Larger Seeds are Dispersed Farther: the Long-Distance Seed Disperser ant *Aphaenogaster famelica* Prefers Larger Seeds

by

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ABSTRACT

Seed dispersal by ants (myrmecochory) is an important life phase in flowering herbs of temperate deciduous forests, and long dispersal is basically preferable in terms of plant fitness. Although it is known that larger ants transport seeds farther, previous studies conflicted whether larger seeds are preferred by large ants. We examine how seed size and ant body size influence removal rate of seeds and their dispersal distance by ants. The obligate myrmecochorous, large-seed bearing *Viola hondoensis* and ant-ballistic diplochorous, small-seed bearing *Viola* spp. and *Corydalis pallida* were comparatively investigated. 1) Ant preference: attraction rate and transportation rate of large *V. hondoensis* seeds by ants were consistently high regardless of the disperser ant size whilst those rates of small diplochorous seeds were conspicuously lower for large *Aphaenogaster* ants than for small *Pheidole* ants, suggesting small seeds are not adapted to be carried by large ants. 2) Dispersal distance: large *V. hondoensis* seeds were transported for a long distance because they were carried by both large and small ants while the small diplochorous seeds were transported for a short distance because they were carried only by small ants. These results suggest that large seeds are advantageous to attract large ants and to be farther dispersed as far as ant-mediated dispersal is concerned. On the other hand, the diplochorous small seeds do not attract large ants, and so are transported short by ants although they disperse their seeds by ballistic dispersal.

Key words: Myrmecochory, plant-animal interaction, *Pheidole*, seed dispersal, *Viola*

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INTRODUCTION

Seed dispersal by ants is called myrmecochory. Myrmecochorous plants have seeds that have caruncles or arils that act as elaiosomes which attract ants. Ant workers take such seeds and transport them to their nest. After the elaiosome part is consumed by the ants, the seed itself may remain in the nest or be discarded on the midden or farther away (Gorb & Gorb 2003). The seed, thus, can disperse from their mother plant. Howe & Smallwood (1982) reviewed advantages to seed dissemination and grouped them into three hypotheses. First, escape hypothesis proposes that dispersal may represent escape from disproportionately high seed and seedling mortality near the parent due to competition and/or predation. Second, colonization hypothesis assumes that seed dissemination may allow parent plants to establish their offspring in vacant sites. And the third hypothesis is termed directed dispersal, in which animals are assumed to consistently deliver seeds to special sites critical for establishment. In the cool temperate forests of Japan, the first and second hypotheses have been shown important (Higashi et al. 1989; Ohkawara & Higashi 1994). Apparently, the two hypotheses predict that long distance dispersal is essentially advantageous in terms of plant fitness.

To get seeds dispersed farther, myrmecochorous plants have two alternative tactics. One is to associate with 'good' long-distance transporting ants, and the other is to adopt diplochorous (ballistic long-distance and ant-mediated short-distance) dispersal (Ohkawara & Higashi 1994). Ness et al. (2004) demonstrated that seed dispersal distance generally increases with ant body size in three ant subfamilies that include most of seed-dispersing ants. So, as far as ant dispersal distance is concerned, plants should hire large ants as seed dispersers for long-distance dispersal. Then, what kind of seed character is desirable in order to attract larger ants? Beattie et al. (1979) found that large-sized ant species tended to take larger diaspores, and Hughes & Westoby (1992b) reported that diaspore size increased removal rates of seeds by *Pheidole* ants. On the contrary, Boulay et al. (2007) showed the seed size did not affect ant visitation rate to a myrmecochorous plant, *Helleborus foetidus*.

In Japanese temperate deciduous forests, two types of myrmecochorous herbs are encountered: obligate myrmecochorous herbs and ant-ballistic diplochorous herbs. As the former have no way to disperse their seeds other than ant dispersal, we hypothesize that they have 'desirable' seeds to attract large, 'good' disperser ants.

The aim of the present study is to investigate the effect of seed size on attraction rate, transportation rate, and dispersal distance by ants in both obligate myrmecochorous and ant-ballistic diplochorous herbs. Additionally, we compared the effect between small-sized and large-sized seed disperser ant species. Our hypothesis is that larger seeds are attractive to large ants and are transported farther. To address this issue, we investigated (1) preference of two different-sized ant species for seed size, and (2) relationship between dispersal distance and seed size.

MATERIALS AND METHODS

Study site and materials

The study site is a temperate deciduous forest at Fujii glen, Nagano, central Japan (alt.: c.700m). It is an oak-pine forest, the dominant tree species being *Quercus acutissima, Q. serrata* and *Pinus densiflora*. Field work was carried out from April to October 2005 along footpaths through the forest (c. 10m x 400m). The potential seed-dispersing ants of the forest were *Aphaenogaster famelica*: body length 3.5-8mm (large), *Pheidole fervida*: 2-2.5mm (small), *Formica japonica:* 4.5-6mm (large), *Lasius japonicus:* 2.5-3.5mm (small), *Camponotus obscuripes:* 7-12mm (large), *Pristomyrmex pungens:* 2-2.5mm (small), and *Paratrechina flavipes:* 2-2.5mm (small), among which *A. famelica, P. fervida,* and *P. flavipes* were conspicuously abundant and were selected for the study material. The density of ants (all the ant species included) was c. 10-30 walking ants and several ant nests per 1m^2 forest floor.

Myrmecochorous herbs of the study site include *Viola* spp., *Corydalis pallida* and *Erythronium japonicum.* These plants are encountered at similar microhabitats in the forest floor. Generally, *Viola* and *Corydalis* are diplochorous plants that disperse their seeds in two ways, ballistic and ant dispersal. A matured capsule opens and ejects seeds in a moment. Additionally, tiny elaiosomes are attached to the pole of the diaspores and attract ants when the seeds are dropped on the ground. The myrmecochorous plants we studied are as follows. *Viola hondoensis*: obligate myrmecochorous, have especially large seeds. They do not disperse the seeds ballistically, seed size: 2.6-4.1mm (large), elaiosome size: 1.0-1.8mm. *V. mirabilis*: ant-ballistic diplochorous dispersal, seed size: 2.7-3.3mm (large), elaiosome size: 0.6-1.1mm. *V. tokubuchiana:* ant-ballistic diplochorous dispersal, seed size: 2.0-2.2mm (small), elaiosome size: 0.6-0.8mm. *V. yedonensis*: ant-ballistic diplochorous dispersal, seed size: 1.5-2.0mm (small), elaiosome size: 0.4-0.5mm. *Corydalis pallida*: ant-ballistic diplochorous dispersal, seed size 1.5-2.2mm (small).

Ant preferences for seed size

We observed the behavior of ants that collected myrmecochorous seeds at 13 depots situated randomly within the study area of 10m x 400m on the forest floor. Each depot included fifteen seeds: six depots contained "large" seeds (*V. hondoensis*) and seven depots "small" seeds (mixtures of *C. pallida*, *V. tokubuchiana*, and *V. yedoensis* seeds). The "small" seeds were grouped together irrespective of the species because they showed no recognizable differences in ant preferences. We observed each depot for 15 minutes and recorded behavior of each ant walking near the depot (nearer than the ant body length). Within the 15 minutes, no depletion of the seeds or no aggressive behavior of the ants for the seeds was observed. Ant behavior was classified as follows. (1) Ignore: ignore or do only antennation, (2) Try to transport: try to grasp the seed with mandible but failed, and (3) Succeed to transport: succeed to transport the seed at least five times longer than the ant body length.

From the frequencies of each behavior, we calculated 'attraction rate', 'success rate', and 'transportation rate'. Attraction rate is the proportion of ants that was attracted to the depot and tried to transport a seed among total observed ants. Success rate is the proportion of ants succeeded to transport a seed among the attracted ants. Transportation rate is the proportion of ants succeeded to transport a seed among total observed ants. Transportation rate is a product of attraction rate x success rate. Simple equations can be written thus:

$$\text{Attraction rate} = (T + S) / (I + T + S) \quad [1]$$
$$\text{Success rate} = S / (T + S) \quad [2]$$
$$\text{Transportation rate} = S / (I + T + S) \quad [3]$$

where I, T, and S are the frequencies of observation for Ignore, Try to transport, and Succeed to transport, respectively.

Dispersal distance

From June to August 2005, we collected ripen seeds of *V. hondoensis*, *V. tokubuchiana*, *V. yedoensis, V. mirabilis* and *C. pallida* from the plants, measured major axis of the seeds (including elaiosomes), put them one by one (up to 15 seeds per observation) at the base of the plants, and waited for ants to transport them. Thirteen observations were carried out at randomly selected sites in the forest floor (10m x 400m). The fallen seeds around the plants were cleared away beforehand. When an ant began to carry a seed, it was tracked until it brought the seed into the ant nest. All the transporting ants under observation were not lost in the way. Then the dispersal direct distance was measured. Totally, 72 tracking observations were made. Again, the "small" seeds were grouped together irrespective of the species because they showed no recognizable differences in dispersal distance.

RESULTS

Ant preferences for seed size

To small seeds (*Viola* spp. and *C. pallida*), large ant species, *A. famelica,* was consistently less attracted than small ant species, *P. fervida*, i.e., large ants tried to carry the small seeds less often (Table 1). Large ants were also less successful in transporting the small seeds than small ants, i.e., even when large ants tried to carry, they failed to transport the small seeds (Table 1). On the contrary, to large seeds (*V. hondoensis*), the large ants and the small ants were similarly attracted, and they were similarly successful in transportation (Table 1).

Dispersal distance

Large *A. famelica* ants transported seeds for a long distance, while small *P. fervida* ants did for a short distance (Fig. 1). The dispersal distance of large ant species (128.6±13.3 cm, mean ± SE) was longer than that of small ants (21.8±2.5 cm, t- test, $P<0.01$). Large ants exclusively transported large seeds while small ants transported both large and small seeds (Fig. 1).

DISCUSSION

The results indicate that the interaction between myrmecochorous herbs and ants in Japanese deciduous forest is asymmetric, i.e., large ants are associated only with large seeds whilst large seeds of obligate myrmecochorous plants

Table 1. Response of ants to myrmecochorous seeds. Large ant: *Aphaenogaster famelica* (body length: 3.5-8mm), Small ant: *Paratrechina fervida* (2-2.5mm), Large seed: *Viola hondoensis* (seed size: 2.6-4.1mm), Small seed: *Corydalis pallida* (c. 1.8mm), *V. tokubuchiana* (2.0-2.2mm), and *V. yedoensis* (1.5-2.0mm). Attraction rate is the proportion of ants attracted to the depot among total observed ants. Success rate is the proportion of ants succeeded to transport a seed among the attracted ants. Transportation rate is the proportion of ants succeeded to transport a seed among total observed ants. I, T and S indicate Ignore, Try to transport, Succeed to transport, respectively. *P*–values are based on chi-square test. * $P<0.05$; ** $P<0.01$; NS not significant.

	Seed size	Large ant	Small ant	*P*
attraction rate	Large	0.75 (9/12)	1.00 (5/5)	NS
(T + S) / (I+ T + S)	Small	0.40 (19/48)	1.00 (23/23)	*
success rate	Large	0.67 (6/9)	0.40 (2/5)	NS
S / (T+ S)	Small	0.26 (5/19)	0.96 (22/23)	*
transportation rate	Large	0.50 (6/12)	0.40 (2/5)	NS
S / (I + T + S)	Small	0.10 (5/48)	0.96 (22/23)	**

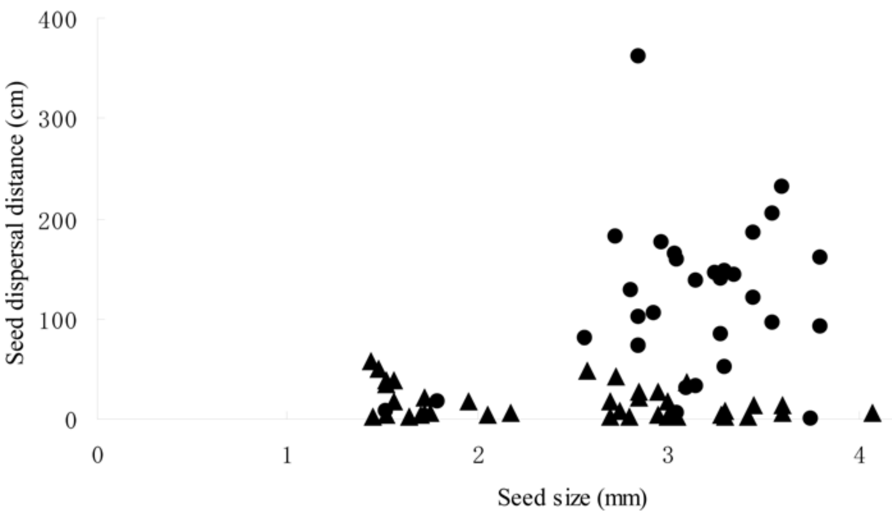

Fig. 1. Scatterplots of the relationship between seed dispersal distance by ants and seed size. Each ccircle indicates an ant individual of large ant species (*A. famelica*), and each triangle indicates that of small ant species (*P. fervida*). Large seeds of *V. hondoensis* (2.5mm-4mm) were transported by large and small ants, while small seeds of *Viola* spp. and *C. pallida* (1.5mm-2.5mm) were transported by only small ants.

are associated with large and small ants. This study confirms that these size-dependent specificities in seed-disperser interaction are maintained through ant preference for seed size.

Ant preferences for seed size

Attraction rate, success rate, and transportation rate of large seeds (*V. hondoensis*) were consistently high regardless of the disperser ant size (Table 1). Contrarily, those rates of small seeds (*C. pallida*, *V. tokubuchiana* and *V. yedoensis*) were low for large ants, suggesting they are not adapted to be carried by large ants (Table 1). Additionally, in the result of the dispersal distance experiment (Fig. 1), the small seeds were, again, rarely transported by large ants.

An ant usually grips the elaiosome when it transports a myrmecochorous seed (Byrne & Levery 1993). When large *A. famelica* ants tried to carry small seeds, they could not grip it because the elaiosome of small seeds appeared too small for large ants to grip. Gorb & Gorb (1995) similarly observed that elaiosome and seed bodies of small seeds are too small for manipulation by large ant (*Formica polyctena*), especially by large individuals. In addition, small seeds are usually hard and spherical, which makes their manipulation even more difficult for large ants. On the other hand, large seeds were carried by both large and small ants. Large ants usually lift a large seed whereas small ants usually drag one.

Hughes & Westoby (1992b) emphasized the elaiosome/seed ratio rather than just the seeds size as the trait that might have been selected to increase removal rate by beneficial ant partners. However, we observed that the large ants often slipped past small seeds without checking their elaiosome/seed ratio even when they walked within c. 1cm of the seeds (Table 1, counted as 'I: ignore'). This suggests that absolute size of seed is primarily important to attract large ants. Of course, once ants notice a seed, they may assess whether it is worth transporting or not. The assessment may depend on the cost and benefit of the transportation, i.e., the elaiosome/seed ratio and absolute elaiosome size (Hughes & Westoby 1992b). As ants need a larger inducement to remove larger seeds, elaiosome size should increase with seed size (Edwards et al. 2006). For the plants studied here, the seed size of each plant species well reflects the elaiosome size (see Materials and Methods) so that the assessment by the ants is supposed to depend basically on seed size.

Dispersal distance

Large ants dispersed seeds farther (Fig. 1), which is consistent with the results of Gómez & Espadaler (1998) and Ness et al. (2004). As larger ant species usually have larger territories, they can transport seeds for a longer distance on average. Such long-distance dispersing partner is basically advantageous for plants because they disperse far enough to reduce distance-related and density-dependent costs for the plants such as parent-offspring conflict and sibling competition.

As the overall fitness gain for plants resulting from myrmecochory may depend on the identity of the seed disperser, plant traits that affect the attraction of beneficial dispersers are likely to be under strong selection pressure (Giladi 2006). In this study, as large seeds attracted large ants and enabled longer dispersal on average (Fig. 1), large seeds are supposed to be selected for, especially in the obligate myrmecochorous *V. hondoensis*, which depends their seed dispersal entirely on ants. The large seeds of *V. hondoensis,* however, were not necessarily dispersed for a long distance (Fig. 1). There are two reasons for this. First, large seeds were attractive not only to large ants but also to small, short-distance disperser ants (Table 1). Second, dispersal distance can not be longer than the distance between the seed and ant nest (Gómez & Espadaler 1998). Even when a large ant disperses a seed, dispersal distance will be short if the seed is near the ant nest. For these two reasons, large seeds are not necessarily dispersed for a long distance. On average, however, the dispersal distance is longer in large seeds than in small ones.

For ant-ballistic diplochorous, small-seed bearing *Viola* spp. and *Corydalis pallida,* there may be a tradeoff for the seed size. Smaller seeds are supposed to disperse farther by ballistic dispersal mechanisms, while they disperse shorter afterwards by ant dispersal. Although we did not measure the distance by ballistic dispersal, Ohkawara & Higashi (1994) reported that diplochorous *V. selkirkii* and *V. verecunda* ejected their seeds for 38cm and 56cm on average, respectively. This means that the small seeds are already dispersed far enough, and they need not be dispersed for a long distance by ants. To get in ant's nests may be the sole purpose for the diplochorous small seeds to bear elaiosomes because deposition of seeds in nests or refuse piles by ants reduces losses to aboveground seed predators (Ohkawara & Higashi 1994; Pizo & Olivia 2001; Vander & Longland 2004).

Factors affecting seed size

Attractiveness and dispersal distance of myrmecochorous seeds were size-dependent: small seeds were not attractive to large ants, and so were not carried for a long distance while large seeds were carried by large and small ants so were dispersed farther (Table1, Fig. 1). As the obligate large seeds of *V. hondoensis* do not disperse ballistically, they must rely on ants for long distance dispersal. As large ants consume much energy to work per se (Fewell et al. 1996), they would prefer large foods to collect. Therefore, not only the elaiosome/seed ratio but also absolute seed (elaiosome) size is important to attract large ants (Mark & Olesen 1996).

On the other hand, ant-ballistic diplochorous plants do not need to have large seeds in order to be dispersed farther by ants. The distance of ballistic dispersal is negatively correlated with the weight of the seed (Lisci & Pacini 1997), and seed size and number are negatively correlated (Stearns 1992). So, ant-ballistic diplochorous plants may have rather been selected for to have many small seeds.

Interactions between myrmecochorous plant species and disperser ant species were size-specific (Table 1). Our results agree with the prediction of Giladi (2006) and Boulay et al. (2007), that myrmecochory is not as diffuse as it is frequently presented. Such size-dependent specificity is explained by the dichotomy of the plant strategy to get dispersed for a long distance: produce large seeds to be transported by large ants, or produce small seeds to flip farther. Then, which strategy should a plant embrace? Predation pressure of vertebrate and invertebrate predators may influence the choice of plant strategy. Ballistic and ant dispersal diplochorous plants must lift their fruits previous to flip the seeds for a long distance. Such lifted fruits are conspicuous so that seed predators like birds often eat them (Takahashi S personal observation, although there is a possibility that the seeds are capable of surviving ingestion by birds and are 'dispersed' by birds). On the other hand, obligate ant dispersed seeds of *V. hondoensis* bear fruits under the leaves which are not found by birds but easily accessible by ground beetle predators and mammals. Comparison of seed mortality between diplochorous plants and obligate ant dispersed plants will shed light on the evolution of dichotomy of plat dispersal strategies.

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