Effects of feeding on reproduction and overwintering in female adults of *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae)

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Abstract

Effects of feeding on longevity, reproduction and overwintering were examined in female adults of the wasp, *Ooencyrtus nezarae*, an egg parasitoid of phytophagous heteropterans. There were no significant differences in longevity among females fed on honey, glucose, galactose or sucrose, although fecundity was significantly higher in females fed on honey than those fed on these sugars. Lactose was less nutritious with respect to both longevity and fecundity. Most females fed on extrafloral nectar of *Vicia angustifolia* and some of them laid eggs after feeding on it only once. Most or all females fed on honeydew of the aphid, *Aphis craccivora*, and flower nectar of *Erigeron annuus*, and some females laid eggs when one of these foods was continuously supplied. Some females began to feed on flower nectar of *Solidago altissima* within 15 min after the flowers were supplied. When this food was continuously supplied, all females laid eggs and their fecundity was not significantly different from that of females feeding on honey. Therefore, these 4 foods may be natural foods of *O. nezarae*. However, when kept from autumn under natural photoperiod and temperature with aphid honeydew or flowers of *E. annuus* or *S. altissima*, no females survived the winter.

Key words: Parasitoid wasp, *Ooencyrtus nezarae*, adult feeding, ovarian development, longevity

INTRODUCTION

Adult feeding has been shown to be important for reproduction and survival of parasitoid wasps (Doutt, 1959; Leius, 1961a, b; Jervis and Kidd, 1986; Jervis et al., 1996). Without feeding in the adult stage, some parasitoid wasps cannot lay eggs, or show reduced fecundity and/or longevity (Leius, 1961a, b; Jervis et al., 1996). *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) is an egg parasitoid of phytophagous heteropterans (Takasu and Hirose, 1985). We have examined the effects of environmental factors on reproduction and diapause in female adults of this species both under constant conditions in the laboratory (Numata, 1993) and under natural photoperiod and temperatures (Teraoka and Numata, 1995). In these experiments, adult wasps were continuously supplied with honey (Numata, 1993; Teraoka and Numata, 1995). Without feeding in the adult stage, females can neither lay eggs nor overwinter (Teraoka and Numata,

unpublished). However, honey may be more nutritious than the adult food available in the field, and therefore it is possible that our previous results did not completely reflect reproduction and diapause in the field. To clarify the seasonal occurrence of reproduction and diapause of this species more precisely, it is necessary to determine the natural food of adult *O. nezarae*.

In many other parasitoid wasps, floral or extrafloral nectar, honeydew of aphids and pollen have been shown to be adult foods (Leius, 1960, 1967; Rogers, 1985; Bugg et al., 1989; Jervis et al., 1993, 1996; Olson and Nechols, 1995). In the present study, we selected 4 candidates as natural foods of adult *O. nezarae*: (1) extrafloral nectar of *Vicia angustifolia* L. (Fabales: Fabaceae), (2) honeydew of the aphid, *Aphis craccivora* Koch (Homoptera: Aphididae), (3) flower nectar of *Erigeron annuus* (L.) (Asterales: Asteraceae), and (4) flower nectar of *Solidago altissima* L. (Asterales: Asteraceae). These were common in and around crop fields

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in Kyoto and Osaka, the natural habitat of this species. We examined the feeding behavior of female adults of *O. nezarae* on natural food and the effects of feeding on their longevity, fecundity and overwintering.

MATERIALS AND METHODS

Insects. A laboratory culture of *O. nezarae* originating from parasitized eggs of *Riptortus clavatus* (Thunberg) (Heteroptera: Alydidae) collected in Kyoto City, Japan, was maintained under 16L-8D at $25 \pm 1^\circ\text{C}$ (Teraoka and Numata, 1995), and relative humidity was kept at 95% with saturated solution of Na_2HPO_4 . Adults were kept in glass tubes (9 mm i.d. $\times$ 75 mm) with a droplet of honey as food. Eggs of *R. clavatus* within 24 h after oviposition were provided as hosts. In this paper, adult emergence does not mean pupal-adult ecdysis but egression of adult wasps from host eggs.

Longevity and fecundity on various sugars. Female wasps within a day after adult emergence were placed individually in glass tubes and supplied with a host egg and various kinds of food at 95% r.h. under 16L-8D at 25°C . The host egg was replaced every day and dissected under a stereoscopic microscope to count the number of parasitoid eggs. These procedures were repeated daily until the death of the females. Various sugars or honey were given in agar gel. Honey, D-glucose, D-galactose, D-sucrose or D-lactose (15 g) was dissolved in 3% agar solution (15 ml) to form a gel. A 5 mm-cube block of the gel was supplied to each female. Because female adults of this species feed on host eggs before oviposition (Takasu and Hirose, 1993), the longevity without host eggs was also recorded.

Feeding behavior on natural food. Female wasps within a day after adult emergence were placed individually in glass tubes (15 mm i.d. $\times$ 100 mm) with a putative source of food, i.e., 2 leaves of *V. angustifolia* with extrafloral nectaries, 2 leaves of *V. angustifolia* without extrafloral nectaries infested with *A. craccivora*, one flower of *E. annuus*, and 5 flowers of *S. altissima*, or a droplet of honey. The leaf surface of *V. angustifolia* infested with *A. craccivora* was covered with aphid honeydew. These plants were collected between 2:00 and

4:00 p.m., and immediately used for the experiment. The behavior of females was observed at 25°C .

When a female did not feed for 15 min, this was regarded as no feeding. When feeding was terminated, the females were individually transferred to new glass tubes, and kept with a piece of filter paper (5 $\times$ 10 mm) soaked with water under 16L-8D at 25°C . Two days after feeding, a host egg was given to each female and removed after 24 h. The host egg was dissected and the number of parasitoid eggs laid was counted. The females that had not laid any eggs were dissected under a stereoscopic microscope and the developmental state of their ovaries was determined.

Longevity and fecundity on natural food. According to the above procedures for various sugars, the longevity and fecundity of female wasps were examined on putative sources of food, i.e., about 10 leaves of *V. angustifolia* infested with *A. craccivora* together with 5 adult aphids, one flower of *E. annuus*, and 10 flowers of *S. altissima*, or a droplet of honey. These plants were collected from the field and their cut end was soaked in water in glass vials. The plants were given to female wasps and replaced every other day.

Overwintering with natural food. Parasitized host eggs were placed in an outdoor cage on the Campus of Osaka City University on 20 September, 1 October and 11 October in 1995, and emergence of parasitoid adults was checked daily. Female wasps were placed individually in glass tubes within a day after adult emergence and maintained with: a droplet of honey; water only; 10 leaves of *V. angustifolia* infested with *A. craccivora* together with 5 adult aphids; a flower of *E. annuus*; or with 10 flowers of *S. altissima*. These plants were collected from the field and their cut end was soaked in water in glass vials, and replaced every other day. Survival after overwintering was examined on 15 April 1996.

RESULTS

Effects of various sugars on longevity and fecundity

First, we examined whether sugars were sufficient foods for inducing reproduction in

females. When host eggs were provided, the median of longevity in females fed on honey, glucose, galactose or sucrose was 31–38 days (Table 1). No significant differences were observed in longevity among these 4 groups. Females fed on lactose and those with only water survived only a few days, and the longevity of these 2 groups was significantly shorter than that of the other 4 groups. Females taking only water did not lay eggs at all. All females fed on honey or glucose laid eggs, and more than 80% of females laid eggs when fed on galactose or sucrose. The total number of eggs per female was significantly higher on honey than on any other food. When fed on lactose, however, only about 20% of females laid eggs. The number of eggs laid by females fed on lactose was less than that of females fed on the other three sugars, although the difference was not statistically significant.

There was no significant difference in the longevity between females with and without host eggs in each diet group, and therefore feeding on host eggs had no effect on longevity (Table 1).

Feeding behavior on natural food

As feeding on sugars induced oviposition in *O. nezarae*, we looked for sugar sources in and

around crop fields, the natural habitat of this wasp, in Kyoto City and Osaka City throughout the season. We found extrafloral nectaries of *V. angustifolia* from April to June, honeydew of *A. craccivora* from April to June and from September to November, and flowers of *E. annuus* and *S. altissima* in September and October. Therefore, we examined whether female wasps can feed on these sugar sources, and whether feeding induced ovarian development.

Upon arrival at the food source, the female contacted it first with the antennae, and then with the mouthparts. Thereafter the female stopped moving and fed on the food. The contact to the food with the mouthparts and the separation from it were recorded as the beginning and termination of feeding, respectively. Most females took water when provided (Table 2). After 2 days, one of 5 surviving females showed ovarian development. All females fed on honey survived for the 2 days after feeding, and showed ovarian development. When extrafloral nectaries of *V. angustifolia* were provided, most females fed on the extrafloral nectar. Two days after feeding, most of the surviving females had mature ovaries and 50% of them laid eggs. All females fed on aphid honeydew, and about 70% of the surviving females had mature ovaries 2 days after feeding,

Table 1. Longevity and fecundity in female adults of *Ooencyrtus nezarae* fed on various sugars and honey

Food	<i>n</i>	Longevity ^a (<i>d</i> , median [range])	No. of females laying eggs	No. of eggs laid ^a (median [range])
With host eggs				
Water	14	2.5 [1–3] a	0	—
Honey	20	31 [10–61] b	20	19 [7–39] b
Glucose	24	38 [18–60] b	24	8.5 [1–28] a
Galactose	24	35 [3–52] b	20	6.5 [1–21] a
Sucrose	25	37 [9–67] b	23	5 [1–24] a
Lactose	22	3 [2–5] a	5	3 [2–5] a
Without host eggs				
Water	11	3 [2–4] a	—	—
Honey	20	23 [13–61] b	—	—
Glucose	23	35 [10–66] b	—	—
Galactose	25	28 [5–55] b	—	—
Sucrose	24	39.5 [18–63] b	—	—
Lactose	21	5 [3–6] a	—	—

^a Values followed by the same letter in each column are not significantly different at $p=0.05$ by nonparametric Tukey-type multiple comparisons (Zar, 1999, pp. 223–226).

Table 2. Feeding behavior on various foods and its effect on reproduction in female adults of *Ooencyrtus nezarae*

Food	n	Searching time ^a (s, median [range])	No. of feeding females	Feeding time ^a (s, median [range])	No. of surviving females ^b	Reproductive state ^c		
						–	+	++
Water	20	29 [2–454] a	17	27 [8–78] a	5	4	1	0
Honey	16	139 [8–400] bc	16	806 [616–1083] e	16	0	7	9
Extrafloral nectar of <i>Vicia angustifolia</i>	18	183 [2–609] bc	15	134 [59–241] d	14	2	5	7
Honeydew of <i>Aphis</i> <i>craccivora</i>	21	38 [5–194] ab	21	72 [13–285] c	18	6	12	0
Flower nectar of <i>Erigeron annuus</i>	17	96 [11–727] abc	15	47 [8–179] b	8	3	5	0
Flower nectar of <i>Solidago altissima</i>	20	366 [4–641] c	7	111 [25–226] cd	7	4	2	1

^a Values followed by the same letter in each column are not significantly different at $p=0.05$ by nonparametric Tukey-type multiple comparisons (Zar, 1999, pp. 223–226).

^b Number of adults surviving for 2 days with only water after feeding.

^c –, immature ovaries; +, mature ovaries without oviposition; ++, oviposition.

but none laid eggs. When a flower of *E. annuus* was provided, most females fed on the flower nectar. However, about 50% of them died within 2 days. Five of 8 surviving females had mature ovaries, but none laid eggs. When flowers of *S. altissima* were provided, only 35% of the females fed on the flower nectar, although all of them survived for the 2 days after feeding. Three of the 7 females had mature ovaries and one laid eggs.

The food searching time was short for water and honeydew, although it varied between individuals for any food. The duration of feeding for water was usually less than 1 min and significantly shorter than that for any other food. In contrast, all females fed on honey for longer than 10 min, and the duration was significantly longer than that for any other food. The median duration of feeding for the other 4 foods, i.e., putative natural foods for adult *O. nezarae*, varied between 47 and 134 s (Table 2).

Effects of natural food on longevity and fecundity

The above experiment showed that natural food could induce not only feeding behavior but also ovarian development in female adults of *O. nezarae*. However, feeding was permitted only once, and this might be the reason why not all females laid eggs. In this experiment, there-

fore, we examined longevity and fecundity, with natural food continuously available. When the experiment was conducted in September 1995, *V. angustifolia* had no extrafloral nectaries and we could not examine their effect. Leaves of *V. angustifolia* without extrafloral nectaries were also given to females as a control for honeydew, because honeydew was supplied with leaves of *V. angustifolia*.

When kept with leaves of *V. angustifolia* with aphid honeydew, about 50% of females laid eggs, whereas no females laid eggs with the leaves only (Table 3). When kept with a flower of *E. annuus*, only 30% of females laid eggs. When kept with flowers of *S. altissima*, however, all females laid eggs. The longevity was longest on this food, although there were no significant differences in longevity among all groups. The number of eggs laid was significantly more for females kept with flowers of *S. altissima* than for the other 2 groups with oviposition. There was no significant difference in the number of eggs between the females kept with flowers of *S. altissima* (Table 3) and those fed on honey (see Table 1) ($p>0.05$, by Mann-Whitney test).

Effects of natural food on overwintering

Thus, natural food can induce oviposition in adult *O. nezarae*. We then examined the effects

Table 3. Longevity and fecundity in female adults of *Ooencyrtus nezarae* on various foods available in nature

Food	<i>n</i>	No. of females laying eggs	Longevity ^a (<i>d</i> , median [range])	No. of eggs laid ^b (median [range])
Water	14	0	2 [1–3]	
Honeydew of <i>Aphis craccivora</i> ^c	17	9	3 [1–5]	2 [2–4] a
Leaves of <i>Vicia</i> <i>angustifolia</i> ^d	16	0	2 [1–2]	
Flower nectar of <i>Erigeron annuus</i>	10	3	3 [2–4]	1.5 [1.5–6.5] a
Flower nectar of <i>Solidago altissima</i>	10	10	5 [4–6]	20.5 [6–27] b

^aThere were no significant differences among all groups ($p > 0.05$, by Kruskal-Wallis test).

^bValues followed by the same letter are not significantly different at $p = 0.05$ by nonparametric Tukey-type multiple comparisons (Zar, 1999, pp. 223–226).

^cHoneydew was supplied with leaves of *Vicia angustifolia*.

^dLeaves without extrafloral nectaries were supplied.

of natural food on overwintering. Because *V. angustifolia* has no extrafloral nectaries in autumn, we used aphid honeydew and flowers of *E. annuus* and *S. altissima*. In autumn, these flowers were dominant around crop fields in Kyoto City and Osaka City.

When parasitized host eggs were placed outdoors on 20 September, adults emerged on 20 October. All these adults died before winter even when kept with honey. Adults emerged on 31 October from parasitized host eggs placed outdoors on 1 October. Six of 18 females kept with honey survived the winter, although all of 21 females kept with aphid honeydew died before the winter. When parasitized host eggs were placed outdoors on 11 October, adults emerged on 23 November. Eight of 20 females kept with honey overwintered, although all females kept with aphid honeydew, a flower of *E. annuus* or flowers of *S. altissima*, died within 20 days ($n = 19$ –21).

DISCUSSION

Longevity was not significantly shorter in female adults of *O. nezarae* fed on glucose, galactose or sucrose than in those fed on honey, which has been used as an adult food for this species in the laboratory (Takasu and Hirose, 1991, 1993; Numata, 1993; Teraoka and Numata, 1995). On these diets, most or all females laid eggs, and there were no significant

differences in fecundity among females fed on glucose, galactose and sucrose. However, longevity was significantly shorter in females fed on lactose than in those fed on the other sugars, and not significantly different from control wasps kept with only water.

Some sugars such as melezitose have been reported to have negative effects in some parasitoid wasps (Leius, 1961a, b; Avidov et al., 1970). In our experiments, however, lactose did not show negative effects on longevity. Furthermore, lactose appeared to be nutritionally effective, because a small proportion of females fed on this sugar laid eggs. Fecundity was significantly lower on glucose, galactose or sucrose than on honey. Vitamins or proteins in the honey probably increased fecundity, as shown in some parasitoid wasps (Bracken, 1965).

It has been reported that host feeding increases both longevity and fecundity in some parasitoid wasps (Bracken, 1965; Jervis and Kidd, 1986). *O. nezarae* also feeds on host egg fluid before oviposition (Takasu and Hirose, 1993). In the present study, however, longevity was not significantly different between females kept with and without host eggs. Therefore, no effect of host feeding on longevity was detected in this species.

By observing the natural habitat of *O. nezarae* throughout the season, we selected honeydew of *A. craccivora* and extrafloral nec-

tar of *V. angustifolia* as possible adult food from April to June. In the laboratory experiment, most or all females fed on these. Some females laid eggs 2 days after feeding on the extrafloral nectar. Two days after feeding on the honeydew, some females had mature ovaries, but none laid eggs. When the honeydew was continuously available, some females laid eggs. We assumed, therefore, that both honeydew of *A. craccivora* and extrafloral nectar of *V. angustifolia* can induce oviposition in female adults of *O. nezarae* from April to June in the field. Furthermore, we concluded that the extrafloral nectar is a better food source than honeydew, even though we did not examine the longevity or fecundity in wasps given the extrafloral nectar continuously. It is likely that female adults of *O. nezarae* primarily feed on the extrafloral nectar from April to June.

Some parasitoid wasps change the adult food throughout the season (Syme, 1977; Jervis et al., 1993; Idris and Grafius, 1995). Because *V. angustifolia* had no extrafloral nectaries after July in our observations, it is possible that *O. nezarae* also changes adult food as the season advances. *A. craccivora* disappeared in July, but was observed again in September and October. In addition to the aphid honeydew, we selected the flowers of *E. annuus* and *S. altissima* as candidates for adult food sources in September and October. Some females of *O. nezarae* showed feeding behavior on the flowers of these 2 species, and laid eggs when these flowers were continuously supplied. Therefore, these flowers can be used as adult food sources by this wasp in September and October in addition to the aphid honeydew. The proportion of females feeding within 15 min was higher on *E. annuus* than on *S. altissima*. When the food was continuously supplied, however, both the proportion of oviposition and fecundity were lower on *E. annuus* than on *S. altissima*. There is a relationship between morphology and foraging ability of parasitoid wasps and floral architecture (Jervis et al., 1993; Patt et al., 1997). Patt et al. (1997) classified flowers into 5 types based on the architecture and the position of nectar glands. The flowers of *E. annuus* and *S. altissima* belong to type 1 (umbels with exposed nectaries) and type 5 (capitula with hid-

den nectaries), respectively. Parasitoid wasps arrive at the nectaries of type-1 flowers more easily than those of type-5 flowers (Patt et al., 1997). Therefore, it may take more time for adults of *O. nezarae* to find the flower nectaries of *S. altissima* than those of *E. annuus*, although the nectar of *S. altissima* may be nutritionally better than that of *E. annuus*.

Teraoka and Numata (1995) showed that female adults of *O. nezarae* overwinter outdoors when fed on honey. In the present study, the female adults emerging on 31 October and 23 November survived winter when fed on honey. However, all females kept with a presumed natural food died within 20 days. Therefore, these flower nectars and the aphid honeydew were effective for inducing ovarian development but nutritionally deficient for increasing longevity and acquiring the ability to overwinter. Bugg et al. (1989) suggested that extrafloral nectar of the faba bean, *Vicia faba* L., is used as adult food for overwintering in several species of Ichneumonidae, although they presented no direct evidence. There is no experimental evidence concerning the natural adult food of parasitoid wasps for overwintering. The natural overwintering site of *O. nezarae* is also unclear. To clarify the natural adult food for overwintering, field observations during autumn and winter are necessary.

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REFERENCES

- Avidov, Z., M. Balshin and U. Gerson (1970) Studies on *Aphytis coheni*, a parasite of the California red scale, *Aonidiella aurantii*, in Israel. *Entomophaga* 15: 191–207.
- Bracken, G.K. (1965) Effects of dietary component of the parasitoid *Exeristes comstockii* (Cress.) (Hymenoptera: Ichneumonidae). *Can. Entomol.* 97: 1037–1041.
- Bugg, R.L., R. T. Ellis and R. W. Carlson (1989) Ichneumonidae (Hymenoptera) using extrafloral nectar of faba bean (*Vicia faba* L., Fabaceae) in Massachusetts. *Biol. Agric. Hortic.* 6: 107–114.
- Doutt, R.L. (1959) The biology of parasitic Hymenoptera. *Annu. Rev. Entomol.* 4: 161–182.
- Idris, A.B. and E. Grafius (1995) Wildflowers as nectar sources for *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Yponomeutidae). *Environ. Entomol.* 24: 1726–1735.
- Jervis, M.A. and N. A. C. Kidd (1986) Host-feeding strategies in hymenopteran parasitoids. *Biol. Rev.* 61: 395–434.

- Jervis, M. A., N. A. C. Kidd, M. G. Fitton, T. Huddleston and H. D. Dawah (1993) Flower-visiting by hymenopteran parasitoids. *J. Nat. Hist.* 27: 67–105.
- Jervis, M. A., N. A. C. Kidd and G. E. Heimpel (1996) Parasitoid adult feeding behavior and biocontrol—a review. *Biocont. News Inf.* 17: 11–26.
- Leius, K. (1960) Attractiveness of different foods and flowers to the adults of some hymenopterous parasites. *Can. Entomol.* 92: 369–376.
- Leius, K. (1961a) Influence of food on fecundity and longevity of adults of *Itopectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). *Can. Entomol.* 93: 771–780.
- Leius, K. (1961b) Influence of various foods on fecundity and longevity of adults of *Scambus buolianae* (Htg.) (Hymenoptera: Ichneumonidae). *Can. Entomol.* 93: 1079–1084.
- Leius, K. (1967) Influence of wild flowers on parasitism of tent caterpillar and codling moth. *Can. Entomol.* 99: 444–446.
- Numata, H. (1993) Induction of adult diapause and of low and high reproductive states in a parasitoid wasp, *Ooencyrtus nezarae*, by photoperiod and temperature. *Entomol. Exp. Appl.* 66: 127–134.
- Olson, D. L. and J. R. Nechols (1995) Effect of squash leaf trichome exudates and honey on adult feeding, survival, and fecundity of the squash bug (Heteroptera: Coreidae) egg parasitoid *Gryon pennsylvanicum* (Hymenoptera: Scelionidae). *Environ. Entomol.* 24: 454–458.
- Patt, J. M., G. C. Hamilton and J. H. Lashomb (1997) Foraging success of parasitoid wasps on flowers: interplay of insect morphology, floral architecture and searching behavior. *Entomol. Exp. Appl.* 83: 21–30.
- Rogers, C. E. (1985) Extrafloral nectar: entomological implications. *Bull. Entomol. Soc. Am.* 31: 15–20.
- Syme, P. D. (1977) Observations on the longevity and fecundity of *Orgilus obscurator* (Hymenoptera: Braconidae) and the effects of certain foods on longevity. *Can. Entomol.* 109: 995–1000.
- Takasu, K. and Y. Hirose (1985) Seasonal egg parasitism of phytophagous stink bugs in a soybean field in Fukuoka. *Proc. Assoc. Pl. Prot. Kyushu* 31: 127–131 (in Japanese with English summary).
- Takasu, K. and Y. Hirose (1991) Host searching behavior in the parasitoid, *Ooencyrtus nezarae* (Hymenoptera: Encyrtidae) as influenced by non-host food deprivation. *Appl. Entomol. Zool.* 26: 415–417.
- Takasu, K. and Y. Hirose (1993) Host acceptance behavior by the host-feeding egg parasitoid, *Ooencyrtus nezarae* (Hymenoptera: Encyrtidae): host age effects. *Ann. Entomol. Soc. Am.* 86: 117–121.
- Teraoka, T. and H. Numata (1995) Induction of adult diapause in a parasitoid wasp, *Ooencyrtus nezarae* under natural conditions. *Entomol. Exp. Appl.* 76: 329–332.
- Zar, J. H. (1999) *Biostatistical Analysis*. 4th ed. Prentice Hall, Upper Saddle River. 919 pp.