beattie 1985; Koptur
19 1992; Lengyel et al 2010). In many ecosystems, these interactions involve multiple interacting
20 species (Bentley 1976; Beattie 1985; Koptur 1992; Katayama and Suzuki 2005).

21 Many plant species depend on ants for their seed dispersal (myrmecochory).
22 Myrmecochory has been reported in over 11,000 plant species in 334 genera (Lengyel et al
23 2010). The geographical and taxonomic distributions of myrmecochory suggest that it has
24 independently evolved many times, possibly as a response to different selection pressures
25 operating under different ecological circumstances (Westoby et al. 1991; Dunn et al. 2007).

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

14 Although it is generally believed that specialized associations in ant-mediated seed
15 dispersal are rare, there is increasing evidence of more specialized relationships in ant-seed
16 interactions (Zelikova et al. 2008; Manzaneda and Rey 2009). Several studies reported plant
17 elaiosomes that attract specific guilds of ants (Marshall et al. 1979; Lanza et al. 1992; Giladi
18 2006). However, most previous studies only examined seed removal rate, and studies evaluating
19 actual seed fate after ant dispersal are lacking. In this study, we performed three laboratory
20 experiments and one chemical analysis to demonstrate species-specific seed dispersal by ants in
21 the spotted spurge, *Chamaesyce maculata* in Japan, and to evaluate fate of seeds carried into ant
22 nests. *Chamaesyce maculata* (Euphorbiaceae) is a prostrate annual weed, and produces small
23 seeds (about 0.8 mm and 0.15 mg fresh) from summer to autumn in western Japan (Suzuki and
24 Teranishi 2005; Suzuki and Ohnishi 2006). It has two different modes of seed dispersal
25 depending on season (Ohnishi et al. 2008). The seeds produced in summer are dispersed farther

1 from the parents by automatic mechanical seed dispersal (autochory). However, no autochory
2 occurs in the seeds produced in autumn, and some seeds are instead dispersed by ants. Although
3 18 species of ants have been recorded visiting *C. maculata* plants at 50 sites in western Japan,
4 only two, *Tetramorium tsushimae* and *Pheidole noda*, have been observed carrying the seeds of
5 *C. maculata* into their nests (Ohnishi et al. 2008). In this study, we investigated the fate of *C.*
6 *maculata* seeds in nests of *T. tsushimae* and *P. noda*. Our results showed that only *T. tsushimae*
7 carried seeds out of their nest after removing the seed coat. Second, we demonstrated that
8 removal of the seed coat is potentially beneficial for the seeds by decreasing the infection rate
9 by fungi. Third, we observed ant behavior in response to an aqueous extract of the seed coat,
10 which suggested that the seed coat was important in inducing *T. tsushimae* to carry the seeds
11 into their nest. Finally, we carried out a chemical analysis which revealed that the aqueous
12 extract contained at least four sugars and one unknown substance.

13

14 **Materials and methods**

15 *Study species*

16 *Chamaesyce maculata* is distributed in North America and East Asia. In Japan, the plants grow
17 in open areas such as waste grounds, footpaths, and rock crevices from early June to November.
18 *Chamaesyce maculata* does not produce typical elaiosomes on its seeds (Ohnishi and Suzuki
19 2011). However, we found that its seeds were covered with a thin coat. We randomly collected
20 over 2,000 seeds of *C. maculata* produced in autumn from several mature plants on the
21 campuses of Saga University (city of Saga, 33°14'N, 130°18'E) in western Japan for use in the
22 following experiments.

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

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

5

6 *Experiment 1: Fate of seeds in ant nests*

7 To evaluate the fate of seeds in the nests of *T. tsushimae* and *P. noda*, we carried out a laboratory
8 experiment. We made five artificial nests of *T. tsushimae* and *P. noda*, respectively (Appendix
9 Fig. S1). Five hundred workers, 100 larvae, and one queen were put in a plastic cage (13.5×6.2
10 $\times 3.5$ cm). The bottom of each cage was covered with plaster, 2.5 cm deep, to maintain a
11 suitable humidity, and the top of each cage was covered with a red cellophane film to maintain
12 darkness for ants while allowing for observation of ant behaviors in the nest.

13 Ants were starved for four days prior to the beginning of the experiment, and we
14 connected one nest to a plaster stage (2.5 cm deep) in a plastic cup (diameter 10 cm, depth
15 4.5 cm) by a vinyl chloride tube (6 mm in inner diameter and 10 cm long). The inside of the
16 plastic cup was coated with talc powder to prevent ants from escaping. Hereafter, the plaster
17 stage in the plastic cup is referred to as the arena. We placed 100 *C. maculata* seeds on the
18 arena and counted the number of seeds carried into each nest within 12 hours. After 12 hours,
19 we removed the remaining seeds from the arena and then we counted number of seeds that were
20 carried back to the arena from the nest once a day. Then we checked the seed status (damaged
21 or undamaged, and with or without seed coat), and calculated the proportion of damaged seeds
22 to the total seeds brought back to the arena. After three days, we opened the ant nest, and
23 checked the status of seeds in the nest. Based on the number of undamaged seeds remaining, we
24 estimated the number of consumed seeds. The trials were replicated one time for each of the *T.*
25 *tsushimae* and *P. noda* nests.

1

2 *Experiment 2: Benefit for seeds by removing seed coat*

3 To evaluate the benefits of seed coat removal for *C. maculata*, we measured the fungal infection
4 rates of seeds with and without seed coats. We collected 40 seeds from five nests of *T. tsushimae*
5 after the previous experiment. Then, we placed them on wet plaster in a plastic cup (diameter
6 10 cm, depth 4.5 cm, i.e., 40 seeds per cup) at 25°C and 0L24D photoperiod conditions. All of
7 the seeds that had been placed in the ant nest for three days were undamaged, but the seed coats
8 had been removed by *T. tsushimae*. After three days, we recorded the proportion of seeds that
9 were infected by fungi. As a control, we put 43 seeds with seed coats on wet plaster in another
10 plastic cup under same condition mentioned above, and recorded the proportion of seeds
11 infected by fungi.

12

13 *Experiment 3: Ant behavior responses to seed coat extract*

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

1

2 *Sugar analysis*

3 We analyzed sugar contents in the seed coat. Two hundred seeds were placed in a 1.5-mL tube
4 along with 1 mL distilled water. After five min, the water extract was transferred to another
5 1.5-mL tube, and was freeze-dried for 24 hours. The sample was dissolved in 10 μ l of
6 Milli-Q-Water, and sugar contents in the sample were analyzed by high-pressure liquid
7 chromatography (HPLC), using a Wakosil 5NH₂-MS packed column (4.6 x 150 mm; Wako Pure
8 Chemical, Osaka, Japan) and 78% acetonitrile mobile phase at room temperature. Peak size for
9 the various sugars present in the sample was calculated directly using a refractive index detector
10 (RID; Shimadzu Corp., Kyoto, Japan). The sample was optimized using two sugar standards
11 containing five or six sugars, with each sugar at a concentration of 5 μ g μ L⁻¹ (standard 1:
12 fructose, glucose, sucrose, maltose, and melezitose, standard 2: xylose, galactose, melibiose,
13 mannitol, lactose, and raffinose). The composition of each sample was determined by
14 comparison of retention times with those from a standard sample measured on the same day. We
15 replicated this analysis five times.

16

17 *Statistical analysis*

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

25

1 **Results**

2 *Fate of seeds in ant nests*

3 Over the course of 12 hours, workers of both ant species carried most seeds into their nests. The
4 number of seeds carried into the nest did not differ between ant species ($Z = 0.645$, $P = 0.517$,
5 Fig. 1a). However, the ant's behavior after 12 hours differed. Workers of *T. tsushimae* brought
6 68% of the seeds that were carried into the nest back to the arena within three days (Fig. 1b).
7 Workers of *P. noda* rarely carried seeds (11%) out of their nest ($Z = -2.514$, $P = 0.012$, Fig. 1b).
8 Inside the nests, *T. tsushimae* consumed 32% of the seeds remaining in the nest, while *P. noda*
9 consumed most seeds (96%) in the nest ($Z = 2.514$, $P = 0.012$, Fig. 1c). Out of the nests of *T.*
10 *tsushimae* and *P. noda*, 14% and 46% seeds were damaged, respectively ($Z = -2.087$, $P = 0.034$,
11 Fig. 1d, Fig. 2a). In total, 22% and 90% of seeds were damaged or consumed by *T. tsushimae*
12 and *P. noda*, respectively. The total proportion of seeds damaged or consumed by *T. tsushimae*
13 was significantly smaller than the proportion damaged or consumed by *P. noda* ($Z = 2.514$, $P =$
14 0.012). Before the experiment, thin seed coats were present on all seeds (Fig. 2b). However, the
15 seed coats of the seeds carried out of *T. tsushimae* nests and of those remaining in the nests were
16 removed (Fig. 2cd).

17

18 *Benefit for seeds by removing seed coat*

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

25

1 *Ant responses to seed coat extract*

2 The extract of the seed coat induced *T. tsushimae* to carry treated filter papers into their nest
3 (Fig. 4a). *Tetramorium tsushimae* workers carried more pieces of filter paper with the extract
4 than those that had been treated only with distilled water (one-way repeated measures ANOVA;
5 treatment: $F_{1,18} = 5.438$, $P = 0.032$; time: $F_{8,11} = 2,503$; $P = 0.080$, treatment \times time: $F_{8,11} =$
6 2.944 ; $P = 0.050$). *Pheidole noda* workers carried some pieces of the filter paper during the
7 experiment: however the seed coat-extract did not induce *P. noda* ants to carry the paper into
8 their nest (treatment: $F_{1,16} = 1.381$, $P = 0.257$, time: $F_{9,8} = 2.175$, $P = 0.144$, treatment \times time:
9 $F_{9,8} = 0.498$, $P = 0.840$, Fig. 4b).

10

11 *Sugar analysis*

12 Four sugars (fructose, glucose, sucrose, and maltose) were detected in the seed coat (Appendix
13 Fig. S2). Amounts of each sugar per seed are as follows: fructose: 46.9 ± 6.6 ng, glucose: $65.3 \pm$
14 17.9 ng, sucrose: 10.1 ± 4.8 ng, maltose: 18.3 ± 3.6 ng. In addition to these sugars, one peak
15 was detected ahead of the peak of fructose although the retention time did not overlap with any
16 of those of 11 reference sugars. The amount of the unknown substance was calculated using
17 fructose as a reference; it was 106.1 ± 14.4 ng per seed.

18

19 **Discussion**

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

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

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

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

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

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

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

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

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

21

22

23 Acknowledgments. We are grateful for the assistance provided by the members of the
24 Laboratory of System Ecology, Saga University and Center for Ecological Research, Kyoto
25 University. We thank S. Teranishi, M. Ushio and K. Hashimoto for comments on this paper. We

1 also thank D. Hembry and M. Whitaker for correcting the English of the text. This study was
2 partly supported by the Global COE program A06 of Kyoto University, and by Grants-in-Aid
3 from the Ministry of Education, Science, Sports and Culture of Japan (grant no. 14540582 and
4 19570022).
5

1 **References**

- 2 Auld TD (1986) Population dynamics of the shrub *Acacia suaveolens* (Sm.) Willd.: dispersal
3 and the dynamics of the soil seed-bank. *Aust J Ecol* 11:235–254
- 4 Bascompte J, Jordano P, Melián CJ, Olesen JM (2003) The nested assembly of plant–animal
5 mutualistic networks. *Proc Natl Acad Sci USA* 100:9383–9387
- 6 Beattie AJ (1985) *The evolutionary ecology of ant-plant mutualisms*. Cambridge University
7 Press, New York
- 8 Beattie AJ, Culver DC (1983) The nest chemistry of two seed-dispersing ant species. *Oecologia*
9 56:99–103
- 10 Bentley BL (1976) Plants bearing extrafloral nectaries and the associated ant community:
11 interhabitat differences in the reduction of herbivore damage. *Ecology* 57:815–820
- 12 Bond WJ, Yeaton R, Stock WD (1991) Myrmecochory in Cape Fynbos. In: Huxley CR, Cutler
13 DF (eds) *Ant–plant interactions*. Oxford Science publications, Oxford, pp 448–462
- 14 Dawkins R, Krebs JR (1979) Arms races between and within species. *Proc R Soc Lond B*
15 205:489–511
- 16 Dunn RR, Gove AD, Barraclough TG, Givnish TJ, Majer JD (2007) Convergent evolution of an
17 ant–plant mutualism across plant families, continents, and time. *Evol Ecol Res*
18 9:1349–1362
- 19 Garrido JL, Rey PJ, Cerdá X, Herrera CM (2002) Geographical variation in diaspore traits of an
20 ant-dispersed plant (*Helleborus foetidus*): are ant community composition and diaspore
21 traits correlated? *J Ecol* 90:446–455
- 22 Giladi I (2006) Choosing benefits or partners: a review of the evidence for the evolution of
23 myrmecochory. *Oikos* 112:481–492
- 24 Gorb SN, Gorb EV (1995) Removal rates of seeds of five myrmecochorous plants by the ant
25 *Formica polyctena* (Hymenoptera, Formicidae). *Oikos* 73:367–374

- 1 Guimarães Jr PR, Rico-Gray V, dos Reis SF, Thompson JN (2006) Asymmetries in
2 specialization in ant–plant mutualistic networks. *Proc R Soc B* 273:2041–2047
- 3 Handel SN, Beattie AJ (1990) Seed dispersal by ants. *Sci Amer* 263:58–64
- 4 Horvitz CC (1981) Analysis of how ant behaviors affect germination in a tropical
5 myrmecochore *Calathea microcephala* (P. & E.) Koernicke (Marantaceae): Microsite
6 selection and aril removal by neotropical ants, *Odontomachus*, *Pachycondyla*, and
7 *Solenopsis* (Formicidae). *Oecologia* 51:47–52
- 8 Hughes L, Westoby M (1992) Effect of diaspore characteristics on removal of seeds adapted for
9 dispersal by ants. *Ecology* 73:1300-1312
- 10 Janzen DH (1979) How to be a fig. *Ann Rev Ecol Syst* 10:13-51
- 11 Katayama N, Suzuki N (2005) The importance of the encounter rate between ants and
12 herbivores and of ant aggressiveness against herbivores in herbivore exclusion by ants on
13 *Vicia angustifolia* L. (Leguminosae) with extrafloral nectaries. *Appl Entomol Zool*
14 40:69-76
- 15 Kato M, Takimura A, Kawakita A (2003) An obligate pollination mutualism and reciprocal
16 diversification in the tree genus *Glochidion* (Euphorbiaceae). *Proc Natl Acad Sci USA*
17 100:5264-5267
- 18 Kobayashi Y (2009) The significance of seed dispersal by ants in the prostrate annual,
19 *Chamaesyce maculata* (L.) Small (Euphorbiaceae) and benefits of carrying the seeds for
20 ants. PhD thesis, Kagoshima University, Kagoshima (in Japanese with English summary)
- 21 Koptur S (1992) Extrafloral nectary-mediated interactions between insects and plants. In:
22 Bernays E (ed) *Insect–plant interaction*, vol IV. CRC Press, Boca Raton, pp 81-129
- 23 Lanza J, Schmitt MA, Awad AB (1992) Comparative chemistry of elaiosomes of three species
24 of *Trillium*. *J Chem Ecol* 18:209-221
- 25 Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR (2010) Convergent evolution of seed

- 1 dispersal by ants, and phylogeny and biogeography in flowering plants: A global survey.
2 Persp Plant Ecol Evol Syst 12:43-55
- 3 Manzaneda AJ, Rey PJ (2009) Assessing ecological specialization of an ant–seed dispersal
4 mutualism through a wide geographic range. Ecology 90:3009–3022
- 5 Mark S, Olesen JM (1996) Importance of elaiosome size to removal of ant-dispersed seeds.
6 Oecologia 107:95-101
- 7 Marshall DL, Beattie AJ, Bollenbacher WE (1979) Evidence for diglycerides as attractants in an
8 ant-seed interaction. J Chem Ecol 5:335-344
- 9 Nathan R, Muller-Landau HC (2000) Spatial patterns of seed dispersal, their determinants and
10 consequences for recruitment. Trends Ecol Evol 15:278-285.
- 11 Ohkawara K, Higashi S, Ohara M (1996) Effects of ants, ground beetles and the seed-fall
12 patterns on myrmecochory of *Erythronium japonicum* Decne (Liliaceae). Oecologia
13 106:500–506
- 14 Ohnishi KY, Suzuki N (2009) Preferential removal of non-injured seeds by an omnivorous ant,
15 *Tetramorium tsushimae* Emery, in the seed dispersal of *Chamaesyce maculata* (L.) small.
16 Ecol Res 24:1155-1160
- 17 Ohnishi KY, Suzuki, N (2011) Seed predator deterrence by seed-carrying ants in a
18 dyszoochorous plant, *Chamaesyce maculata* L. Small (Euphorbiaceae). Popul Ecol
19 53:441-447
- 20 Ohnishi Y, Suzuki N, Katayama N, Teranishi S (2008) Seasonally different modes of seed
21 dispersal in the prostrate annual, *Chamaesyce maculata* (L.) Small (Euphorbiaceae), with
22 multiple overlapping generations. Ecol Res 23:299-305
- 23 Pellmyr O, Leebens-Mack J (1999) Forty million years of mutualism: Evidence for Eocene
24 origin of the yucca-yucca moth association. Proc Natl Acad Sci USA 96:9178–9183
- 25 Peters M, Oberrath R, Bohning-Gaese K (2003) Seed dispersal by ants: are seed preferences

- 1 influenced by foraging strategies or historical constraints? *Flora* 198: 413-420
- 2 Pizo MA, Oliveira PS (1998) Interaction between ants and seeds of a nonmyrmecochorous
3 neotropical tree, *Cabralea canjerana* (Meliaceae), in the Atlantic forest of southeast
4 Brazil. *Am J Bot* 85:669–674
- 5 Pizo MA, Oliveira PS (2001) Size and lipid content of nonmyrmecochorous diaspores: effects
6 on the interaction with litter-foraging ants in the Atlantic rain forest of Brazil. *Plant Ecol*
7 157:37-52
- 8 Retana J, Picó X, Rodrigo A (2004) Dual role of harvesting ants as seed predators and
9 dispersers of a non-myrmecochorous Mediterranean perennial herb. *Oikos* 105:377–385
- 10 Suzuki N, Ohnishi Y (2006) Significance of the simultaneous growth of vegetative and
11 reproductive organs in the prostrate annual *Chamaesyce maculata* (L.) Small
12 (Euphorbiaceae). *Ecol Res* 21:91-99
- 13 Suzuki N, Teranishi S (2005) Phenology and life cycle of the annual, *Chamaesyce maculata* (L.)
14 Small (Euphorbiaceae), with multiple overlapping generations in Japan. *Ecol Res* 20:
15 425-432
- 16 Thompson JN (2005) *The geographic mosaic of coevolution*. University Chicago Press,
17 Chicago
- 18 Westoby M, French K, Hughes L, Rice B, Rodgerson L (1991) Why do more plant species use
19 ants for dispersal on infertile compared with fertile soils. *Aust J Ecol* 16:445-455
- 20 Whitney KD (2002) Dispersal for distance? *Acacia ligulata* seeds and meat ants *Iridomyrmex*
21 *viridiaeneus*. *Austral Ecol* 27:589–595
- 22 Wilson MF (1993) Dispersal mode, seed shadows, and colonization patterns. *Vegetatio*
23 107/108:261–280
- 24 Youngsteadt E, Baca JA, Osborne J, Schal C (2009) Species-specific seed dispersal in an
25 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

1 **Figure legends**

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

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

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

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

14

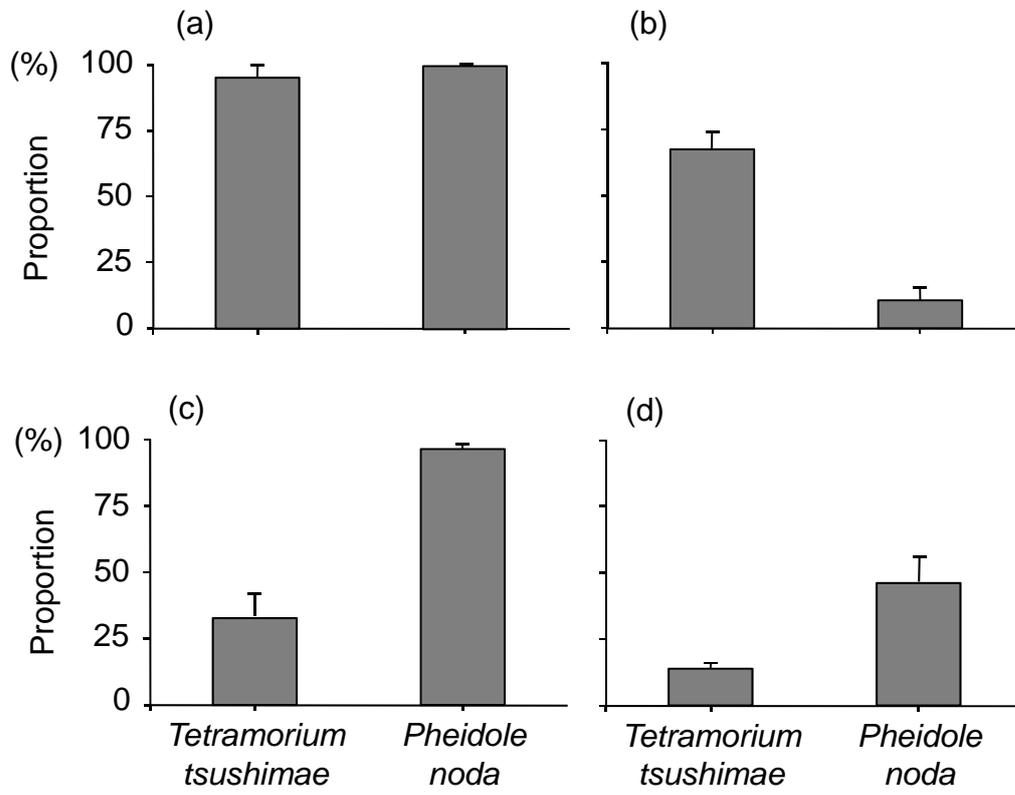


Fig. 1



Fig. 2

(a)

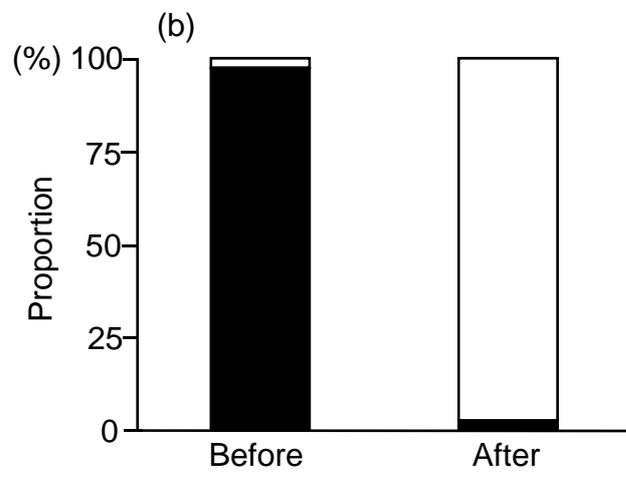


Fig. 3

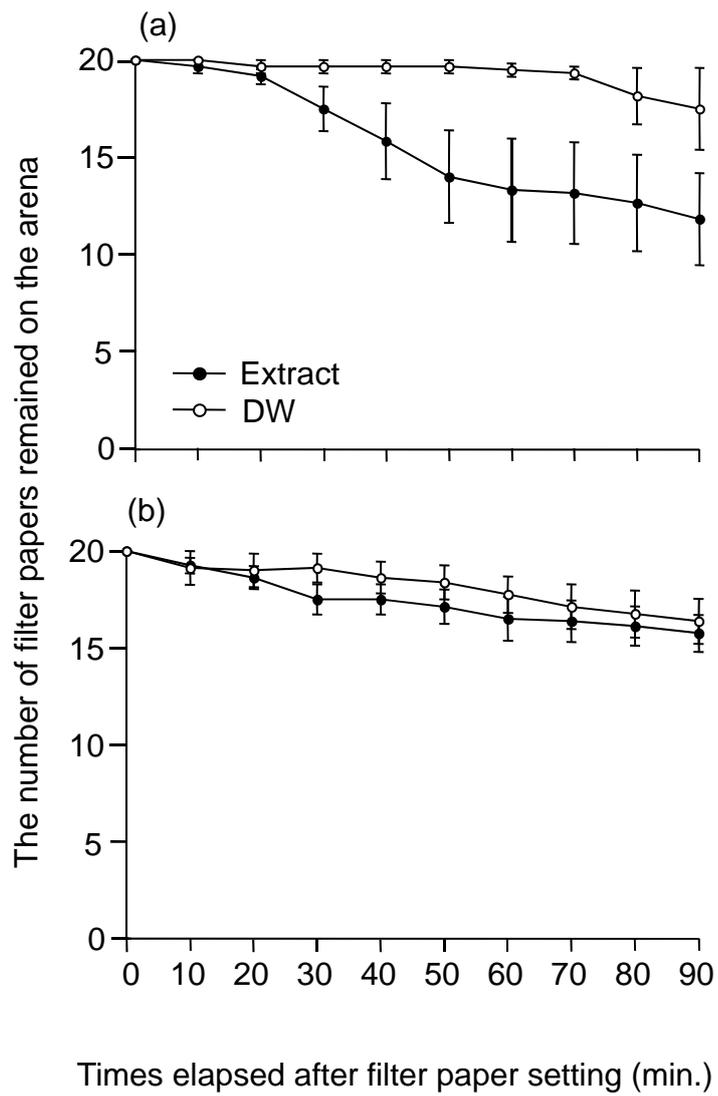


Fig. 4

1 **SUPPLEMENTARY MATERIALS**

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

4 Yoshihiro K. Ohnishi, Noboru Katayama, and Nobuhiko Suzuki

5

6 Appendix: Fig. S1. Illustration of experimental setting.

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

8

9

10 ***Corresponding author:*** Noboru Katayama

11 *Field Science Center for Northern Biosphere*

12 *Hokkaido University*

13 *Toikanbetsu 131*

14 *Horonobe-cho, Hokkaido 098-2943*

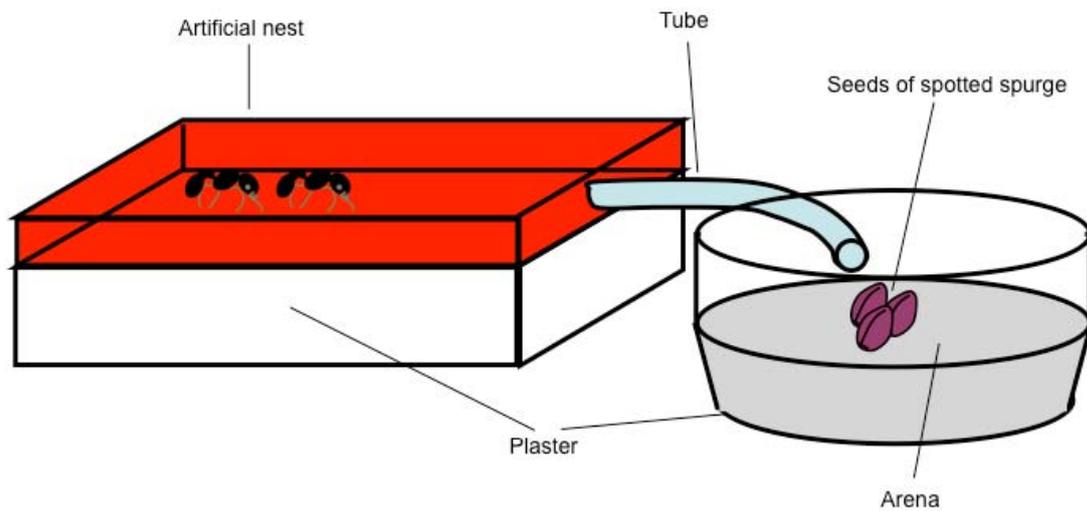
15 *Japan*

16 E-mail: noborukata1913@gmail.com

17 Phone: +81-1632-6-5211

18 Fax: +81-1632-6-5003

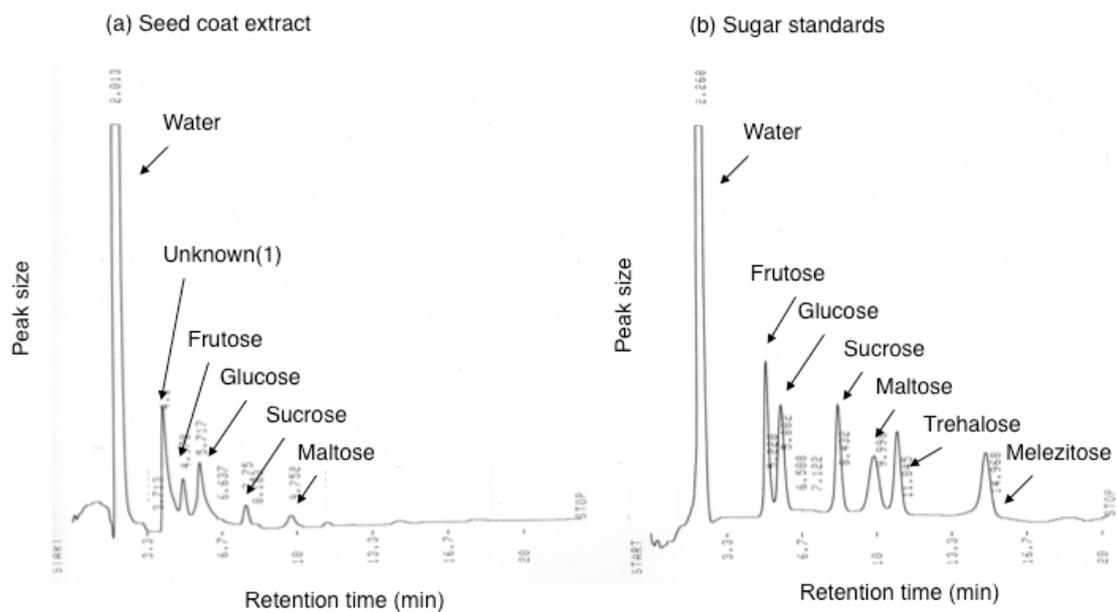
19



1

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

7



1

2 **Fig. S2** Chromatographs of (a) water extract of *Chamaesyce maculata* seeds and (b)

3 water standard containing fructose, glucose, sucrose, maltose, trehalose and melezitose.

4 Four sugars (fructose, glucose, sucrose, and maltose) were identified in the seed coat by

5 comparison of retention times with those from a standard sample. One peak was

6 detected ahead of the peak of fructose although the retention time did not overlap with

7 those of any reference sugars.

8