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Yamawo, Akira

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Seed dispersal by ants in the fully mycoheterotrophic plant *Sciaphila secundiflora* (Triuridaceae)

Kenji Suetsugu[1*], Takuto Shitara[2], Akira Yamawo[3]

[1]Department of Biology, Graduate school of Science, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe, 657-8501, Japan
[2]Graduate School of Life and Environmental Sciences, Ibaraki, Japan
[3]Faculty of Agriculture and Life Science, Hirosaki University, Aomori, Japan

[*]Correspondence author. *Email address:* kenji.suetsugu@gmail.com (K. Suetsugu)

Short title: Myrmecochory in a full mycoheterotroph

ABSTRACT

The tiny seeds, or dust seeds, of heterotrophic plants are usually dispersed by wind. However, most mycoheterotrophic plants grow in the understory of densely vegetated forests, where the wind is less reliable and wind dispersal is likely less efficient. In the present study, we found that seeds of the fully mycoheterotrophic plant *Sciaphila secundiflora* (Triuridaceae) possess elaiosomes, and that at least one species of ant, *Nylanderia flavipes*, functions as a dispersal agent of the plant's seeds. This is the first demonstration of myrmecochory in mycoheterotrophic plants, as well as the first report of zoochory in Triuridaceae.

Keywords: elaiosome; myrmecochory; mycoheterotroph; seed disperser

Introduction

Non-photosynthetic, or heterotrophic, plants have long attracted the curiosity of biologists, both because such plants contradict the notion that autotrophy is synonymous with the Plant Kingdom and because these plants are relatively rare (Leake, 1994). However, until recently, the ecological and evolutionary interactions between heterotrophic plants and the other organisms have been poorly understood (Bidartondo, 2005; Suetsugu, 2014).

One of the most significant common characteristics of heterotrophic plants is the extreme reduction of the size and complexity of their seeds (Leake, 1994), and the resulting tiny and numerous seeds, or dust seeds, are usually dispersed by wind (Leake, 1994; Eriksson and Kainulainen, 2011). However, some heterotrophic species have evolved adaptive strategies to utilize animals for zoochorous seed dispersal (Bresinsky, 1963; Suetsugu, 2015). For example, some heterotrophic plants produce indehiscent fleshy fruits specifically adapted for endozoochorous dispersal (Amico and Aizen, 2000; Klooster et al., 2009; Suetsugu et al., 2015).

The family Triuridaceae comprises ca. 50 species of mycoheterotrophic plants distributed throughout the tropical areas as well as the subtropical and temperate regions of Argentina, Brazil, Paraguay, and Japan (Maas-van de Kamer and Weustenfeld, 1998; van de Meerendonk, 1984). Molecular phylogenetic studies have shown that Triuridaceae constitutes a monophyletic clade within Pandanales (Davis et al., 2004; Mennes et al., 2013). Triuridaceae is of particular interest for plant anatomists and evolutionary biologists, owing to their unusual subterranean organs (Imhof, 2003; Imhof, 2004) and unique floral structures (Rudall, 2003; Rudall, 2008; Nuraliev et al., 2016).

In contrast to other aspects of their biology (reviewed by Maas and Rübsamen, 1986), seed dispersal in Triuridaceae has not been studied in detail, but it has been assumed that, like in other mycoheterotrophic plants, the wind disperses the seeds of these species (Rübsamen-Weustenfeld, 1991). However, the structure of diaspores in these species differs from that of typical anemochorous seeds, as they are larger and possess a reticulate epidermal pattern on their fruitlet wall. In addition, the seeds of Triuridaceae themselves are smooth and ellipsoid to ovoid in shape, rather than fusiform with wing-like structures (Rübsamen-Weustenfeld, 1991). In some species, the water-resistant diaspores can float, which suggests that they utilize hydrochory. Yet, the diaspores of some other species are coated in sticky slime that is produced by the receptacles and likely facilitates epizoochory (Fleischer, 1929; Rübsamen-Weustenfeld, 1991).

In addition, Yamawo and Hada (2008) reported that seeds of *Sciaphila nana* Blume possess appendages that are morphologically similar to elaiosomes, structures generally associated with dispersal by ants. The elaiosome, which usually contains nutritional substances such as oleic acids and essential amino acids, provides a food source for the ants (Beattie, 1985). Seed-dispersing ants usually take the seeds with elaiosomes to their nests, and after consuming the elaiosomes, discard the intact seeds with other nest wastes, either inside or outside of their nests (Beattie, 1985).

We noticed that the seeds of *Sciaphila secundiflora* Thwaites ex Benth. also possess appendages that are morphologically similar to elaiosomes. The objective of the current study was therefore to test whether seed dispersal in *S. secundiflora* is mediated by ants. In addition, even if the seeds of *S. secundiflora* can be carried in an ants' nest, it is important to determine whether there are any negative fitness effects, such as seed consumption. Therefore, we also investigated the fate of the seeds carried by the ants under laboratory conditions.

Materials and Methods

Study organism

Sciaphila secundiflora is a small mycoheterotrophic perennial herb that possesses a simple, although sometimes branched, stem up to 15 cm long. The species produces unisexual flowers, and six perianth segments of the male flower are equal in size. The fruitlet is a one-seeded follicle, longitudinally ventro-dorsally dehiscing from apex to base, as in other *Sciaphila* species (Maas and Rübsamen 1986). Because the fruitlet wall tightly surrounds the seed, the fruitlets have approximately the same size and shape as the seeds. The seeds are often not dispersed immediately after the dehiscence of the fruitlet, but stay attached to the open fruitlet by their bases up to the late fruiting stage.

Field observations

The field study was conducted in two populations separated by ca. 1 kilometer of Kohzu Village on Kohzu Island, Tokyo Prefecture. Two populations are located in the understory of humid, dense forests dominated by *Cryptomeria japonica* D.Don, *Castanopsis sieboldii* (Makino) Hatus. and *Machilus thunbergii* Siebold & Zucc. Each study site, approximately 15 m by 10 m in area, contained only ca. 10 fruiting individuals. For each population, 10 fully ripened seeds with elaiosome-like appendages (hereafter diaspores) were collected in total from fruiting plants. The diaspores were then placed at the base of a single fruiting plant, and the manipulations of ants with the diaspores were observed by sitting close to them at population 1 between 10:30~11:30 a.m. on 18 August 2015 and population 2 at 11:00~12:00 a.m. on 19 August 2015.

Feeding experiments

Laboratory experiments were performed to confirm that the *S. secundiflora* diaspores removed by the ants were actually dispersed, rather than consumed. We used *Pheidole fervida* Smith and *Nylanderia flavipes* Smith workers, which were observed removing *S. secundiflora* diaspores in the field (see results). One colony of each species was collected from Mt. Kaibuki (40°57'N, 140°66'E, elev. 300–350 m), Hirosaki City, Aomori Prefecture, in June 2015.

After collection, each colony was adjusted to include 500 workers, one queen, and 300 brood, and then confined to a glass test tube (1.5 cm × 15 cm), which served as an artificial ant nest. An entrance was constructed using a vinyl chloride tube (0.6 cm × 10 cm) and was connected to the opening of each tube using a cotton mass. Each artificial nest was placed in a plastic container (35 cm × 15 cm × 5 cm), the inner surfaces of which had been covered with talc (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to prevent the ants from escaping. Ants were kept at 25°C under a photoperiod of 12 hours. The ants were fed with two mealworms every two days and sucrose water ad libitum. Diaspore offering experiments were conducted within 24 hours from ant feeding with mealworms.

The diaspores of *S. secundiflora* were collected from Kohzu Island, Tokyo Prefecture on 30 August 2015 and were kept in a refrigerator at 4°C. The diaspores were pressed in wet cotton for 12 hours before the experiments commenced. During September 2015, we placed seven diaspores at 8 cm from the nest entrance of each colony in the laboratory, and the number and status of the diaspores were recorded after 24 hours and 7 days. When diaspores were not found in the container after 7 days, the inside of the artificial ant nest was checked carefully under a microscope to determine whether the diaspores had been consumed.

Results

Field observations

The *S. secundiflora* seeds are ellipsoidal, ca. 1.5 mm in length, with a reticulate surface and a large elaiosome-like appendage (Figure 1). During the 1 hour observation period, the diaspores of population 1 were visited by workers of *Pristomyrmex punctatus* Smith (n = 5) and *Nylanderia flavipes* (n = 11), both of which investigated the diaspores using their antennae. Although the workers of *P. punctatus* left the diaspores after their preliminary inspection, four workers of *N. flavipes* collected the diaspores, grasping them by their mandibles, and carried them away (Figure 2). Meanwhile, the diaspores of population 2 were visited by workers of *Pheidole fervida* (n = 17), five of which collected diaspores, using their mandibles, and carried them away to their nest (Figure 2).

Feeding experiments

In the laboratory colonies, all diaspores were collected and transported into the nest within 24 hours, regardless of ant species. After 7 days in the colony of *N. flavipes*, three intact seeds were carried outside of the nest, and four intact seeds were deposited with other garbage within the nest. The elaiosome-like appendage has been removed in all seven diaspores. Meanwhile, after 7 days, in the *P. fervida* colony, one intact seed without elaiosome was moved outside of the nest, and two destroyed seeds lacking elaiosome were deposited with other waste in the nest. The four other diaspores were not found in either the container or nest.

Discussion

While myrmecochory is usually regarded as a generalized seed dispersal strategy, there is growing evidence that specialization occurs more often than expected (Giladi, 2006; Zelikova et al., 2008; Manzaneda and Rey, 2009; Youngsteadt et al., 2009). The present observation that the elaiosomes of *S. secundiflora* fail to elicit collection by *P. punctatus* may also suggest that seed dispersal in *S. secundiflora* is at least somewhat specialized, rather than generalized. This point remains to be investigated in future studies.

Furthermore, the feeding experiments suggested that *P. fervida* consumes seeds of *S. secundiflora*, which indicates that this ant species can function as a seed predator. At the same time, our results do not preclude the possibility that *P. fervida* contributes to seed dispersal in the same manner that granivorous ants do. More specifically, previous studies have shown that even though the majority of seeds collected by granivorous ants are consumed, a small proportion escapes predation, either because they are dropped on the way to the nest or because they are mistakenly taken to refuse piles (Pizo and Oliveira, 1998; Retana et al., 2004). Indeed, our results suggested that *P. fervida* consumes most of *S. secundiflora*'s seeds, while one intact seed without elaiosome was removed outside of the nest in the present study. Actually, the dual role of the genus *Pheidole* as seed predator and seed disperser has also been demonstrated for other angiosperms (Levey and Byrne, 1993).

In contrast to other ants studied, *N. flavipes* attracted by *S. secundiflora* seeds, selectively consumed elaiosome-like appendages. Therefore, elaiosome-like appendages should facilitates seed dispersal by ants and can therefore be considered a true elaiosome. Because *N. flavipes* consumed the elaiosomes of *S. secundiflora* and then constantly discarded the intact seeds with other nest waste either inside or outside of their nests, the *N. flavipes*-*S. secundiflora* interaction represents a genuine example of myrmecochory. Therefore, our findings show that at least one species of ant, *N. flavipes*, functions as a seed dispersal agent for *S. secundiflora*.

Zoochory is unusual for heterotrophic plants, which usually produce wind-dispersed dust seeds (Leake, 1994; Eriksson and Kainulainen, 2011). However, wind dispersal is likely a less-efficient dispersal strategy in the understory of densely vegetated forests, where the wind is less reliable (Givnish et al., 2005). Given that mycoheterotrophy is often considered an adaptation that facilitates the invasion of dark forest understory habitats, where there are few autotrophic competitors, it is perhaps not surprising that the transition to such habits might simultaneously favour the development of zoochorous seed dispersal (Suetsugu et al., 2015).

Although this is the first documented example of myrmecochory in a fully mycoheterotrophic plant, and the first example of zoochory in the Triuridaceae, it is possible that myrmecochory is a more common seed dispersal strategy than has been recognized previously, at least within the genus *Sciaphila.* Indeed, the observation that some other *Sciaphila* species also produce seeds with elaiosome-like appendages would support this hypothesis (Yamawo and Hada, 2008; Suetsugu, unpublished data). In contrast, the closely related *Seychellaria* apparently lacks elaiosomes (Nuraliev et al., 2016). It is worth investigating whether seeds with elaiosome-like appendages are common in the family Triuridaceae and if so, whether elaiosome-like appendages actually work as the attractants for ant species.

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Figure legends

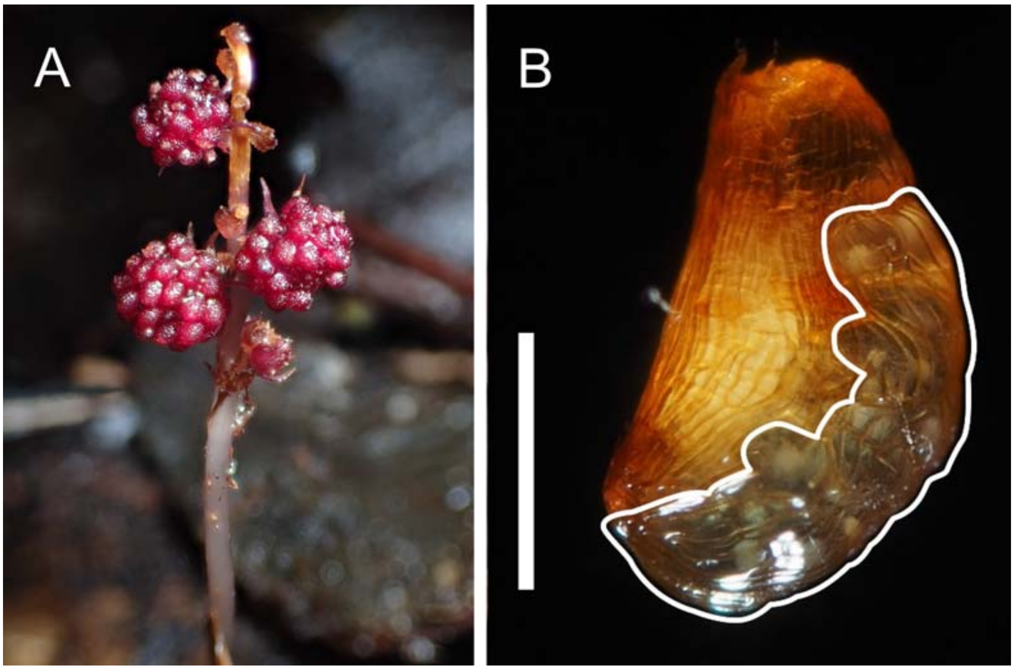

Fig. 1. (A) Fruiting plant of *Sciaphila secundiflora*. (B) Seed of *S. secundiflora* with the

elaiosome. The elaiosome is enclosed by a white line. Bar = 1 mm

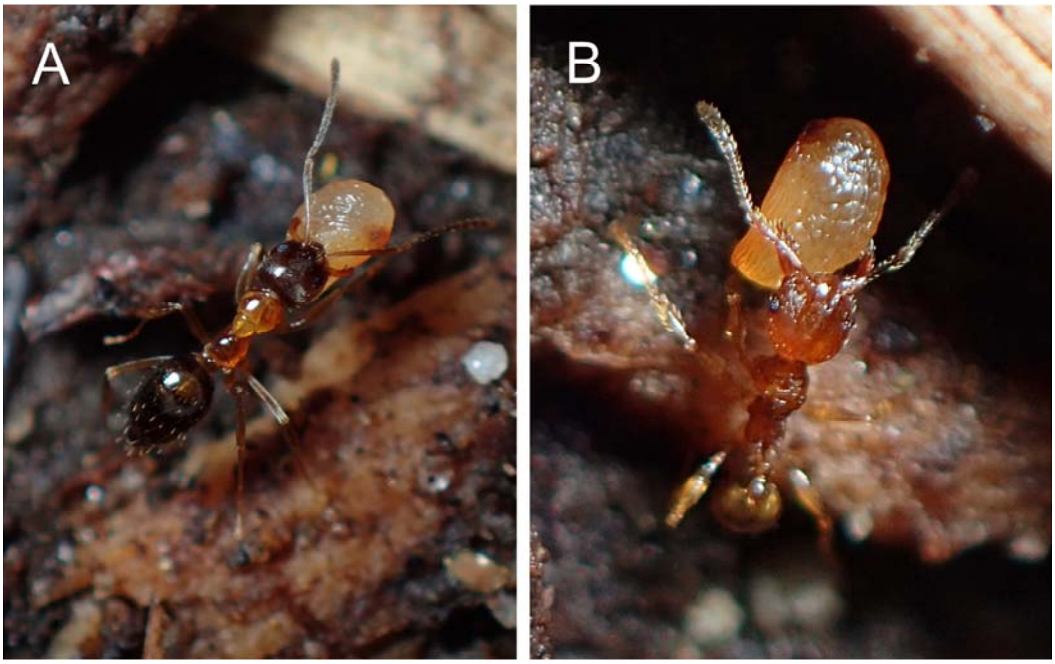

Fig. 2. (A) Worker of *Nylanderia flavipes* carrying a *S. secundiflora* diaspore. (B) Worker of *Pheidole fervida* carrying a *S. secundiflora* diaspore.