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Research Article
Ecological Significance of Mucilage in Catchfly (*Silene armeria*): Impact on the Behavior of Nectar Robbers and Pollinators

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Abstract

Background and Objective: *Silene armeria* is also known as catchfly because it is characterized by a mucilaginous area under the leaves on the upper part of the stem. It has been observed that insects are trapped by this sticky substance. This study investigated whether ants visit flowers when catchfly mucilage is removed. **Materials and Methods:** The plant specimens with and without mucilage were prepared and the response of insects to these two types of plant specimens were compared. **Results:** This study did not observe ants visiting flowers even when the mucilage on the stem was removed. However, it was observed that stems with mucilage tended to have more pollinators than stems without mucilage. Furthermore, it was observed that pollinators such as bees, hoverflies and Syrphinae, prefer stems with mucilage to stems without mucilage. Also observed was that the fruit set rate and seed size of the stems without mucilage was lower when compared to plants with intact mucilage. When comparing plants without mucilage to plants that were covered with net (bagged) the fruit set rate and the seed size are comparable, showing that self-fertilization took place. **Conclusion:** Consequently, it is suggested that plants without mucilage had a low rate of cross-pollination. The results of this study, suggest that catchfly (*Silene armeria*) mucilage affects pollinator attraction.

Key words: Catchfly, *Silene armeria*, mucilage, ant, pollinator, evolutionary advantage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Silene armeria is an annual plant species in the family Dianthus[1]. While this plant is native to Europe, it is now distributed throughout the warmer parts of the world[2,3] and is utilized as an ornamental plant[4,5]. Catchfly was introduced into Japan from Europe for horticultural use in the Edo period. At present, they occur wild on roadsides, vacant lots and riverbanks[4,6-8].

Silene armeria is characterized by a mucilage-secreting area located under the leaves and it is often observed that small bugs are caught in this mucilage. Therefore, *S. armeria* is named catchfly in English. In Japanese, it is called "Mushitori-nadeshiko", meaning "the flowers that catch bugs" or "Haetori-nadeshiko" which means "the flowers that catch flies". However, their mucilage does not digest bugs and *S. armeria* is not a carnivorous plant.

Silene armeria has been studied as a model plant and a horticultural plant for its photoperiodism[9-11] and its reactivity to plant hormones[9,12]. Furthermore, the use of extracts from *S. armeria* has been studied[2,8,13-16]. However, to the best of our knowledge, no study has reported the ecological significance and evolutionary advantage of mucilage in *S. armeria*. *S. armeria* is characterized by the partial secretion of stickiness on part of the stem rather than the entire plant. It is commonly said that this mucilage prevents nectar robbing ants from climbing the stem. However, ants are rarely observed attached to the adhesive area, but flies are observed often. Thence the name "catchfly". Moreover, the Japanese "Haetori-nadeshiko" also refers to flies and not ants. Therefore, it is doubtful whether the adhesive area serves to prevent ants from obtaining nectar.

This study, investigated whether the mucilage of *S. armeria* functions as a protective measure against ants. Furthermore, as a result of examining alternative evolutionary advantages other than the defence against ants, also reported that the mucilage of catchfly may have a role in attracting pollinators rather than defence against ants.

MATERIALS AND METHODS

Study area: The experiments were conducted from March 9 to June 25, 2021, in a field located in The Center for Education and Research in Field Sciences, Shizuoka University, Japan. (34.905216N, 138.272125E)

Plant material: Twenty plastic pots with a diameter of 15 cm containing potting soil "*Hanachan-baiyodo*" and "*Akadama-tsuchi*" (1:1) was prepared. Rosette seedlings of *S. armeria* with a plant height of about 5 cm were planted in the pots on March 9, 2021. These were cultivated in a greenhouse at Shizuoka University's Farm (Kariyado, Fujieda City, Shizuoka pref., Japan) and used for the experiments.

Two types of *S. armeria* were prepared: (1) Plants with mucilage (Mucilage+) and (2) Plants without mucilage (Mucilage-). "Mucilage-" was prepared by covering the area of the stem with mucilage by sandwiching it with white clover leaves and then wrapping it with green coloured tape.

Field observation: The community of *S. armeria* established on the riverbanks of the Abe River (Shizuoka City, Shizuoka pref., Japan) was selected as the survey site. On May 11, 2021, seventy plants were investigated and the number of adhesive areas and insects attached to these adhesive areas were noted.

Recording pollinators by the fixed camera: Five Mucilage+ and 5 Mucilage- pots were placed in a plant community, dominated by *Trifolium repens*, *Lamium amplexicaule*, *Sisyrinchium rosulatum*, *Triodanis perfoliata* and *Taraxacum officinale* on Shizuoka University's farm (Kariyado, Fujieda City, Shizuoka pref., Japan). A time-lapse camera (TLC 200, Brinno, Taiwan) was set and aimed at each plant. Photographs were taken once every 30 sec from sunrise to sunset. The survey was conducted from April 20-27 and May 2-4, 2021.

Flower visits by ants and other pollinators: A flowerbed on-site at Mukoshikiji (Shizuoka city, Shizuoka pref., Japan) was used as the survey site. Two Mucilage+ and 2 Mucilage- pots were buried at the depth of the ground surface and the ground surface was covered with fallen leaves on May 7th, 2021. The number of flower clusters was adjusted to 6 per individual. Sugar was sprinkled between the plants to create a path for ants. It was confirmed that ants (*Camponotus japonicus*, *Monomorium intrudens*) passed between the plants. The survey dates were May 8, 9, 12, 15 and 16 and the types and number of organisms that visited the flowers were observed ten times per day at, 7:30, 8:30, 9:30, 10:30, 11:30, 12:30, 13:30, 14:30, 15:30 and 16:30 for 10 min (100 min in total).

Preferences of medium-sized pollinators

Bees and hoverflies: Two Mucilage+ pots and 2 Mucilage- pots were set and covered by a net. One honeybee (*Apis mellifera*) and one hoverfly (*Eristalomyia tenax*) were placed in the net and observed for pollinator behaviour on flowers of *S. armeria*. The test was conducted from 13:30-14:30 on April 30, 2021 and from 08:00-11:30 on May 7, 2021.

Preference of small pollinators, Syrphinae: A captured hoverfly (Syrphinae) was set at the entrance of the T-shaped tube and test samples were placed at both ends of the T-shaped tube to investigate the preference of Syrphinae.

The test set three conditions, (1) Mucilage+ stems vs. Mucilage- stems, (2) Mucilage+ stems vs. flowers, (3) Mucilage+ and flowers vs. Mucilage- stems and flowers, test 1 and 2 were repeated 20 times and test 3 was repeated 37 times on April 30 and May 6, 2021.

Fruiting rate of *S. armeria*: Five Mucilage+ and 5 Mucilage- pots, as well as five *S. armeria* plants covered with a net (Bagging) to obstruct pollinators, were set in Shizuoka University's Farm (Kariyado, Fujieda City, Shizuoka pref., Japan) on May 7, 2021. The fruiting rate was investigated on June 11, 2021. In addition, the diameter of 10 seeds obtained from each test plot was measured. Ten seeds of each obtained were placed in a petri dish with a diameter of 90 mm and the germination rate was investigated. Three replicates were performed by repeating the procedure 3 times at the 25°C and a 12:12 light: Dark regime, from June 11-25, 2021.

Statistical analysis: Bell curve for Excel 5.0 software (Social Survey Research Information Co., Ltd., Tokyo) was used for statistical analyses. The chi-square test was used for the frequency of pollinator visits. T-test was used for the number of pollinators. Tukey's multiple range test with a probability of 95% ($\alpha = 0.05$) was used to compare the seed diameter.

RESULTS AND DISCUSSION

Field observation: Table 1 shows the results of field observation to clarify whether the mucilage of catchfly traps ants. In the 70 catchfly strains investigated, 431 areas with mucilage were observed. Therefore, there were about 6 mucilaginous parts per plant. No insects were observed in mucilaginous parts except 2 flies and 7 aphids. The evolutionary advantage of mucilage in *S. armeria* is unclear. Generally, it is assumed that mucilage of *S. armeria* prevents nectar robbing ants from climbing the stem. However, in this survey, no ants were observed to adhere to any mucilaginous part. Another hypothesis is that it prevents pest insects that feed on flowers. However, this survey, did not observe any flower-eating organisms such as caterpillars, hornworms or slugs. Alternatively, it is reported that pollinators avoid flowers in which predators such as spiders are present[15-18]. From this, it could be hypothesized that the mucilage prevents the spider from climbing the stem. However, did not observe any trapped spiders in the mucilaginous area. Although the sticky substance might trap organisms, it has not been confirmed that the mucilage acts as a trap to catch these harmful organisms in this survey.

Recording pollinators by the fixed camera: Figure 1 shows the results of evaluation using a camera to determine whether ants visit flowers after removing the mucilage. Even "Mucilage-" stems where there was no exposed mucilage, no ground organism, such as hornworms, ants, or spiders visited the flowers of *S. armeria* (Data not shown). However, there are differences in the number of pollinators that were observed between "Mucilage+" and "Mucilage-" and the total number of pollinators of "Mucilage+" was significantly higher than that of "Mucilage-" ($p = 0.0011$). This result suggests that mucilage may contribute to the attraction of pollinators.

Flower visits by ants and flying pollinators: Figure 2 shows the effect of removal of mucilage on the flower visits of ants and flying pollinators. No ants were observed to climb the stems of either "Mucilage+" or "Mucilage-" plants within the survey time (Data not shown). However, it was observed that

Table 1: Types and population of insects that were sticking to the sticky part of *Silene armeria* in the Abe River community (n = 70), Shizuoka, Japan

	Numbers	Frequency (%)
Small household fly	2	0.5
Aphid	7	1.6
Bee	0	0.0
Hoverfly	0	0.0
Ants	0	0.0
Spider	0	0.0

Number of plants surveyed (n = 70) and number of sticky parts (n = 431)

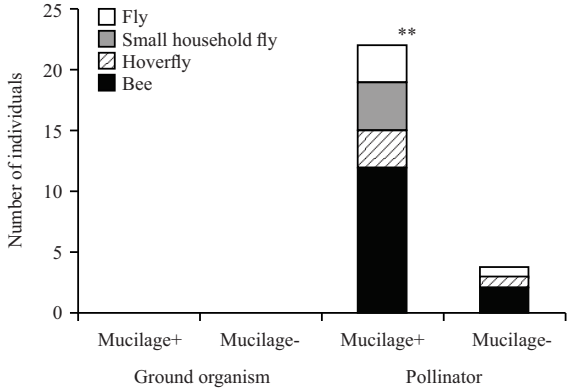

Fig. 1: Number of ground organisms and flying pollinators observed on Mucilage+ (with mucilage) and Mucilage- (without mucilage) *Silene armeria* plants

**Significant difference between the Mucilage+ and Mucilage- according to the t-test at a confidence level of 1%

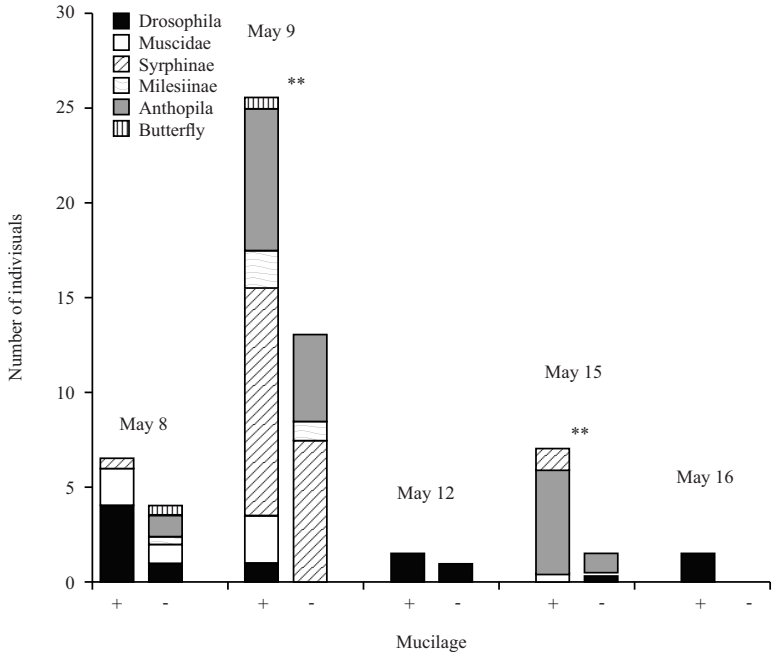

Fig. 2: Type and number of pollinators on Mucilage+ (with mucilage) and Mucilage- (without mucilage) on *Silene armeria* plants
**Significant difference between the Mucilage+ (with mucilage) and Mucilage- (without mucilage) according to the t-test at a confidence level of 1%

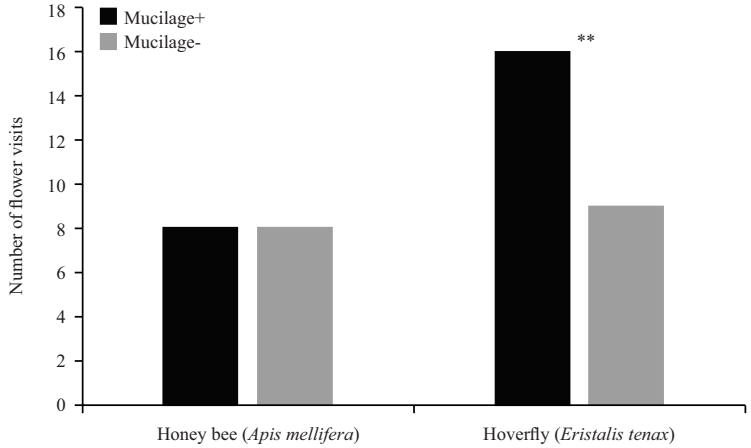

Fig. 3: Number of honeybees (*Apis mellifera*) and hoverfly (*Eristalis tenax*) to visit Mucilage+ (with mucilage) and Mucilage- (without mucilage) *Silene armeria* flowers
**Significant difference between the Mucilage+ (with mucilage) and Mucilage- (without mucilage) according to the chi-square test at a confidence level of 1%

one ant and one fly were caught in the mucilaginous area at the time of the investigation on May, 15. Consequently, although, rarely, as are rarely caught in the mucilaginous part, it could not be said that it does not affect preventing ants.

During the observation period, pollinators such as Drosophila, Muscidae, Syrphinae, Milesiinae, Anthophila and butterflies visited the flowers of *S. armeria*. The temperature varied from 15.5-24.5°C on 8 May, 21-28.5°C on 9 May, 18-20.5°C on 12 May, 20.2-25.7°C on 15 May and 18.9-21.3°C on 16 May. There were few pollinators on 12 May and 16 May, because of the low temperature. "Mucilage+" tended to have more pollinators than "Mucilage-" on 8, 9 and 15 May. This result also supports the possibility that mucilage may assist in the attraction of pollinators.

Preference of medium-sized pollinators, bees and hoverflies: Figure 3 shows the effect of removal of mucilage on flower visits by bees and hoverflies. Honeybees visited "Mucilage+" flowers 8 times and "Mucilage-" flowers 4 times

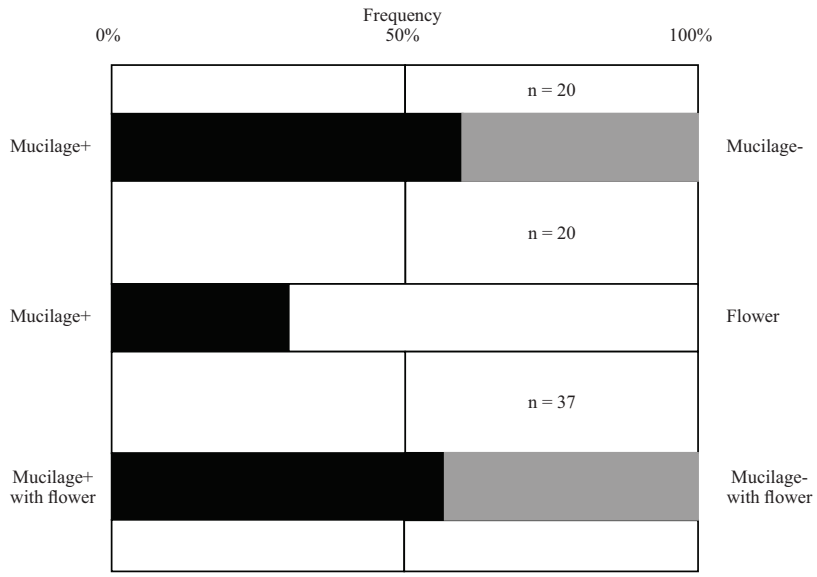

Fig. 4: Results of the preference tests of Syrphinae regarding *Silene armeria* flowers and stems with or without mucilage conducted using a T-shaped pipe

Table 2: Effects of mucus of stem on fruiting rate, seed diameter and germination rate in *Silene armeria*

	Fruiting rate (%)	Seed diameter (mm)	Germination rate (%)
Mucilage +	85.6	1.01[a]	100.0
Mucilage -	64.9	0.99[ab]	93.3
Bagging	65.4	0.94[b]	83.3

Different letters indicate significantly different at $p<0.05$ (Turkey's multiple range test)

($p = 0.24$). Although it was not statistically significant, more honeybees interacted with "Mucilage+". Hoverflies visited "Mucilage+" flowers 16 times and "Mucilage-" plants 10 times ($p = 0.002$). In this case, it was a statistically significant difference. From this, speculate that honeybees and hoverflies tended to select individuals with mucilage.

Preference of small pollinators, Syrphinae: Figure 4 shows the results of the analysis to determine whether mucilage attracts Syrphinae members. Syrphinae selected "Mucilage+" stems 12 times, but only 8 times with "Mucilage-" stems ($p = 0.37$). Although it was not statistically significant, the Syrphinae selected "Mucilage+" stems more often. When comparing the selection of the Syrphinae for "Mucilage+" stems or flowers, they chose the flowers 14 times and the stems 6 times ($p = 0.07$). When comparing the "Mucilage+" stems with flowers and "Mucilage-" stems with flowers, the Syrphinae selected "Mucilage+" stems 21 times and "Mucilage-" stems 16 times ($p = 0.4111$). Although it was a slight difference, "Mucilage+" stems were preferred. This result suggests that mucilage on the stem may be attracting pollinators, but its effect is smaller than that of flowers.

Fruiting rate of *S. armeria*: Table 2 shows the effect of the presence or absence of mucilage on fertility. The fruit set rate of the flowers covered with net (Bagging) was 65.4%. Therefore, it is possible that *S. armeria* can self-pollinate. The fruit set rate of "Mucilage+" high, 85.6%. Consequently, although *S. armeria* can self-fertilize, it is presumed that the fruit set rate will be higher with outcrossing (allogamy). In contrast, the fruit set rate of "Mucilage-" was 64.9%. This is much lower than that of the "Mucilage+" but comparable with that of Bagging. Therefore, we speculate that "Mucilage-" had a low rate of outcrossing and tended to self-fertilize. Furthermore, the seed diameter of "Mucilage+" was larger than that of the seeds from the plants covered by a net (Bagging). The germination rate was also higher in the "Mucilage+" than in the seeds obtained from the bagged flowers. Consequently, it can be inferred that the seeds obtained by cross-pollination had a heavier seed weight and a higher germination rate than the seeds obtained from self-fertilization. In other words, *S. armeria* produces better seeds from cross-breeding than by self-fertilization. The seed diameter of "Mucilage-" was between the "Mucilage+" and Bagging. In addition, the germination rate of "Mucilage-" was also midway between that of "Mucilage+" and Bagging. This

result further strengthened the impression that Mucilage-" had a low rate of out crossing and tended to self-fertilize. Consequently, it is demonstrated that the mucilage of *S. armeria* may attract pollinators and increase the rate of cross-breeding compared to stems without mucilage.

There are many studies on the ecological role of seed mucilage. In contrast, there are few studies on the ecological role of stem mucilage, as it is generally only thought to protect against pests and pathogens[19-22]. This study showed the possibility that the mucilage exuding from the stem can also affect pollinators.

CONCLUSION

It is generally supposed that the mucilage produced on the stems of *S. armeria* prevents nectar robbing ants, from climbing the stem. However, current results showed that ants did not visit flowers even if the obstructive mucilage was eliminated, enabling the ants to climb the stem. Therefore, it is unlikely that catchfly mucilage of *S. armeria* is for protection from ants. However, all tests indicate the tendency of pollinators to prefer stems with mucilage to stems without mucilage. This suggests that mucilage of *S. armeria* have a role in the attraction of pollinators, although no clear proof was found. In tests using t-tubes and Syrphinae, Syrphinae prefers flowers to stems with mucilage. Consequently, it is suggested that the mucilage of *S. armeria* does not attract pollinators by itself strongly but merely assists in attracting the pollinators to the flowers.

SIGNIFICANCE STATEMENT

This study suggests that the mucilage present on the stems of catchfly (*Silene armeria*) may attract pollinators. The findings of the study can help researchers to identify the role of mucilage, which may be critical, for plants.

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