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EGG CANNIBALISM BY NEWLY HATCHED LARVAE OF THE SMALL WHITE BUTTERFLY, *PIERIS RAPAE CRUCIVORA* (PIERIDAE), ON AN ARTIFICIAL DIET

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ABSTRACT. Newly hatched larvae of the small white butterfly, *Pieris rapae crucivora*, wandered over an artificial diet without feeding for ca. 2 hrs after eating their own egg shells. When they encountered unhatched conspecific eggs, egg cannibalism occurred. Throughout the first instar, larvae fed on eggs and intermittently on the artificial diet. The duration of the first instar was significantly shorter for cannibals than for non-cannibals. As later first instars, the cannibals wandered randomly and only nibbled unhatched eggs. Egg cannibalism may help larvae exclude potential rivals from competing for nutrients when the host plant in the field is a limited resource. Because females lay eggs singly and seldom return to oviposit on the same host plant, siblicide in the field is presumably rare or absent.

Additional key words: artificial diet, devouring, larval duration, nibbling, starved larvae.

Females of the small white butterfly, *Pieris rapae crucivora* L., deposit their eggs on the exposed leaves of cruciferous plants. Parasitic wasps, bugs, mites and ants have been recognized as major agents of egg mortality for pierid butterflies on cabbage (e.g., Harcourt 1966, Parker 1970, Feltwell 1982) and on field cress, *Rorippa indica* Hiern (Yamaguchi & Watanabe 1993). Courtney (1986) pointed out that cannibalism is also a major cause of mortality in most Pierinae.

In general, the newly hatched larvae of pierid butterflies eat their own egg shell before eating their host plants. However, the earliest hatched larvae often devour unhatched eggs on the same leaf (Rausher 1979, Watanabe & Yamaguchi 1993). Brower (1961) stated that eating egg shells may simply represent opportunistic egg cannibalism. Since egg cannibalism has been observed in high egg density, the behavior of larvae cannibalizing eggs has been regarded as abnormal (e.g., Feltwell 1986, Warren 1992). However, cannibalism can strongly affect population density when resources are limited (e.g., Fox 1975), and show a density-dependent effect on population dynamics (Polis 1981, Elgar & Crespi 1992). The cannibalistic behavior of newly hatched larvae that occurs under crowded conditions in the absence of sufficient food has been reported in many species (e.g., Dempster 1983). When reared under crowded conditions, larvae of the orange tip, *Anthocaris cardamines* L., showed cannibalistic behavior (Feltwell, 1986).

Egg cannibalism by larvae has been observed under conditions of nutritional deprivation in the laboratory (Hayes 1982). Stenseth (1985) concluded that cannibalism may evolve as the result of individual selec-

tion even in cases where food resources are not in extreme shortage. However, Shapiro (1981) found that egg cannibalism frequently occurred for *P. protodice* Boisd., in which a mechanism of avoiding oviposition on the same leaf surface may have evolutionary implications. Watanabe & Yamaguchi (1993) found that intra- and inter-specific cannibalism among pierid butterflies involved eggs and newly hatched larvae on the same leaf in the field.

The present study was designed to provide insight into the mechanism of egg cannibalism by *P. rapae* under constant substrate conditions.

MATERIALS AND METHODS

Pieris rapae females were collected mainly in Nagano Prefecture, in the cool-temperate zone of Japan, during the summer of 1993. Mated females were obtained from the field and were allowed to deposit eggs on cabbage leaves. Eggs were laid during 2 h around noon on each sampling day.

About 24 h after oviposition, each egg was placed on a medium containing artificial diet (Sato 1974) in a petri dish kept at room temperature (ca. 25–30°C). Wet filter paper approximately 8 cm in diameter was placed on the floor of each dish to reduce desiccation. Some eggs were placed on the wet filter paper for subsequent comparison of larval behavior with that on the artificial diet. All of the eggs were in late developmental stages, as identified by their egg color (yellowish orange). A detailed description of the developmental stages of eggs is given by Watanabe et al. (1993).

Eggs offered to newly hatched larvae were derived from females placed on leaves on subsequent days. None of these eggs hatched earlier than the hatched larvae. Every egg was placed vertically on the food medium or filter paper like a naturally deposited egg. The arrangement of these eggs on the artificial diet in a petri dish is shown in Fig. 1. The number of eggs offered as food was 36 for each egg cannibalizing experiment. Twenty hatched larvae were tested. Since Watanabe & Yamaguchi (1993) found that the average distance between eggs deposited on a leaf of field cress, *R. indica*, was ca. 8 mm in the field, in this experiment all the eggs were placed 8 mm apart from each other.

The position of each cannibalized egg was determined by counting the number of 'steps' from the previously cannibalized egg. A step represents the space between eggs i.e., the distance measured in number of 8 mm units, because we did not know the actual distance of the route of the larva during 10 min observation intervals. For the first egg cannibalized, the location was the number of steps from the original point where the larva hatched.

A stereoscopic microscope was used to observe each larva every 10

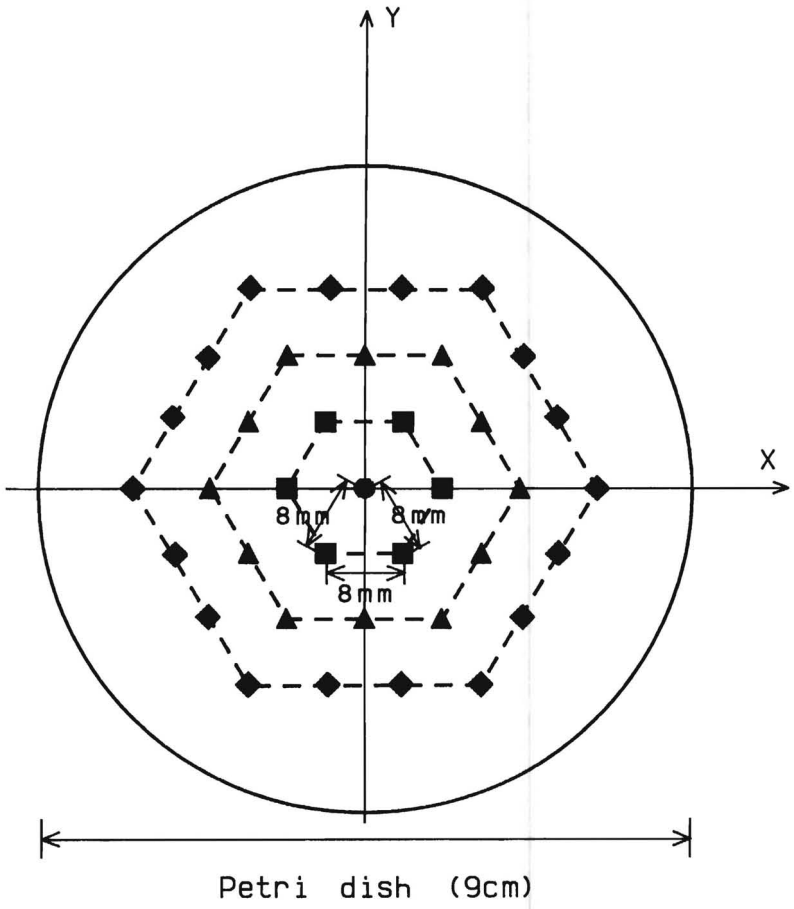

FIG. 1. Arrangement of eggs placed on artificial diet in 9 cm diam. petri dishes. Filled circle indicates an egg that will hatch first and become the cannibal. Squares, triangles and diamonds represent eggs 1, 2 and 3 steps away from the cannibal, respectively.

min, from hatching to the first molting. Records were made of larvae feeding on their own egg shells, on the artificial diet, and on other eggs. Time spent moving between eggs and resting was also recorded. Larval position was recorded every 10 min, in relation to the position of the egg from which the larva hatched.

RESULTS

Immediately after hatching, all 20 larvae began to eat their own egg shells. This normally took about 90 min (Table 1). The larvae then moved out onto the artificial diet.

TABLE 1. Time elapsed for each feeding behavior from the time of hatching, for cannibal and non-cannibal larvae of *Pieris rapae*. Values are minutes ± SE, and are based on 21 cannibal and 5 non-cannibal larvae. Asterisks denote significance at $p < 0.05$ for cannibals vs non-cannibals.

Feeding behavior	Cannibals	Non-cannibals
end of feeding on own egg shell	97 ± 11.1	88 ± 18.5
start of feeding on first egg	179 ± 20.3	—
start of feeding on artificial diet*	387 ± 56.7	174 ± 17.8
duration of first instar*	3701 ± 179.8	4863 ± 133.8

Larvae that were not presented with eggs (i.e., solitary larvae) began to feed on the artificial diet about three hours after hatching. The yellow-brown mid-gut became greenish due to the color of the artificial diet. The larvae then wandered about on the diet, feeding intermittently, during the first instar stage. The duration of the first instar was about 82 h (=3.4 days).

Newly hatched larvae that were presented with eggs began to eat the first egg three hours after hatching. This was not significantly different from the starting time of feeding on artificial diet by solitary larvae (=non-cannibals). The mid-gut remained yellowish brown for some time because they had not yet fed on the artificial diet. There was a delay before the start of feeding on the artificial diet. The mid-gut of cannibal larvae did not become greenish until 6.5 h after hatching. This was significantly longer than the onset of greenish color in solitary larvae ($P < 0.05$ by F-test). Cannibal larvae wandered about with less feeding than solitary larvae. The duration of the first instar of the cannibals was significantly shorter ($P < 0.05$ by F-test).

The newly hatched larvae fed on neighboring eggs (= 1 step) and then fed on eggs 2 steps away. As shown in Fig. 2, one of 20 larvae fed on 2 eggs during the first instar stage, while another fed on 19 eggs (larvae fed on 8.3 ± 4.7 eggs (SE) on average). Because the number of eggs offered as food was stable and they were evenly spaced, it can be seen from the data that, within the limitations of our method, cannibalism may not be a mortality factor in relation to density of unhatched eggs.

Egg cannibalism first occurred 179 ± 20 min after hatching (n = 14). All of the larvae ate eggs within one step. The second egg was eaten by the larvae 421 ± 80 min after hatching (n = 15), and thereafter the third 532 ± 89 min (n = 13), the fourth 773 ± 112 min (n = 13), and the fifth 1108 ± 204 min (n = 13). The time elapsed between each cannibalism event was thus 100–300 min. The tenth egg cannibalism was observed 1618 ± 95 min after hatching (n = 4). Larvae that were more than one day old displayed short intervals between cannibalism events. They moved out one step, and attacked adjacent eggs. The most active larva

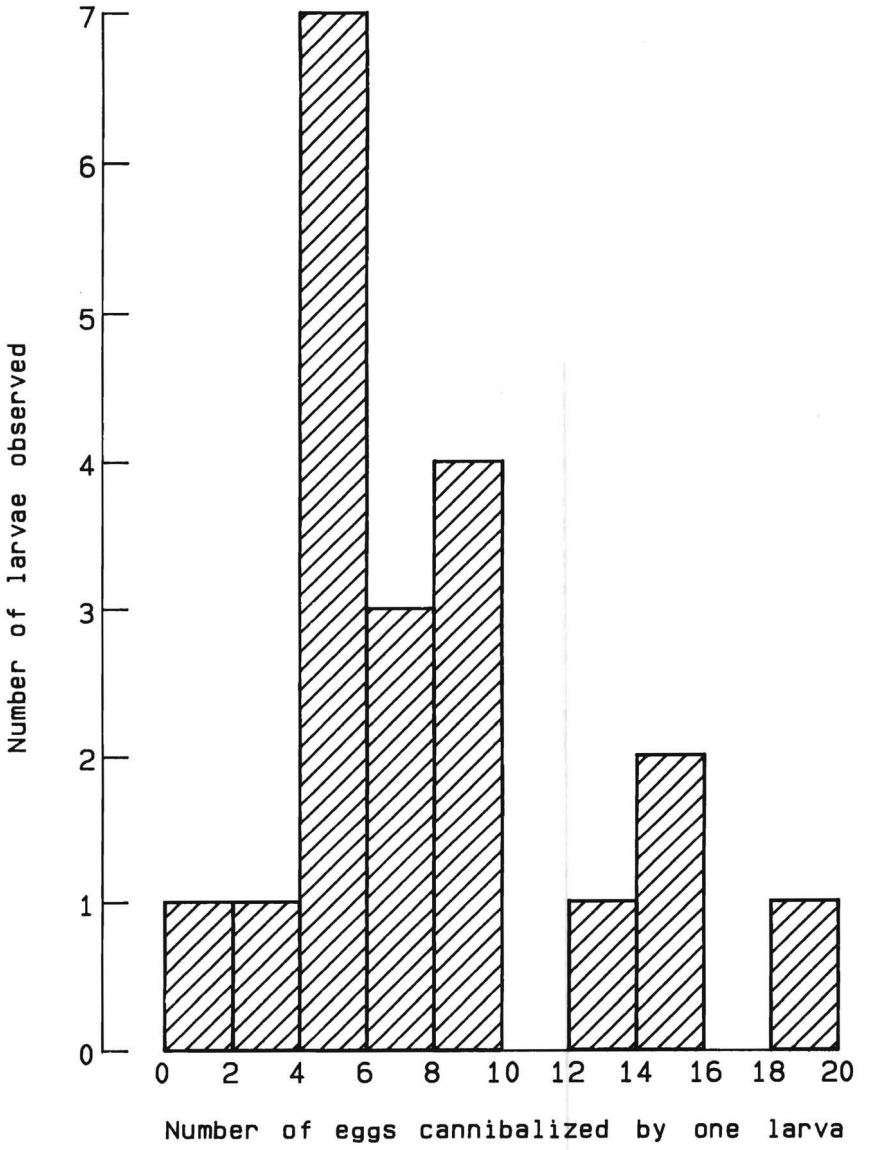

FIG. 2. Frequency distribution of the number of eggs cannibalized.

ate 19 eggs in 3520 min after hatching. The results show that larvae can and do eat conspecific eggs throughout the first instar stage.

After eating their own egg shells, movement by solitary larvae increased to 13 mm/min (Fig. 3). Slower movement reflected the time

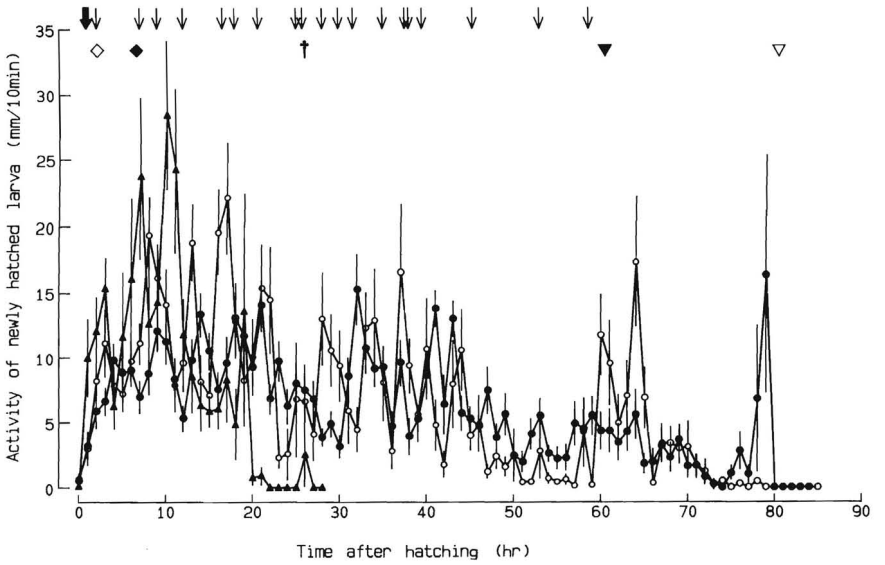

FIG. 3. Changes in movement by first instar larvae on artificial diet (±SD). Dots, open circles and small triangles represent cannibals, non-cannibals and starved larvae, respectively. Thick arrow shows average time required for larvae to eat their own egg shells. Each thin arrow indicates the average of each starting time for cannibals. Closed and open diamonds show the average starting times for feeding on artificial diet by cannibals and non-cannibals, respectively. Closed and open triangles indicate the average molting time to the second instar for cannibals and non-cannibals, respectively. Cross shows average time of death of starved larvae.

taken for feeding on the diet and intermittent resting. Movement by larvae over 45 h old decreased to 5 mm/min. The older larvae often rested, and then molted into the second instar. The speed of movements of cannibal larvae was generally similar to that of solitary larvae, but there were several high peaks of movement by cannibal larvae (Fig. 3). On average, the cannibal larvae were more active than solitary larvae.

Larvae hatched on the wet filter paper began to wander in the petri dish after eating their own egg shells. Their movement was the fastest. They were most active until 12 h after hatching. Their speed decreased gradually thereafter, and they became inactive after about 20 h. The average longevity of the starved larvae was about 26 h (=1.1 days).

The distance moved by larvae increased with time after hatching. As shown in Fig. 4, the change in the cumulative distance differed for cannibal and solitary larvae (Kolomogorov-Smirnov test, $0.05 > P > 0.01$). The cannibals moved further than the non-cannibals during the first 20 h after hatching. However, both moved about 3000 mm during the first instar. A rapid increase in distance moved by the starved larvae was ob-

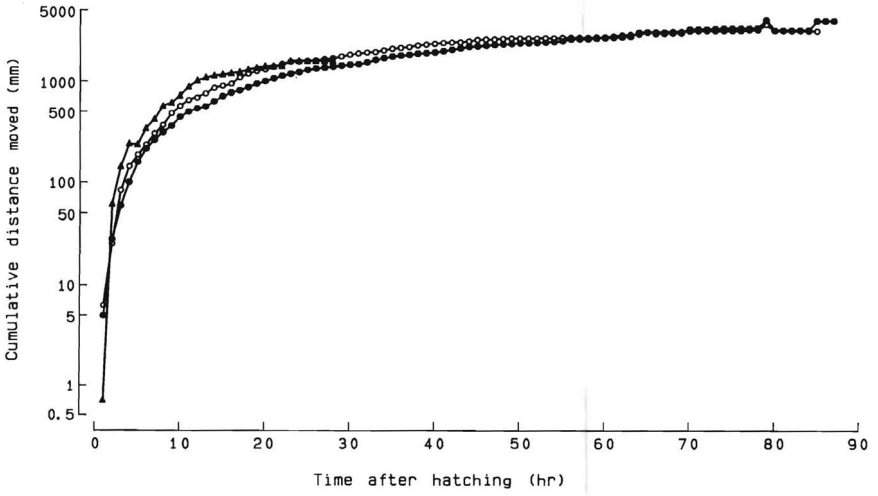

FIG. 4. Changes in the accumulated distance moved for first instar larvae on artificial diet. Dots, open circles and small triangles represent cannibals, non-cannibals and starved larvae, respectively.

served on the wet filter paper. The cumulative distance moved differed significantly (Kolomogorov-Smirnov test) from the distances for cannibal larvae ($P < 0.01$) and the larvae on artificial diet ($P < 0.01$). The starved larvae moved 1000 mm during their life span.

The duration of feeding on a single egg also varied. It took a cannibal larva more than 20 min to consume an entire egg. Some larvae devoured the entire egg with intermittent resting, which increased the time required for complete consumption. As shown in Fig. 5, there were a few larvae that finished eating the first egg within 20 min, but most spent much more than 20 min to consume the egg. Other larvae only nibbled parts of a victim egg shell before beginning to wander. Partial nibbling on an egg usually lasted less than 15 min. Fig. 5 also shows that most cannibal larvae did not spend much time to contact more than 10 eggs. There was a significant tendency for time spent cannibalizing to decrease with the number of unhatched eggs encountered. Therefore, most cannibal larvae tended to devour entire eggs at the onset of feeding, and then nibble eggs later. All eggs that were nibbled did not develop further and did not produce larvae.

Between the first to the fifth eggs cannibalized, larvae usually devoured or nibbled an egg that was nearest (Fig. 6). The large average step number means that larvae moved long distances while feeding on eggs, and apparently sometimes ignored neighboring eggs while moving out randomly on the artificial diet.

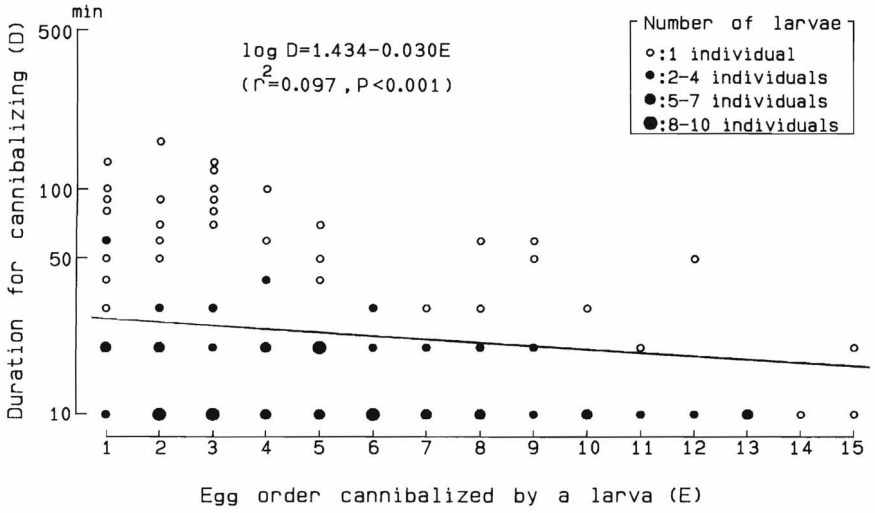

FIG. 5. The relationship between the egg order cannibalized and the duration of cannibalizing.

DISCUSSION

The present experiments show that larvae of *P. rapae crucivora* have a high propensity for egg cannibalism under laboratory conditions throughout the first instar. The newly hatched larvae wandered over the artificial diet without feeding, or on wet filter paper, for two hours after eating their own egg shells (newly hatched larvae also tend to wander actively on host plant leaves in the field i.e., they seem to search for eggs on the leaves similar to their behavior on the artificial medium in this experiment; Watanabe, unpubl. data). While wandering on artificial medium, larvae attack eggs that are encountered. If the larvae cannot find eggs, they begin to feed on the artificial diet. Watanabe and Yamaguchi (1993) found that larvae on leaves with conspecific eggs behaved as cannibals before starting to eat leaves in the field.

Larvae did not feed on the artificial food medium for two hours after hatching, but wandered. They may waste energy during this period. However, starved larvae were able to wander actively on wet filter paper for 12 h. Therefore, newly hatched larvae may not be adversely affected by failing to feed during their first two hours, as this time may be for locating eggs in the field.

Two kinds of egg cannibalism were seen: consumption of the entire egg, and nibbling of part of the egg shell. Both kinds of cannibalism were fatal to unhatched larvae. The former presumably provides the cannibals with nutrients, whereas nibbling may exclude unhatched con-

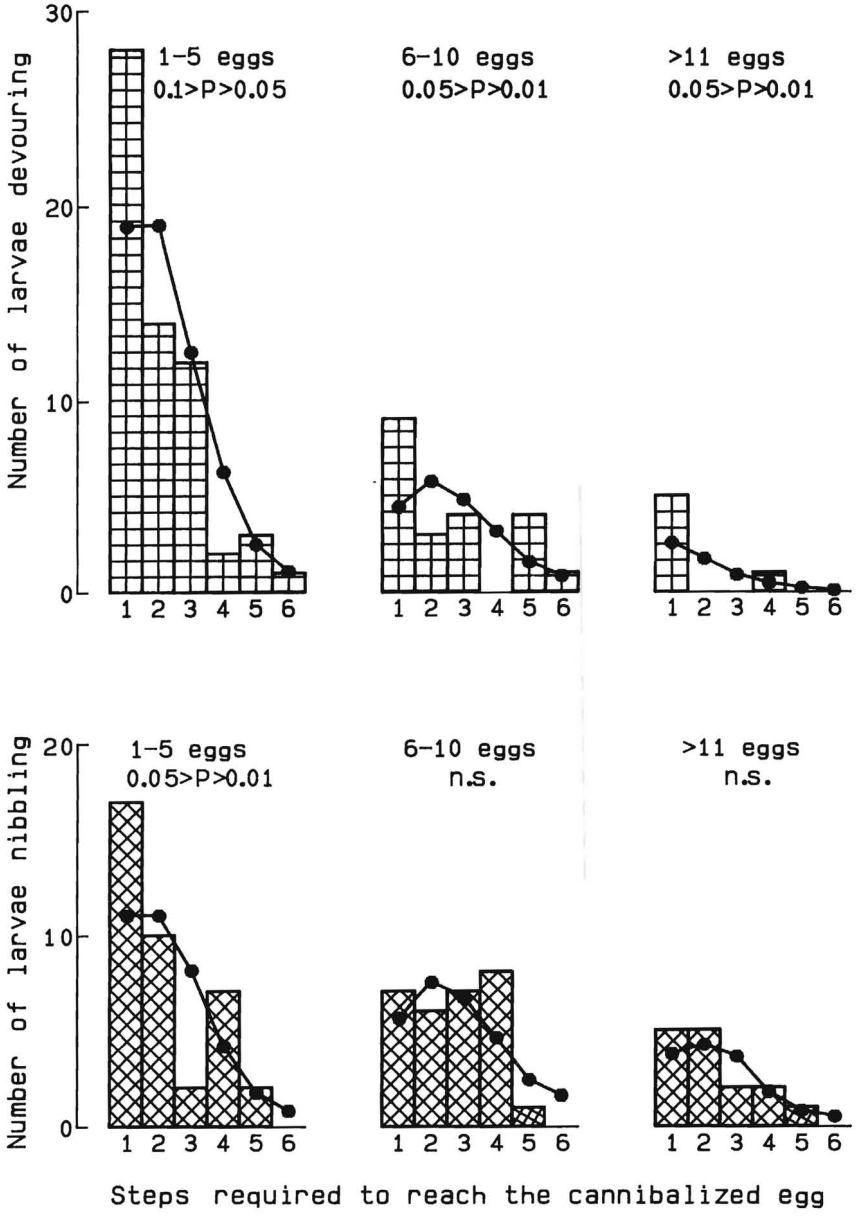

FIG. 6. Frequency distributions of the step-number of eggs cannibalized. 1–5, 6–10, >11 eggs mean the order of eggs cannibalized. Dots show the Poisson distribution. See text for further elaboration.

specifics that would otherwise be competing for food. Therefore, possible advantages for the cannibal include both nutrient gain and the elimination of competitors (Baur & Baur 1986).

Eggs of *P. rapae crucivora* contain amino acids and organic compounds that are not directly derived from leaves (Porter 1992). Such materials might facilitate larval development during the first instar. Cannibalism increases the growth rate of larvae that eat eggs (e.g., Dickinson 1992, Agarwala & Dixson 1992). Osawa (1992) stated that first instar larvae of the lady beetle, *Harmonia axyridis* Pallas, developed faster after eating conspecific eggs. In *P. rapae crucivora*, however, there were no significant differences between cannibalizing and non-cannibalizing larvae over the whole larval period with regard to adult size and weight, or female fecundity (Watanabe, unpubl. data). We observed no egg cannibalism by second, third, fourth and fifth instar larvae, despite Yamamoto's (1981) finding that the eggs of *P. rapae crucivora* and *P. napi* L. were eaten by older larvae.

Courtney & Courtney (1982) stated that cannibalism is concentrated upon particular host individuals, because of contagious egg distributions. Brower (1961) stated that egg cannibalism is density-dependent in the case of the Queen butterfly, *Danaus gilippus*. Polis (1981) noted that cannibalism can also be a tactic to gain exclusive use of resources that serve as both food and habitat. For larvae, there are advantages in being single: more food, and less chance that other members of the same family group will become parasitised or eaten at the same time (Feltwell 1986). The proportion of eggs surviving was a function of larval density in an Australian population of *P. rapae* (Jones & Ives 1979). Watanabe & Yamaguchi (1993) observed that, as a rule, a single larva settled on a single leaf of the field cress, *R. indica*, suggesting that newly hatched larvae may have consumed unhatched eggs on the same leaf.

Egg cannibalism in *P. rapae crucivora* may be advantageous for larval survival on limited resources as well as for the intake of nutrients. While devoured eggs were usually those nearest to the larva, nibbled eggs (>6th eggs) were distributed randomly. The first instar larvae seemed to wander not to take eggs for nutrients, but to kill potential conspecific competitors. Cannibalism helped cannibals when food density was low (e.g., Osawa 1992), because the relatively small host *R. indica* is heavily damaged by a single larva over its complete life cycle (Yano 1993).

Most eggs of *P. rapae* are deposited on the under surface of leaves (Yamamoto 1985 observed that 97% of females deposited eggs on the undersides of leaves of the field cress, *R. indica*). This may induce oviposition by more than one female on the same leaf. In fact, Watanabe and Yamaguchi (1993) counted 781 field cress leaves that had eggs, and found that 25% of them received more than one egg. This presumably

means eggs from more than one female. However, no evidence was obtained for segregation of females on different host plants.

Ohtani & Yamamoto (1985) found that females of *P. rapae* have no site fidelity, emigrating from their emergence site. Since the females lay eggs singly and seldom return to deposit on the same host plant (Watanabe & Yamaguchi, unpubl. data), eggs on the same leaf may be deposited by two or more females. Porter (1992) stated that single-egg-laying females distribute eggs over a large number of host plants, and that this spreads the risks of predation and cannibalism, and reduces competition with other larvae. Rothschild & Schoonhoven (1977) concluded that *P. rapae* discriminated between a cabbage leaf from which conspecific eggs had been removed and a clean control leaf. Few accounts of butterfly cannibalism have considered kin relatedness (e.g., Courtney 1986), though Jones (1982) reviewed cannibalism in relation to kin selection.

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