# Role of extrafloral nectaries of *Vicia faba* for attraction of ants and herbivores exclusion by ants.

Noboru KATAYAMA and Nobuhiko SUZUKI

*Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, Saga, Japan*

**Abstract**

We evaluated directly the role of extrafloral nectary (EFN) for ant attraction and herbivore exclusion by experimental removal of EFN in laboratory. When EFN of *Vicia faba* Linnaeus (Leguminosae) was artificially removed, the number of workers of *Tetramorium caespitum* Linnaeus (Hymenoptera: Formicidae) visiting a plant decreased and the efficiency of herbivore exclusion by ants was also decreased. The herbivore exclusion by ants was mostly ineffective on a plant when less than four workers visited a plant, but when more than four workers visited a plant the residence time of a herbivore on a plant rapidly decreased with increasing numbers of ants on a plant. Thus, the efficiency of herbivore exclusion from plant is determined by the number of ants visiting a plant, and EFN plays an important role for ant attraction.

**Key words:** biological defence, broad bean, facultative mutualisms, *Tetramorium caespitum*.

## INTRODUCTION

Plants have evolved various anti-herbivore strategies (Howe & Westley 1988). These are mainly categorized as physical, chemical, and biological defenses. In the realm of biological defense, some plant species depend on natural enemies of herbivores, such as parasitoids (Turlings & Fritzsche 1999) and ants (e.g. Koptur 1992). Because ants generally remove a great variety of insect species from plants (Risch & Carroll 1982; Beattie 1985), many plant species attract ants by the extrafloral nectary (EFN), the food body and so on for their biological defense.

It is known that plants with EFN occur at least in 93 families (Koptur 1992). EFN is commonly found on leaves, stems, petioles, stipules, and so on, and their structures are greatly diverse (Bentley 1977; Koptur 1992). However, the adaptive significance of EFN has been controversial for decades (Bentley 1977; Beattie 1985; Koptur 1992) because associations between ants and plants with EFN are not specific, and are not shaped by tight coevolutionary interactions between them, especially in temperate regions. The costs and benefits of EFN for plants are affected by several factors such as the intensity of herbivore pressure (Inouye & Taylor 1979; Barton 1986; de la Fuente & Marquis 1999), environmental conditions (Kelly 1986), the composition of the local ant fauna (Horvitz & Schemske 1990), the abundance of ants (Bentley 1976; Inouye & Taylor 1979; Koptur 1985; Barton 1986), and the presence of other carbohydrate resources for ants (Buckley 1983; Horvitz & Shemske 1984). Furthermore, it has also been clarified experimentally that the amount and/or contents of extrafloral nectar are variable according to different degrees of plant damage by herbivory and/or artificial injury (Stephenson 1982; Smith *et al.* 1990; Agrawal & Rutter 1998; Heil *et al.* 2000, 2001; Ness 2003). Changes in the amounts and contents of extrafloral nectar influence the ant's activities on the plants (Bentley 1977; Stephenson 1982; Swifl *et al.* 1994; Agrawal & Rutter 1998; Heil *et al.* 2001; Ness 2003).

In many studies, it has been reported that plants with EFN suffer high herbivory when ants were excluded from the plants experimentally (Stephenson 1982; Barton 1986; Koptur *et al.* 1998; Del-Claro *et al.* 1996; de la Fuente & Marquis 1999). Furthermore, it is likely that the efficiency of herbivore exclusion will depend on the number of ants visiting a plant. For example, it has been reported that plants which were visited by more ants suffer less seed damage and showed more fruit production (Inouye & Taylor 1979; Oliveira *et al.* 1999). Therefore, in order to understand the significance of EFN, we must measure EFN's attractiveness to ants and the effectiveness of herbivore exclusion in relation to the number of ants visiting a plant.

In many studies seeking to clarify the benefits of EFN, ants were removed by adhesives such as a Tanglefoot. However, the efficiency of herbivore exclusion cannot be accurately evaluated by adhesives, because adhesives are likely to exclude other insects including herbivores. Thus in order to more accurately evaluate the benefits derived from EFN, we should artificially remove EFN from a plant and directly measure the efficiency of herbivore exclusion following by attractiveness of ants by EFN.

In this study, we examined ants' attraction to the EFN of *Vicia faba* Linanaeus (Leguminosae) and the efficiency of herbivore exclusion by an ant, *Tetramorium caespitum* Linnaeus (Hymenoptera: Formicidae), in laboratory experiments. We then addressed following questions: (1) How does EFN removal influence the number of ants visiting a plant, and (2) how does the efficiency of herbivore exclusion change in relation to the number of ants visiting a plant?

## MATERIALS AND METHODS

### Organisms

*Vicia faba* is an annual legume herb having EFN on each stipule. The plant grows vegetatively in late winter to early spring and begins to bear flowers in early spring, and produces pods and seeds in April to May in western Japan. In spring, it is often observed that workers of *T. caespitum* visit *V. faba* to collect extrafloral nectar.

Workers of *T. caespitum* are small omnivorous ants approximately 2 mm in body length. They forage mainly on insect carcasses, but also prefer sugars such as the extrafloral nectar of plants and the honeydew of homopteran insects. When a *T. caespitum* worker finds a preferable food resource, she recruits many colony members to that resource.

In July 2000, colonies of *T. caespitum* were collected at Kobe, western Japan (34°41'N, 135°11'E), and were colonized in ten glass test tubes (12 mm in diameter and 120 mm length), each of which contained 300 workers. The bottom of each tube was packed with wet cotton wool about 30 mm deep in order to maintain a suitable humidity level. The tube was covered with aluminum foil to maintain darkness as an ant nest. To form an entrance, each tube was connected to a vinyl chloride tube 6 mm in inner diameter and 100 mm long. The colonies were fed 10% sucrose solution, delivered via a test tube (12 mm in diameter, 120 mm long) plugged with cotton wool.

First instar larvae of the silkworm, *Bombyx mori* Linnaeus (Lepidoptera: Bombycidae), were used as a model organism for herbivores. *B. mori* was regarded as equivalent of *V. faba*'s herbivores such as lepidopteran larvae. Eggs of the silkworm stocked at 5°C were soaked in HCl at 48°C for seven min and after washing, put on wet cotton in petri dishes at 25°C under a photoperiod of LD 24:0. Hatchlings were supplied to the experiments.

**Experiments**

The experiments were carried out at 25°C under a photoperiod of 24L0D in a laboratory. Twenty seedlings of *V. faba* each about 200 mm in height, containing eight to ten EFN, were transplanted individually into water-filled plastic pots, 100 mm in diameter and 45 mm high. Each plastic pot was covered with a petri dish lid with a 15 mm hole in the center to allow penetration by the plant's stem. We removed all EFN from ten plants by cutting each stipule. The plant was kept under a fluorescent lamp (100W) (Toshiba, EFD23EN, Tokyo, Japan) hanging about 200 mm above the plant.

Once in a nest, the ants were starved for four days prior to the experiments in order to increase the sensitivity of their reactions to extrafloral nectar. The entrance of the ant nest was then set on 20 pots of both ten plants with EFN and ten plants without

EFN, giving the ants a chance to visit the plant freely. At that time, one first instar larva of silkworm was released on the plants. We then recorded the residence time of the silkworm larva on the plant until it was excluded from the plant. If the larva remained on the plant for more than 100 min, we finished the observation. Simultaneously the number of ants on the plant was counted at 10 min intervals for 100 min and the average numbers of ants were used for analysis.

## RESULTS

Significantly smaller number of ants visited the plants without EFN than those with EFN (the average number of visiting ants; plants with EFN: $n = 10$, mean ± SE = 10.6 ± 1.4, plants without EFN: $n = 10$, 4.8 ± 1.2; Mann Whitney *U*-test, $z = -2.54$, $P = 0.011$; Fig. 1).

On a plant with EFN, 42% of ants visiting a plant utilized EFN of the plant (the average number of ants utilizing EFN: $n = 10$, mean ± SE = 4.4 ± 2.2). The presence of EFN affected ants' exclusion of silkworm from a plant. Silkworm larvae were removed by ants on ten plants with EFN (10 / 10, 100%) within 100 min, whereas they were removed on four of ten plants without EFN (40%) (Fisher's exact probability test, $P = 0.0108$).

In the trials that silkworm larvae were removed by ants on plants, the residence time of a silkworm larva on a plant did not significantly differ between plants with EFN and without EFN (the average residence time of a silkworm larva; plants with EFN: $n = 10$, mean ± SE = 25.4 ± 6.6 min; plants without EFN: $n = 4$, 33.3 ± 16.6 min; Mann

Whitney *U*-test, $z = -0.28$, $P = 0.78$). Among eight trials in which the average number of workers visited a plant was less than four, a silkworm larva was not excluded from a plant in six trials and resided on a plant for more than 60 min in two trials. On the other hand, in all of the trials (12 trials) in which the average number of workers visiting a plant was more than four, a silkworm larva was excluded within 100 min from the plant either with or without EFN. When the larvae were removed by ants on plants within 100 min, a negative correlation was found between the number of ants on a plant and the residence time of a silkworm larva on a plant (the power regression: n = 14, $Y = 136.64\,X^{-0.932}$, $P = 0.031$, multiple correlation coefficient: $R = 0.794$, Fig. 2). The residence time of a silkworm larva on a plant rapidly decreased with increase of ant number on a plant.

## DISCUSSION

### Ant attractiveness and efficiency of herbivore exclusion by EFN

In this study, we evaluated directly the role of EFN for ant attraction and herbivore exclusion by experimental removal of EFN. The number of ants visiting a plant decreased and the efficiency of silkworm larva exclusion by ants was also decreased when EFN was artificially removed from *V. faba*. Therefore, we considered that the efficiency of herbivore exclusion from a plant is determined by the number of ants visiting a plant. Thus, EFN attracted ants and they excluded herbivores from the plant as reported in Koptur (1979) and Apple and Feener (2001).

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Katayama and Suzuki (2003a) also reported that about half of ants visiting a plant utilized EFN in a similar experiment to that in this study. Thus, we expect that EFN plays an important role for ant attraction.

It has been reported that plants injured by herbivores attracts predators and/or parasitoids of the herbivores by emitting herbivore-induced plant volatiles (Takabayashi & Dicke 1996; Dicke *et al.* 2003). However, the attraction of ants by herbivore-induced plant volatiles has not been reported. Therefore, even if the removal of stipules triggered to emit herbivore-induced plant volatiles, it may be considered that the ant attraction by EFN may be hardly influenced by the removal of stipules.

The herbivore exclusion by ants was mostly ineffective on a plant with less than four workers. Other studies have also reported that herbivores were excluded from a plant when many ants visited a plant (Oliveira *et al.* 1999; Apple & Feener 2001; Giusto *et al.* 2001; Heil *et al.* 2001), because the efficiency of exclusion by ants is likely to depend on the encounter rate with ants (Katayama & Suzuki 2003b).

Furthermore, the aggressiveness of ants may be one of important factors influencing on the efficiency of herbivore exclusion. It has been reported that workers with many nest mates become more aggressive to intruders than workers with a few nest mates (Sakata & Katayama 2001). We observed that workers of *T. caespitum* became more aggressive to herbivores with increased number of ants on a plant (N. Katayama & N. Suzuki, unpublished data, 2003). Therefore, not only high encounter rate between ants and herbivores but also high ant aggressiveness to herbivores by increasing the number of ants visiting a plant may result in high efficiency of herbivore exclusion by ants.

*Vicia faba* is frequently parasitized by several aphid species, such as *Aphis craccivora* Koch, *Acyrthosiphon pisum* Harris and *Megoura crassicauda* Mordvilko (Homoptera: Aphididae) (Katayama & Suzuki 2003a). Among these aphid species, *A. craccivora* is tended by ants (Sakata & Hashimoto 2000; Katayama & Suzuki 2002, 2003a). Therefore, if *V. faba* is parasitized by *A. craccivora*, the ant attraction by EFN decreased with increased number of aphids on a plants (Sakata & Hashimoto 2000; Katayama & Suzuki 2003a). However, high attractiveness by honeydew of aphids facilitates the efficiency of herbivore exclusion by ants (Suzuki *et al.* 2004).

**Facultative mutualism between ants and plants with EFN**

As well as *T. caespitum*, many ant species are ferocious hunters and utilize a great variety of insect species including herbivores on plants as preys (Beattie 1985). Plants have exploited this foraging behavior of ants by intensifying the association to ants. Ant defense for plants has some particular advantages relative to other defensive strategies. Chemical defense (especially qualitative chemical defense) is effective on relatively wide range of herbivore groups (generalists), but often ineffective on some herbivore group (specialists). Ants, on the other hand, attacks regardless of the chemical susceptibilities of herbivores (Beattie 1985). Therefore, ant defense is effective not only on generalist herbivores but also on specialist herbivores (Keeler 1989).

However, ants are not consistently reliable (Howe & Westly 1988) and plants with EFN are not necessarily distributed within the foraging areas of ants. In a field census of insects on *V. faba* plants, we observed that ants visited only 39% of 186 plants investigated (N. Katayama & N. Suzuki, unpublished data, 2000). Therefore, it would be expected that the defensive strategy depending on ants has low level of certainty and consistency in herbivore exclusion, compared with other defensive strategies. This uncertainty may result in ambiguity regarding the efficiency of herbivore exclusion by ants on plants with EFN in the temperate regions, causing controversy concerning the adaptive significance of EFN. Furthermore, it is often difficult to evaluate the ant defense for plants because the relationships between ants and plants with EFN are affected by many factors (e.g. Kelly 1986), and therefore, they are unstable under field conditions. However, we showed that EFN functions certainly as a biological defense strategy and its efficiency is influenced by the number of ants attracted to EFN. In the field, many ant species forage at various places and many plant species with EFN can certainly attract them in universal areas.

In this study, we evaluated the effect of EFN on herbivore exclusion by ants in the simplest experimental unit (i.e. one plant, one ant colony and one herbivore) in laboratory in order to remove the unexpected factors affecting the relationships. In the future, it is necessary to analyze the interactions among ants, herbivores and other organisms on plants with and without EFN under a field condition, and we must evaluate what factors influence the relationships between ants and plants with EFN.

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## REFERENCE

Agrawal AA, Rutter MT (1998) Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos* **83**, 227-236.

Apple JL, Feener Jr DL (2001) Ant visitation of extrafloral nectaries of *Passiflora*: the effects of nectary attributes and ant behavior on patterns in facultative ant-plant mutualisms. *Oecologia* **127**, 409-416.

Barton AM (1986) Spatial variation in the effect of ants on an extrafloral nectary plant. *Ecology* **67**, 495-504.

Beattie AJ (1985) *The Evolutionary Ecology of Ant-Plant Mutualisms.* Cambridge University Press, New York.

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

Bentley BL (1977) Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics* **8**, 407-427.

Buckley R (1983) Interaction between ants and membracid bugs decreases growth and seed set of host plant bearing extrafloral nectarines. *Oecologia* **58,** 132-136.

de la Fuente, MAS, Marquis, RJ (1999) The role of ant-tended extrafloral nectaries in the protection and benefit of a Neotropical rainforest tree. *Oecologia* **118,** 192-202.

Del-Claro, K., Berto, V, Réu, W (1996) Effect of herbivore deterrence by ants on the fruit set of an extrafloral nectary plant, *Qualea multiflora* (Vochysiaceae). *Journal of Tropical Ecology* **12**, 887-892.

Dicke M, de Boer JG, Höfte M, Rocha-Granados MC (2003) Mixed blends of herbivore-induced plant volatiles and foraging success of carnivorous arthropods. *Oikos* **101**, 38-48.

Giusto BD, Anstett M, Dounias E, McKey DB (2001) Variation in the effectiveness of biotic defence: the case of an opportunistic ant-plant protection mutualism. *Oecologia* **129**, 367-375.

Heil M, Fiala B, Baumann B, Linsenmair KE (2000) Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Functional Ecology* **14,** 749-757.

Heil M, Koch T, Hilpert A, Fiala B, Boland W, Linsenmair KE (2001) Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proceedings of the National Academy of Sciences of the United States of America* **98,** 1083-1088.

Horvitz CC, Schemske DW (1984) Effects of ants an ant-tended herbivore on seed production of a neotropical herb. *Ecology* **65,** 1369-1378.

Horvitz CC, Schemske DW (1990) Spatiotemporal variation in insect mutualists of a neotropical herb. *Ecology* **71,** 1085-1097.

Howe HF, Westley LC (1988) *Ecological relationships of plants and animals.* Oxford University Press, New York.

Inouye DW, Taylor Jr OR (1979) A temperate region plant-ant-seed predator system: consequences of extrafloral nectar secretion by *Helianthella quinquenervis. Ecology* **60,** 1-7.

Katayama N, Suzuki, N (2002) Cost and benefit of ant attendance for the aphid *Aphis craccivora* (Hemiptera: Aphididae) with reference to aphid colony size. *The Canadian Entomologist* **134,** 241-249.

Katayama N, Suzuki N (2003a) Changes in the use of extrafloral nectaries of *Vicia faba* (Leguminosae) and honeydew of aphids by ants with increasing aphid density. *Annals of Entomological Society of America* **96,** 579-584.

Katayama N, Suzuki N (2003b) Bodyguard effects for aphids of *Aphis craccivora* Koch (Homoptera: Aphididae) as related to the activity of two ant species, *Tetramorium caespitum* Linnaeus (Hymenoptera: Formicidae) and *Lasius niger* L. (Hymenoptera: Formicidae). *Applied Entomology and Zoology* **38,** 427-433.

Keeler KH (1989) Ant-plant interactions. In: Abrahamson W. G. (ed.) *Plant-Animal Interactions*, pp 207-242. McGraw-Hill, Inc, New York.

Kelly CA (1986) Extrafloral nectaries: ants, herbivores and fecundity in *Cassia fasciculata. Oecologia* **69,** 600-605.

Koptur S (1979) Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. *American Journal of Botany* **66,** 1016-1020.

Koptur S (1985) Alternative defenses against herbivores in *Inga* (Fabaceae: Mimosoideae) over an elevational gradient. *Ecology* **66,** 1639-1650.

Koptur S (1992) Extrafloral nectary-mediate interactions between insects and plants. In: Bernays E (ed). *Insect-plant interaction*, 4, pp 81-129. CRC press, Boca Raton.

Koptur S, Rico-Gray V, Palacios-Rios M (1998) Ant protection of the nectaried fern *Polypodium plebeium* in central Mexico. *American Journal of Botany* **85,** 736-739.

Ness JH (2003) *Catalpa bignonioides* alters extrafloral nectar production after herbivory and attracts ant bodyguards. *Oecologia* **134,** 210-218.

Oliveira PS, Rico-Gray V, Díaz-Castelazo C, Castillo-Guevara C (1999) Interaction between ants, extrafloral nectaries and insect herbivores in Neotropical coastal sand dunes: herbivore deterrence by visiting ants increases fruit set in *Opuntia stricta* (Cactaceae). *Functional Ecology* **13,** 623-631.

Risch SJ, Carroll CR (1982) Effect of a keystone predaceous ant, *Solenopsis geminata.* on arthropods in a tropical agroecosystem. *Ecology* **63,** 1979-1983.

Sakata H, Hashimoto Y (2000) Should aphids attract or repel ants? Effect of rival aphids and extrafloral nectaries on ant-aphid interactions. *Population Ecology* **42,** 171-178.

Sakata H, Katayama N (2001) Ant defence system: a mechanism organizing individual responses into efficient collective behavior. *Ecological Reserch* **16,** 395-403.

Smith LL, Lanza J, Smith GC (1990) Amino acid concentrations in extrafloral nectar of *Impatiens sultani* increase after simulated herbivory. *Ecology* **71,** 107-115.

Stephenson AG (1982) The role of the extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology* **63,** 663-669.

Suzuki N, Ogura K, Katayama N (2004) The efficiency of herbivore exclusion by ants on the vetch *Vicia angustifolia* L. (Leguminosae), mediated by ant attraction to aphids. *Ecological Research* In press.

Swift S, Bryant J, Lanza J (1994) Simulated herbivory on *Passiflora incarnata* causes increased ant attendance. *Bulletin of the Ecological Society America* **75,** 225.

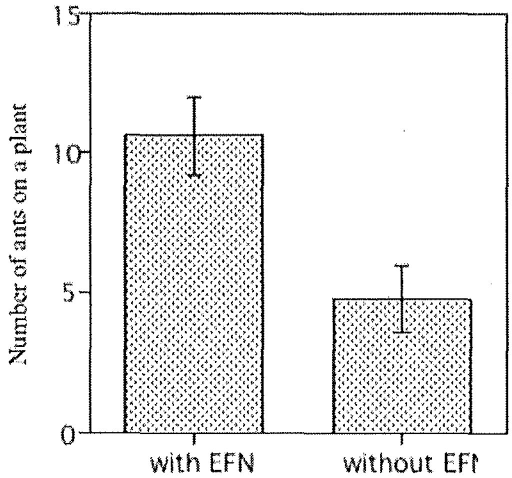
Takabayashi J, Dicke M (1996) Plant-carnivore mutualism through herbivore-induced carnivore attractants. *Trends in Plant Science* **1**: 109-113

Turlings TCJ, Fritzsche ME (1999) Attraction of parasitic wasps by caterpillar-damaged plants. In: Pickett JA (ed.) *Insect-Plant Interactions and Induced Plant Defence*, pp 21-38. Wiley, Chichester.

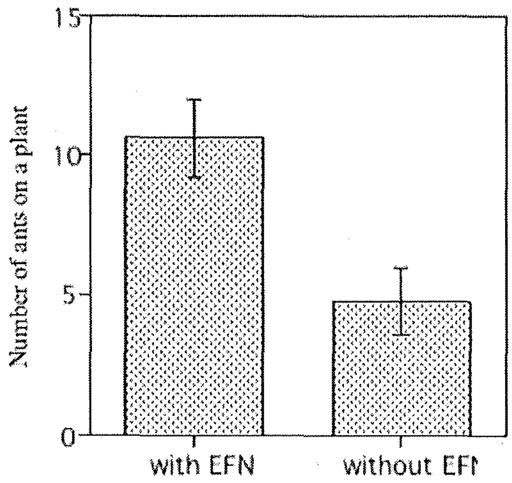
Legends for Figures

**Figure 1** Number of ants visiting a *Vicia faba* plant with EFN and that in which EFN were artificially removed. The bars show SE.

**Figure 2** Relationship between the number of ants visiting a *Vicia faba* plant and the residence time of a silkworm larva on the plant (the power regression: $n = 14$, $Y = 136.64X^{-0.932}$, $P = 0.031$). Solid triangles and open squares indicate the larvae excluded from the plants with and without EFN, respectively. Bars show SD.




(Fig. 1)




(Fig. 1)