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Development and reproduction of an aphidophagous coccinellid, *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae), reared on an alternative diet, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs

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Abstract

To establish a simple method for rearing *Propylea japonica* (Thunberg), we investigated its larval and pupal development and reproduction when offered *Ephestia kuehniella* Zeller eggs as an alternative diet. Larvae raised on *E. kuehniella* eggs matured as well as those raised on *Acyrthosiphon pisum* (Harris), and could be reared at high densities without cannibalism when a sufficient diet was supplied. Although the mean fertility of the adults reared on *E. kuehniella* eggs was about one-third of that of the adults reared on *A. pisum*, 63.7–66.5% of the eggs hatched and the larvae matured normally. These results suggest that *E. kuehniella* eggs are an available alternative diet for the larval and pupal development and reproduction of *P. japonica*, and should make it possible to rear successive generations. However, to obtain large amounts of larvae at the same developmental stage and to collect eggs efficiently, the larvae should be reared on *E. kuehniella* eggs and the adults should be reared on aphids such as *A. pisum*.

Key words: *Propylea japonica*; lady beetle; natural enemy; *Ephestia kuehniella* egg; alternative diet

INTRODUCTION

The lady beetle *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) is a common indigenous natural enemy of the aphids that are serious pests of crops in Japan. Although the amount of predation by larvae and adults is less than that by larger lady beetles such as *Harmonia axyridis* Pallas and *Coccinella septempunctata bruckii* Mulsant, the intrinsic rate of natural increase of *P. japonica* is 1.24 times that of *C. septempunctata* (Kawauchi, 1985a). In addition, *P. japonica* can reproduce under poor diet conditions (Kawauchi, 1985a) and survive a wide range of temperatures, but does not undergo estivation (Kawauchi, 1985b). For these reasons, *P. japonica* is regarded as an important regulating factor for the aphid populations in open fields (Kawauchi, 1992). Therefore, in order to use *P. japonica* as a biological control agent and/or a pesticide test insect, it is important to establish simple rearing methods using alternative diets.

Previously, alternative diets for *P. japonica* investigated include pulverized drone honeybee brood (“Drone Powder”; Matsuka et al., 1972) and a mixture of brine shrimp eggs, yeast, and sucrose (Furuie and Koga, 1999). However, the emerged adults that were reared on these diets were smaller than those reared on aphids, and their reproduction was not investigated. In the present study, we selected the eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as an alternative diet. These eggs have been used for successive rearing of many natural enemies, which include some lady beetles such as *H. axyridis* (Schanderl et al., 1988), *Semiadalia undecimnotata* Schneider (Schanderl et al., 1988), *Pharoscymnus semiglobosus* Karsch (Iperti et al., 1972), *Coccinella decempunctata* Linnaeus (Iperti et al., 1972) and *Hippodamia convergens* Guérin-Méneville (Kato et al., 1999). Therefore, in order to establish simple rearing methods of *P. japonica* using *E. kuehniella* eggs, we investigated the effect of these eggs on the development and reproduction of *P. japonica*.

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MATERIALS AND METHODS

Insects. We collected *P. japonica* adults from a tea field at the National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan, in 2003. These insects were subsequently reared on the pea aphid, *Acyrthosiphon pisum* (Harris), at 20°C under a 16L : 8D photoperiod. Adults and larvae of *P. japonica*, which were stocked from the fourth to eighth generations after the start of rearing, were used in each experiment. *A. pisum* was obtained from a stock culture at the Insect Ecology Laboratory of the National Agricultural Research Center, Tsukuba, Ibaraki, Japan, in February 2003. These aphids had been reared on the seedlings of broad bean (*Vicia faba* L.) in a plastic cage (30×25 cm, 28 cm in height) at 20°C under a 16L : 8D photoperiod. The seedlings were prepared according to Murai (2002).

Alternative diet. Entofood® (frozen eggs of *E. kuehniella*; Arysta Life Science Ltd.) is a supplemental diet source used for some predatory insects. In each experiment, we used these frozen eggs as an alternative diet for *P. japonica*.

Experiment 1: Development of *P. japonica* larvae reared on *E. kuehniella* eggs. First-instar larvae within 24 h after hatching were inoculated individually into plastic Petri dishes (9 cm diam. ×1.5 cm height) in which filter paper had been spread. To prevent the larvae from escaping, we fitted two sheets of filter paper into the cover dish. We added about 50 mg of *E. kuehniella* eggs on a piece of paper towel and water-soaked cotton on a piece of Parafilm® (American National Co. Ltd.) in a Petri dish. The eggs and water were supplied every other day. *A. pisum* were used as the control diet in this experiment. Adults and nymphs of *A. pisum* were shaken into the Petri dishes from the broad bean seedlings. They were supplied to *P. japonica* larvae sufficiently (about 50 individuals) every day. The Petri dishes were kept at 25°C under a 16L : 8D photoperiod. Pupation and emergence were recorded every day. Emerged adults were weighed (to the nearest 0.1 mg) individually within 24 h after emergence.

Experiment 2: Effects of rearing density on the development of *P. japonica* larvae. We reared first-instar larvae on *E. kuehniella* eggs at four larval densities (1, 5, 10, and 20 larvae per dish) in plastic Petri dishes. The larvae were supplied with about 50 mg (density 1), 80 mg (density 5), 150 mg (density 10) or 250 mg (density 20) of *E. kuehniella* eggs and sufficient water every other day. These supplies were not completely exhausted for 2 d in each Petri dish. Other details of the rearing conditions were the same as in Experiment 1.

Experiment 3: Reproduction of *P. japonica* adults reared on *E. kuehniella* eggs. We used two different groups of adults for this experiment. One group (EKE adults) was reared on *E. kuehniella* eggs during their larval period, and the other group (AP adults) was reared on *A. pisum*. We chose some pairs of adults (male and female, within 24 h after emergence) from each group, and supplied one of the two kinds of diet, *E. kuehniella* eggs or *A. pisum*. A pair of adults was inoculated into a plastic Petri dish in which a filter paper had been spread. We added a piece of water-soaked cotton to each Petri dish, and placed a filter paper in the cover dish to serve as an oviposition substrate. We then reared the adults on their respective diets. We supplied sufficient diet and water every day. We replaced any Petri dish with a new one if it became dirty or if eggs were laid on its wall. We recorded the number of eggs laid per female every day for 20 d after adult emergence. Eggs that had been laid on filter paper were transferred into another Petri dish containing a piece of cotton soaked with water. To know the hatchability, these Petri dishes and other Petri dishes in which eggs were laid were incubated for 5 d at 25°C under a 16L : 8D photoperiod. The incubation period was determined by the result of a preliminary experiment (mean egg period was 2.5±0.5 d, n=297, range: 2–4 d). Hatched larvae were removed every day. Hatchability was calculated as percentage of hatched larvae to total number of eggs laid per female for 20 d after emergence.

Statistical analysis. In Experiment 1, we tested the effect of diets on the pupation and emergence rates using the Chi-square test, and on the larval and pupal periods and weights of newly emerged adults using one-way ANOVA. In the comparisons among the four larval densities in Experiment 2, we analyzed the pupation and emergence rates using Fisher's exact probability test with Bonferroni method, and the developmental period and weights of the newly emerged adults using one-way ANOVA with Tukey-Kramer method. In Experiment 3, we conducted two-way ANOVA to

Table 1. Development of *Propylea japonica* reared on *Acyrthosiphon pisum* or *Ephestia kuehniella* eggs

Diet	% Pupation (*n*)	% Emergence (*n*)	Developmental period (d)[a] Mean±SD (*n*)		Weight of newly-emerged adults (mg) Mean±SD (*n*)	
			Larval stage	Pupal stage	Male	Female
A. pisum	74.7 (83) a	85.5 (62) a	7.0±0.7 (62) a	2.8±0.5 (53) a	5.5±0.6 (29) a	6.6±0.7 (24) a
E. kuehniella eggs	78.6 (84) a	97.0 (66) a	7.8±1.1 (66) b	3.0±0.6 (64) a	5.4±0.7 (33) a	6.8±0.8 (31) a

Values in the same column followed by the same letter do not differ significantly at the 5% level. % Pupation and % emergence were analyzed using the Chi-square test and two other traits using one-way ANOVA.

[a] Developmental periods were not divided into male and female because no significant differences were found between sexes at the 5% level by Student's *t*-test.

Table 2. Development of *P. japonica* reared on *E. kuehniella* eggs at different densities

Larval density (Number of individuals per Petri dish)	% Pupation (*n*)	% Emergence (*n*)	Developmental period (d)[a] Mean±SD (*n*)		Weight of newly-emerged adults (mg) Mean±SD (*n*)	
			Larval stage	Pupal stage	Male	Female
1	78.6 (84) a	97.0 (66) a	7.8±1.1 (66) a	3.0±0.6 (64) a	5.4±0.7 (33) a	6.8±0.8 (31) a
5	77.0 (100) a	100 (77) a	8.7±0.5 (77) b	2.5±0.6 (77) b	5.5±1.5 (32) a	6.9±1.1 (45) a
10	77.0 (100) a	96.1 (77) a	8.6±0.5 (77) b	2.7±0.7 (74) b	5.7±0.8 (37) a	7.2±0.9 (37) a
20	66.0 (200) a	98.5 (132) a	8.8±0.4 (132) b	2.5±0.7 (130) b	5.7±0.6 (63) a	6.6±0.8 (67) a

Values in the same column followed by the same letter do not differ significantly at the 5% level. % Pupation and % emergence were analyzed using Fisher's exact probability test with Bonferroni method and two other traits using one-way ANOVA with Tukey-Kramer method.

[a] Developmental periods were not divided into male and female because no significant differences were found between sexes at the 5% level by Student's *t*-test.

analyze the effects of larval and adult diets on the pre-oviposition period, fertility, egg period and hatchability. We arcsine-transformed the hatchability values before analysis. In all cases, significance is indicated at the 5% level. All statistical analyses were performed using StatView version 5.0 (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

The mean larval period of *P. japonica* larvae reared on *E. kuehniella* eggs was about 1 d longer than that of larvae reared on *A. pisum*, but the mean pupal period, pupation rate, emergence rate and the weight of newly emerged adults did not differ significantly between the diets (Table 1). Matsuka et al. (1972) reported that the mean weight of emerged adults (including males and females) of *P. japonica* reared on pulverized drone honeybee brood (Drone Powder) was 4.4 mg. Furuie and Koga (1999) reported that the mean weight of adults reared on a mixture of brine shrimp eggs, yeast, and sucrose (1 : 1 : 1) was 5.5 mg. In our study, the mean weight of emerged adults of *P. japonica* reared on *E. kuehniella* eggs was 6.1 mg and almost the same as that reared on *A. pisum*, which was 6.0 mg. These results show that *E. kuehniella* eggs are a suitable diet for the development of *P. japonica*.

The pupation rate, emergence rate and mean newly emerged adult weights of *P. japonica* did not differ significantly among the four larval densities (Table 2). Although the pupation rate at density 20 was slightly lower than those at other densities, the diets were not completely exhausted and cannibalism was not observed at any density. Takahashi (1987) reported that larval cannibalism of *C. septempunctata* did not occur when a sufficient supply of aphids was provided. These results indicate that *P. japonica* larvae could be reared at high densities using a diet of *E. kuehniella* eggs.

The two-way ANOVA using the adult and larval diets as factors indicated that the adult diet significantly affected the pre-oviposition period, fertility, offspring egg period and hatchability, and the larval diet significantly affected only the egg period (Tables 3 and 4). The mean pre-oviposition period of the adults fed on *E. kuehniella* eggs was about 2 d longer than that of the adults fed on *A. pisum*. Also the mean fertility of those fed on *E. kuehniella* eggs was about one-third of that on *A. pisum*. Thus, it may be possible to assume that there is a qualitative difference in nutrition between *A. pisum* and *E. kuehniella* eggs. However, 66.5% of the eggs laid by the EKE adults that fed on *E. kuehniella* eggs hatched and the larvae reared on *E. kuehniella* eggs matured normally into adults (pupation rate: 86.0% (43/50), emergence rate: 97.7% (42/43), the weight of newly emerged adults: male 5.4±0.6 mg (18), female 6.6±0.6 mg (24), n=50). In addition, some of the EKE adults that fed on *E. kuehniella* eggs laid about 200 eggs in the 20 d after emergence. These results show that *P. japonica* can reproduce by feeding on *E. kuehniella* eggs.

The mean egg period was significantly affected by each of the larval and adult diets of the mother beetle (Table 4). However the differences of the mean values among the treatments were very small (Table 3). It may be possible to think that the difference in the diet have affected egg development only slightly.

Hatchability of the eggs laid by the adults fed on *E. kuehniella* eggs was significantly higher than that by the adults fed on *A. pisum* (Tables 3 and 4). Sasaji (1978) reported that egg cannibalism of *P. japonica* was observed in open fields and hatchability ranged from 60 to 70%. Kawauchi (1985a) also reported that the percentage of egg cannibalism by larvae of *P. japonica* was about 15%. In our experiment, the hatchability ranged from 60.8 to 77.5% and egg cannibalism by larvae was frequently observed, especially for the large egg batches. The fertilities of the adults that fed on *E. kuehniella* eggs were fewer than those of the adults that fed on *A. pisum*, and the egg batches laid by the adults fed on *E. kuehniella* eggs were relatively smaller than those by the adults fed on *A. pisum*. These results suggest that the significant difference of hatchability was caused by egg cannibalism and

Table 3. Reproductive traits of *P. japonica* reared on *A. pisum* or *E. kuehniella* eggs

Adult diet	Pre-oviposition period (d)		Fertility[a]		Egg period (d)		Hatchability (%)[b]	
	Larval diet		Larval diet		Larval diet		Larval diet	
	A. pisum	*E. kuehniella* eggs	*A. pisum*	*E. kuehniella* eggs	*A. pisum*	*E. kuehniella* eggs	*A. pisum*	*E. kuehniella* eggs
A. pisum	3.9±1.4 (15)	3.7±1.5 (19)	184.4±106.8 (15)	210.9±95.9 (19)	2.23±0.42 (1,705)	2.25±0.44 (2,704)	60.8±9.2 (15)	63.7±20.0 (19)
E. kuehniella eggs	6.8±3.6 (13)	5.3±1.4 (15)	58.0±33.8 (13)	76.5±52.1 (15)	2.17±0.39 (570)	2.22±0.44 (783)	77.5±15.5 (13)	66.5±21.0 (15)

Mean±SD. Values in parentheses indicate the number of samples.
[a] Fertility indicates the total number of eggs laid per female for 20 d after emergence.
[b] Hatchability indicates the percentage of hatched larvae to the fertility.

Table 4. Results of two-way ANOVA on effects of adult diet and larval diet on the reproductive traits of *P. japonica*

Source	Pre-oviposition period (d)			Fertility			Egg period (d)			Hatchability (%)[a]		
	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Larval diet	1	2.236	0.140	1	1.160	0.286	1	5.508	<0.05	1	0.963	0.330
Adult diet	1	15.470	<0.001	1	37.881	<0.001	1	9.067	<0.05	1	5.914	<0.05
Larval diet ×adult diet	1	1.302	0.259	1	0.035	0.853	1	1.799	0.180	1	3.958	0.051

[a] The hatchability values were arcsine-transformed before analysis.

not by the difference in the adult diets.

Our results indicate that *E. kuehniella* eggs are suitable for larval and pupal development and available for the reproduction of *P. japonica*. Using these eggs as an alternative diet, it should be possible to rear successive generations. However, the number of eggs laid by the adults fed on *E. kuehniella* was smaller than that laid by the adults fed on *A. pisum*. So further research is needed to clarify more suitable alternative diets for the egg production and/or to select the fertile strain of *P. japonica* that can be reared on *E. kuehniella* eggs.

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