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Short Communication

Repellence of nectar-thieving ants by a physical barrier: Adaptive role of petal hairs on *Menyanthes trifoliata* (Menyanthaceae)

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A B S T R A C T

Some plant species possess petal hairs, the defensive functions of which remain untested. I hypothesized that the petal hairs of an emergent plant species, *Menyanthes trifoliata* (Menyanthaceae), have a function in deterring nectar-thieving ants (*Lasius japonicus*). *Lasius japonicus* individuals were observed to stop moving forward after touching hairs and had difficulty in maintaining their balance when walking on hairs. In order to confirm the effectiveness of the hairs quantitatively, I trimmed hairs with a nose hair trimmer and then observed the behavior of flower-visiting *L. japonicus*. The success rate of *L. japonicus* in entering the floral tubes was significantly lower in the presence of intact hairs than when there were trimmed hairs. Moreover, it took *L. japonicus* twice as long to enter floral tubes with intact hairs, even when they succeeded. These results suggest that hairs on *M. trifoliata* petals are effective as a physical barrier against *L. japonicus* by restricting the movements of the ants.

Introduction

One of the most remarkable examples of biodiversity is the diversity shown by angiosperm flowers; this is confirmed by the high degree of interspecific variation in floral characters, represented by color, size, morphology, and smell. These diversified floral characters have evolved in response to selection pressure both from mutualists (e.g., pollinators) and antagonists (e.g., florivores and nectar-thieves) (Willmer, 2011). Hairs on flowers form a conspicuous feature in some plant species of different lineages (e.g., Menyanthaceae, Asclepiadaceae, and Rubiaceae). Previous studies have shown that hairs on flowers of some plant species are adapted to interact with pollinators in various ways, including by visually attracting pollinators (Faden, 1992), secreting nectar for short-tongued pollinators (Johnson et al., 2007), or providing pollinators with footholds (Inoue and Amano, 1986). In contrast, ecological functions of floral hairs to deter antagonists have not been demonstrated. As hairs on photosynthetic organs (i.e., leaves and stems) function in defense against predation by herbivores (Hanley et al., 2007), floral hairs are expected to function in protecting the plant's reproductive organs from antagonists such as florivores and nectar-thieves. The emergent plant species *Menyanthes trifoliata* (Menyanthaceae) has high-density hairs on its petals (Fig.1a). When observing this species in the wild, I noted that there were many walking ants (*Lasius japonicus*) present on the stems and buds (Fig. 1b) and hypothesized that the hairs function as physical barriers to deter ants from entering the floral tubes.

Although ants positively affect the fitness of many plant species in defense against herbivory (Trager et al., 2010) and in nutrient acquisition (Bazile et al., 2012; Mayer et al., 2014), flowering plants do not usually welcome ants, because ants are ineffective pollinators in at least three respects. First, the small size of ants means that they often exploit nectar without touching the anthers or stigmas (Rostás and Tautz, 2010). Second, antimicrobial chemicals secreted from the metapleural glands of the ants decrease pollen viability (Beattie et al., 1984; Dutton and Frederickson, 2012). Third, wingless ants repeatedly visit the same location and do not therefore facilitate outcrossing (Fowler, 1983). Additionally, the presence of ants on flowers frightens and disturbs more effective pollinators, thus reducing the frequency and duration of their visits (Tsuji et al., 2004; Lach, 2007). Therefore, it would seem to be natural for flowers to have evolved mechanisms to deter ants. Previous studies show that extrafloral nectaries (EFNs), which occur in > 110 angiosperm families (Weber et al., 2015), distract or attract ants away from flowers (Wagner and Kay, 2002; Del-Claro et al., 2016), and the floral nectar of some flowers contains ant-repellent chemicals (Willmer et al., 2009). In addition, physical barriers such as base-thickened corollas, slippery waxy surfaces, trichomes, and hairy structures on corollas or calyces are considered to be effective in deterring ants (Willmer, 2011). However, the functional effectiveness of these physical barriers in deterring walking ants has not so far been tested.

In order to examine whether hairs on *M. trifoliata* petals are effective in repelling ants, I conducted a simple experiment that involved shortening the length of hairs with a nose hair trimmer (Fig. 1a) and

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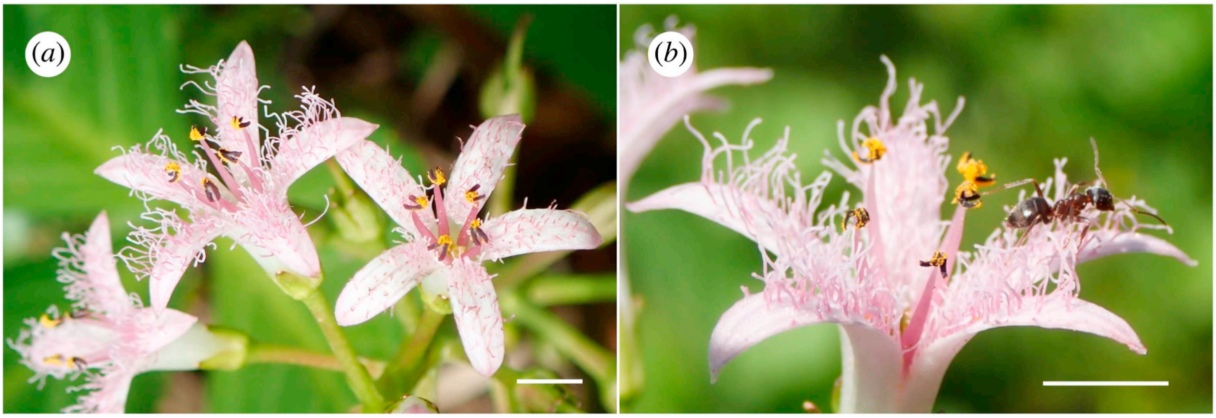

Fig. 1. (a) Flowers of *Menyanthes trifoliata* with hairs on petals. Left is the control flower and right is the treatment flower whose hairs were trimmed. Bar = 5 mm. (b) A nectar-thieving ant species *Lasius japonicus* walking on petals of *M. trifoliata*. Bar = 5 mm.

observing the behavior of the ants. Specifically, I compared the rate at which ants successfully entered floral tubes, and the length of time they took to access and then remain in floral tubes, between hair-trimmed and control flowers.

Materials and methods

Materials and study site

Menyanthes trifoliata, an emergent plant species found in shallow bogs and river margins, produces white-petalled flowers from April to May in western Japan. An individual plant usually has 10–20 flowers in a flowering season, and 2–3 flowers bloom at a time. There were 55.9 ± 9.23 fringed hairs (mean ± s.d., $N = 10$) on the inner side of the petal. The length of hairs on the petals outside of the floral tubes (2.03 ± 0.592 mm, $N = 44$) was significantly longer than those in the floral tubes (1.38 ± 0.55 mm, $N = 28$) (Wilcoxon signed rank test, $P < 0.001$). The hairs in the floral tubes tended to be longer closer to the entrance of the floral tubes (Linear regression, estimate ± s.e. = 0.267 ± 0.0627, $P < 0.001$, $N = 28$), which implied that the function of these hairs is to protect the entrance of floral tubes.

This species has a distylous reproductive system, and successfully uses insect pollination to produce seeds from the different morphs. Over a total field observation period of 14 h, undertaken on sunny days, I noted some Dipteran, Hymenopteran and Lepidopteran species as flower visitors. Large-sized Dipteran species (*Bombylius major* and *Eristalis tenax*) and Hymenopteran species (Halictidae sp.) crawled into the floral tubes and fed on nectar. While feeding, pollen attached to their bodies. Small-sized Dipteran species (*Sphaerophoria interrupta* and *Sphegina* sp.) fed on pollen while standing on petal hairs. Pollen sometimes got attached to their bodies during feeding. A Lepidopteran species (*Parnassius glacialis*) used a long proboscis to sip nectar, and pollen did not attach to its body. Therefore, it is suggested that large-sized Diptera and Hymenoptera are major pollinator species of *M. trifoliata*, while small-sized Diptera are opportunistic pollinators. I also observed *Lasius japonicus* (Formicidae) to be a nectar-thieving ant species. *Lasius japonicus* is ca. 3–4 mm in length and collects nectar from flowers. These ants usually did not touch stamens because of their small bodies; however, a few individuals touched stamens and pollen attached to their bodies when crawling into floral tubes. Even in these cases, frequent grooming by *L. japonicus* prevented pollen from being carried out from the *M. trifoliata* individual. These observations showed that *L. japonicus* was not a pollinator but a nectar-thief. *Lasius japonicus* attacked pollinators on petals, especially small-sized Dipteran species (*Sphaerophoria interrupta* and *Sphegina* sp.). *Lasius japonicus* ants were not found on *M. trifoliata* individuals that were flooded, which suggests that *L. japonicus* cannot move through water to reach *M. trifoliata*.

Experiments were carried out on three sunny days from April to May 2018 in a bog located at Misasa Town, Tottori Pref., Japan. The bog is ca. 2000 m^2 and is surrounded by paddy fields. I designed the experiment very carefully so as not to damage the vegetation, and permission to conduct the study was obtained from the Misasa Town board of education and local residents association.

Experimental design

I trimmed hairs on *M. trifoliata* petals with a nose hair trimmer (G-5; Kawasaki seiki, Japan); experimental groups included hair-trimmed ($N = 30$) and control flowers (N = 30) (Fig. 1a). I walked around the survey plot to observe and note the behaviors of *L. japonicus* trying to enter the floral tubes. In total, I observed 67 and 50 *L. japonicus* individuals trying to enter the floral tubes of control and hair-trimmed flowers, respectively. Then I noted whether or not *L. japonicus* succeeded in entering the floral tube, and also calculated the success rate of *L. japonicus* entering floral tubes for each treatment. For 36 and 40 *L. japonicus* visiting control and hair-trimmed flowers, respectively, I noted the time required for *L. japonicus* to enter floral tubes (defined as the time from when an ant touched the calyces below the corolla to being entirely within the floral tube). In order to quantify the effects of hairs on different plant parts, I used a digital camera (Stylus TG-5; Olympus, Japan) in movie-mode to note the time that 36 *L. japonicus* individuals visiting control flowers spent on the outer side of petals, inner side of petals without hairs, inner side of petals with hairs, and floral tubes. Furthermore, I noted the length of time the ants remained in the floral tubes (defined as the time from entry into the floral tube to reaching the calyces after leaving the corolla) for 17 *L. japonicus* individuals visiting control flowers and 17 individuals visiting hair-trimmed flowers.

Statistical analyses

In order to confirm whether the existence of hairs on petals affects the success rate of ants in entering floral tubes, I performed Fisher's exact probability test. Wilcoxon signed rank tests were carried out to determine differences in the times taken to enter and the lengths of time ants remained in floral tubes, between hair-trimmed flowers and control flowers. All statistical analyses were computed using R ver. 3.1 (R Core Team, 2010). Data are expressed as means ± standard deviation.

Results

Behaviors of Lasius japonicus on petals of Menyanthes trifoliata

Lasius japonicus moved from the outer to inner side of petals, and walked on the inner side of petals to crawl into floral tubes. *Lasius*