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Effect of sugar food type and concentration on feeding behavior and life expectancy of a parasitoid wasp (Hymenoptera: Ichneumonidae)

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Abstract
Many species of insect parasitoids depend on carbohydrate-rich sources to sustain their activity. The present study focused on the effects of sugar-food type (sucrose vs. honey) and concentration on food searching behavior and survival of *Itoplectis naranyae*, a parasitoid wasp attacking lepidopteran pests of rice. While food type, sugar concentration and wasp age did not affect their feeding responses, males fed on sugar food more often than females, and smaller wasps approached more quickly to food. Sex- or size-linked differences in survival were not detected but wasps that had fed sucrose lived longer. Also, wasp age had a great impact on life expectancy after the last meal, and older wasps had lower life expectancy, suggesting that older wasps have to find a suitable sugar source more frequently. The implications of the present results were discussed in the context of the importance of food on parasitoids.

Keywords: Foraging behavior, natural enemies, longevity, host feeding, survival

Introduction
Carbohydrates or sugars are important nutrients driving the activity of insects including natural enemies such as parasitoid wasps [1]. Indeed, many studies have revealed that insect natural enemies such as parasitoids and even predators largely depend on sugar-rich sources such as floral nectar and honeydew to sustain their life and activity [2-4]. However, the availability of sugar sources in the fields should highly be variable depending on the season and environment, and such food sources also should differ in terms of composition and concentration of sugars [5-7]. Then, insect natural enemies should recognize food of different type and quality, changing their feeding behavior, accordingly.

Although the effects of sugar-rich food on the life expectancy and reproduction of parasitoids have been examined extensively [8-10], very few studies have focused on how the type of sugar food and sugar concentration affect their feeding behavior. It is likely that they modify their feeding behavior in response to different quality of food in order to balance the nutritional demand and to maximize their reproductive success; such behavioral responses are known in other insects like dipteran flies and butterflies [11-13]. Given parasitoid wasps in the field should encounter food of different quality, our knowledge and understanding of nutritional ecology of parasitoid wasps would be furthered by examining how they respond to food of different quality.

The parasitoid wasp *Itoplectis naranyae* (Hymenoptera: Ichneumonidae) is a solitary endoparasitoid attacking prepupae and pupae of a wide range of Lepidoptera [14, 15]. This parasitoid wasp is distributed widely in Asia, and is an important natural enemy of lepidotepran pests of rice such as rice leaf folder and rice skipper [14, 15]. Like other parasitoids, *I. naranyae* requires carbohydrate food to sustain its life and to maximize its reproductive potential [16, 17]. Adult *I. naranyae* often is found visiting flowers and/or feeding on honeydew attached on leaves (Ueno, unpublished). In addition, *I. naranyae* is a synovigenic species that typically emerge with no or a limited number of mature eggs and continue to mature eggs during the adult stage [15, 18, 19]; food acquired during the adult wasp stage is important in its reproduction.

In the present study, we examine how sugar-food type (sucrose solution vs. diluted honey) could affect feeding behavior and life expectancy of the parasitoid wasp *I. naranyae*. We also investigate the effect of sugar concentration on its behavioral responses. Based on the results, we discuss the importance of sugar-rich food in parasitoids and the feeding behavior in response to variable food quality.

Materials and Methods

We established a laboratory colony of *Itoplectis naranyae* using adult wasps collected from Fukuoka City, Japan. Basic rearing procedure was described by Ueno and Tanaka (1994) [15]. Female wasps were placed individually in transparent plastic cups (10 cm in diameter, 4.5 cm in height). To provide food, tissue paper saturated with 20% diluted honey was placed in each cup. The tissue paper was replaced twice a week thereafter to give fresh food. The rearing cups were kept in an incubator at 20 ± 1°C under a 16L:8D photoperiod condition. In this condition, the male and female wasps live around 1 month or even more [16]. The parasitoid wasps were reared on pupae of the wax moth *Galleria mellonella*, a laboratory host. This host is highly suitable for rearing *Itoplectis* parasitoids [15]. Wax moth cocoons containing fresh pupae were presented to the female wasps in the cups for parasitization. After parasitization, host cocoons were removed from the cups and were held at 25 ± 1°C with a 16L:8D photoperiod, until adult parasitoid emergence. Newly emerging female wasps were placed individually in the rearing cups together with a male and were maintained as mentioned above.

Test sugar food

In the present study, we used sucrose as an experimental carbohydrate because it is known as the major sugar content in floral nectar [5, 6] and is also a main content in honeydew [20]. We also chose honey for the experiment because it is commonly used in the laboratory rearing systems of many parasitoid wasps. Here three different concentrations were prepared for testing; 5%, 20% and 40%. Sucrose or honey was diluted with distilled water prior to the experiment. A droplet of test sugar solution was placed in an experimental cup (10 cm in diameter, 4.5 cm in height) into which one test wasp was released to observe its behavior.

Test parasitoid wasps

Newly emerged parasitoid wasps were individually placed in transparent plastic cups (10 cm in diameter, 4.5 cm in height) together with tissue paper saturated with 20% diluted honey. Females were allowed to mate with a male; the male was removed after mating had been confirmed. For males, unmated individuals were used for testing. Both males and females were individually reared as mentioned above, and the rearing cups were kept in an incubator maintained at 20 ± 1 °C under a 16L:8D photoperiod until the following experiment. To incorporate the effect of age, wasps of different age were used (2 – 14 days old). Wasps for testing were deprived of food for 6 hours prior to behavioral observations. 84 wasps in all were prepared for the following experiment.

Feeding behavior and life expectancy

Test wasps were individually transferred into an experimental cup in which a test sugar source was placed, and the behavioral observation was initiated. For each test wasp, we made a 5 min observation. The time was measured with a stopwatch. When test wasps located the food source and began to feed on sugar food, the time between the initiations of the observation and feeding event was recorded. When wasps did not respond to a food source, the observation was terminated and was recorded as "no response". After the observation, test wasps used were returned to the original rearing cup and were kept as mentioned above and were again subject to a 6 hours starvation in the next day for the next test. This procedure was repeated for 3 times on the daily basis in consecutive 3 days for each test wasp. Each test wasp was allocated either for sucrose-fed or honey-fed group. Also, each wasp was offered all 3 different concentrations randomly during the 3 days experimental period. After the last feeding test in the 3rd day, all test wasps were reared as mentioned above in order to examine their life expectancy. The condition of the wasps was checked every 6 hours everyday, and, when mortality was confirmed, the hours of survival since the last sugar meal was calculated.

Statistical analyses

The data were analyzed with the aid of JMP software (version 11.0; SAS Institute) and GLM and survival analyses were performed.

Results

In all, the feeding decision was recorded for 245 cases. Among the cases, feeding on test food was observed in 146 cases. Some of test wasps escaped from the cups or died during the experimental period; the data were excluded from the analyses for life expectancy.

Table 1: Generalized linear model fit* to factors affecting the behavioral decision of whether to feed on a sugar source

Factors	df	chi-square	P values
Food type	1	0.008	0.93
Concentration	2	0.20	0.90
Wasp sex	1	3.93	0.04
Wasp age	1	0.28	0.59
Wasp size	1	0.84	0.36

*Link function is logit assuming binomial distribution (n = 245)

First, factors affecting the feeding decision by *I. naranyae* were statistically examined. Among the 245 observations, wasps that had located and fed on the food were found in 147 cases while, in 98 cases, wasps did not respond to an offered sugar source. A generalized linear model (GLM) fit demonstrated that only the wasp sex had a significant effect on the feeding decision and the other factors, *i.e.*, food type, food concentration, wasp age and size, were not statistically significant (Table 1). During the experiments, 51.7 % of females located and fed on test sugar food whereas 64.7 % of males did so, indicating that males were more likely to feed on sugar food than females (Fig. 1).

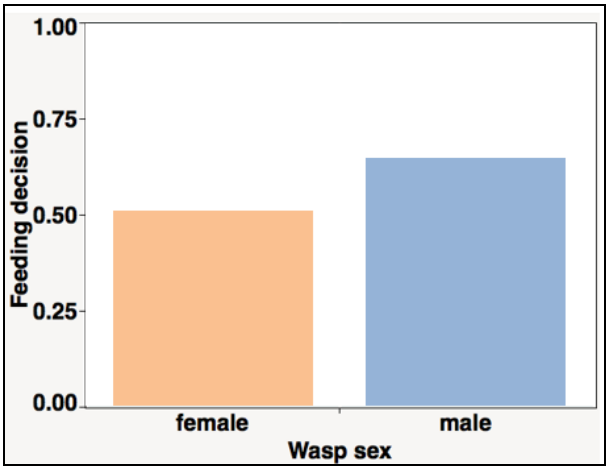

Fig 1: The relationship between wasp sex and feeding decision (whether or not the wasp fed on a test sugar food)

Then, factors involved in the time taken to locate and start feeding (= searching time) were analyzed. Before the analysis, we excluded the data in which test wasps did not feed on sugar food during the 5 min observation. We applied a parametric survival analysis assuming the Weibull distribution because the time data were fit to the Weibull distribution and because such data are known to be suitable for survival analysis rather than GLM or the other parametric or non-parametric procedures.

Table 2: Parametric survival fit* to factors affecting searching time

Factors	df	chi-square	P values
Food type	1	0.11	0.73
Concentration	2	3.99	0.14
Wasp sex	1	0.99	0.32
Wasp age	1	0.07	0.78
Wasp size	1	4.12	0.04

* Survival curve assuming the Weibull distribution is used (n = 147).

The analysis showed that, except wasp size, factors examined such as food type or concentration had no significant effect on searching time (Table 2). Wasp size had a significant positive effect on searching time of *I. naranyae* wasps (Table 2), and the overall relationship between the two are shown in Fig. 2.

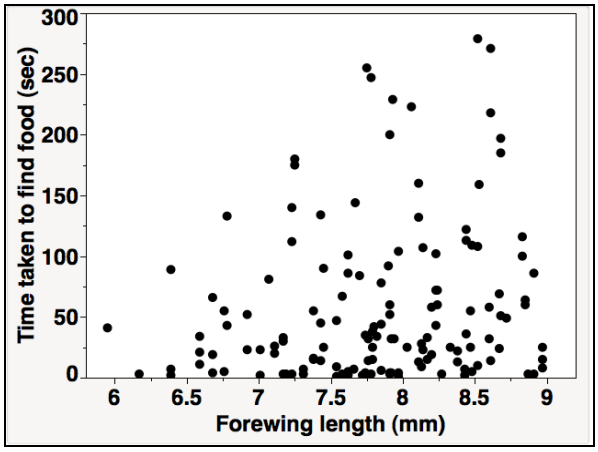

Fig 2: The relationship between wasp size and searching time for food

Lastly, how wasps' life expectancy was influenced by candidate factors was analyzed with a survival analysis assuming the Weibull distribution. The overall mean survival was 101.6 ± 45.7 (± SD) hours. Because during the 3 trials to each test wasp in the experimental period, not all wasps fed on offered sugar food, the number of time that they fed on food was included as a variable in the analysis.

Table 3: Parametric survival fit* to factors affecting life expectancy

Factors	df	chi-square	P values
Food type	1	10.95	0.0009
Concentration	2	0.63	0.43
Wasp sex	1	13.50	0.0002
Wasp age	1	0.22	0.64
Wasp size	1	1.77	0.18

* Survival curve assuming the Weibull distribution is used (n = 79).

The analysis demonstrated that food type and wasp age were both highly significant affecting the survival of the test wasps after the last meal (Table 3). *Itoplectis naranyae* wasps lived longer when the last meal was sucrose than when it was diluted honey (Fig. 3). Also, wasp age had a strong significant impact on the life expectancy, and older wasps lived shorter (Fig. 4). The other factors were not statistically significant (Table 3).

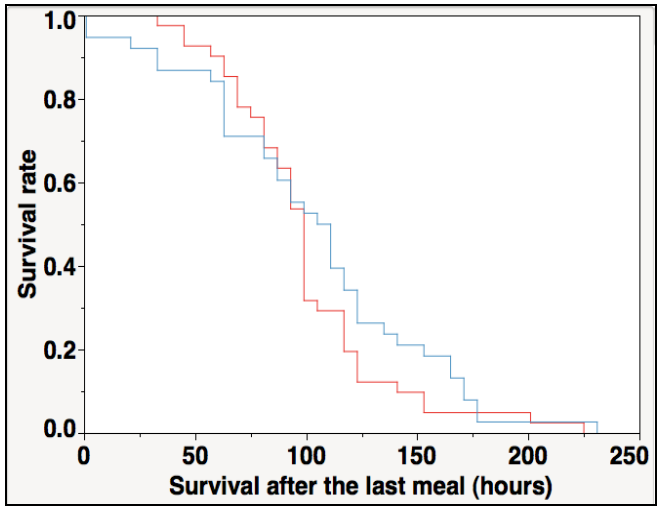

Fig 3: Survival curves of sucrose-fed and honey-fed wasps

Discussion

The primary aim of the present study was to reveal the effects of sugar food type and concentration on the feeding behavior or response of *Itoplectis naranyae.* Overall, the present results suggest the effects, under the experimental condition applied here, are rather very small or weak. First, the two factors did not influence the feeding decision of whether the wasps fed on sugar food though a sexual difference was detected (Table 1). Second, the time taken until the wasps detected a food source during the 5 min observation also did not differ regardless of food type (sucrose versus honey) and concentration (Table 2). Because honey has a specific odor while sucrose solution not, this difference could have an impact on wasp behavior but it is not the case in the present study.

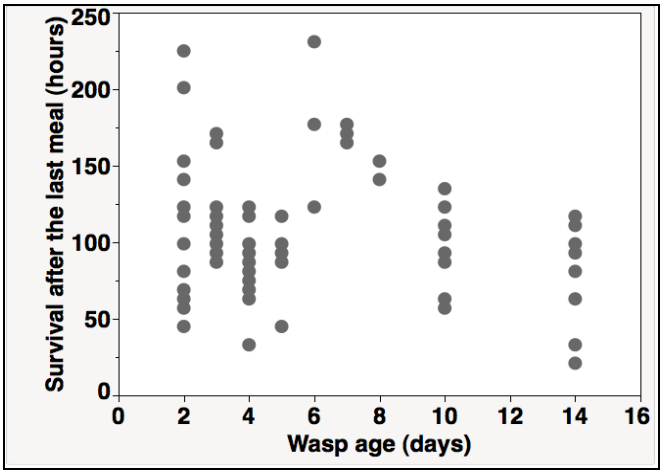

Fig 4: The relationship between wasp age and life expectancy of *I. naranyae*

Both sexes of *I. naranyae* require carbohydrate food for survival and activity [16, 17], as is typical for many parasitoid wasps [2, 8]. When only water is provided during the adult wasp lifetime, they, at 20°C condition, live only 10-15 days on average but provision of sugar food can enhance their lifetime 1 month or more if food is continuously provided [15, 16]. Then, it would be expected that food type and concentration should have some influence on their feeding behavior. However, sugar concentration was found to have no effects in the present study. Very few studies have focused if the concentration of carbohydrate sources could affect parasitoid feeding behavior; our knowledge of how quality of sugar food (type and concentration) affect parasitoid physiology, behavior and reproduction are rather poor. To further our knowledge and understanding, future studies should address on this topic.

Apart from the two factors mainly focused, the other factors influenced the feeding responses of *I. naranyae*. Sexual difference was detected in the behavioral decision of whether or not to feed on a sugar source (Table 1). Given the females of *I. naranyae* are larger than the males [15, 21], and the former can require more nutrition because of egg production and host searching activity, it is likely that the females feed on sugar food more frequently; in fact, males were more frequently observed feeding on sugar food (Fig. 1). This may be because the males are known to actively fly around to locate a newly emerging female via sex pheromone [22].

Also, larger wasps took more time to locate and feed on test sugar food (Fig. 2). In other word, the hunger level of large wasps was weaker than that of small wasps. An interpretation is that smaller wasps have to search a food source more frequently because their nutrition-storing capacity is lower. In parasitoid wasps, body size often reflects the resource storing capability of an individual, and is correlated with life-history parameters such as longevity and reproductive success [2]. Larger *I. naranyae* can store more energy and to produce more eggs, resulting in longer life span and higher reproductive success [18, 21]. Likewise, larger *I. naranyae* may be able to ingest more sugar food in a single meal event, making their hunger level lower and showing a slower response to a food source.

Food type was found to have a significant effect on life expectancy though it had no influence on feeding behavior (Table 3). Strikingly, honey as a sugar source was rather of lower quality than sucrose. In the present study, we detected two major significant factors influencing the life expectancy of *I. naranyae* wasps after the last sugar feeding; food type and wasp age (Table 3). Test wasps lived longer when the last meal had been sucrose than when they fed on diluted honey (Fig. 3). This suggests that honey may contain chemicals that are rather costly to the wasps, reducing their life expectancy.

Honey is well known for its high nutritional values containing amino acids, vitamins, minerals and others [24]. In fact, Harvey *et al*. (2012, 2017) [25, 26] showed that two synovigenic parasitoid wasp species *Lysibia nana* and *Gelis agilis* that had given diluted honey produced more offspring than those given only glucose or honey-mimic sugar solution, suggesting non-sugar constituents in honey could enhance their reproduction. Additionally, they demonstrated that adult female longevity did not differ among test groups that had been offered different sugar foods [25, 26]. It is thus not clear why honey is, in fact, of lower quality for *I. naranyae* than sucrose.

Curiously, in a fruit fly parasitoid wasp, *Diachasmimorpha tryoni*, white sugar results in higher adult female lifespan than honey does [27]. Thus, the effect of sucrose versus honey may depend on parasitoid species. Determining what sugar food is most suitable for rearing parasitoid wasps concerned is crucial to establish the efficient rearing method in the laboratory. In rearing systems, honey is often the standard food that is given to adult wasps. However, sucrose or white sugar may be superior in terms of wasp life span. Further, it is much cheaper and easy to get than honey. Given these, sucrose food is a better food in rearing *I. naranyae*.

Another factor significantly affecting the life expectancy was adult wasp age (Table 3); younger wasps survived longer, regardless of the sexes and food type (Fig. 4). What was determined in the present study was longevity or life expectancy of the wasps after the last meal. In our experimental set up, overall mean survival was around 100 hours. This value suggests a potential maximum time interval within which wasps have to find the next food source otherwise they may be starved to death.

The oldest class of test wasps here was 14 days old while expected lifetime at 20°C condition with unlimited access to sugar source is more than 1 month. Therefore, the shorter survival in older wasps in the present study is unlikely due to senescence itself. A possible reason is that older wasps consume a smaller amount of food at any one time, making their life expectancy lower. If the amount of sugar food taken does not differ among different aged wasps, the difference detected should reflect the difference in nutritional stock of the wasps, which cannot be restored by sucrose or honey food. Also, nutritional stock stored during the larval stage should be greater in younger wasps, resulting in longer survival. In any case, the present study suggests that older wasps have to find a suitable sugar source more frequently. Although very few studies have examined life expectancy of parasitoids after the last meal, the information can provide an insight on their nutrition-storing capacity and how frequently they should find a food source [23].

Conclusion

Feeding behavior of *I. naranyae* was not affected by type and concentration of sugar food though the males fed on sugar food more frequently. Provision of sucrose solution resulted in higher life expectancy of the wasps than diluted honey, demonstrating that sucrose is a better food source for the parasitoid. Older wasps lived shorter after the last sugar meal, suggesting that they have to locate sugar food sources more frequently in the field.

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