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Hatching Synchrony and Cannibalism in the Leaf Beetle, *Gastrophysa atrocyanea* (Motschulsky) (Coleoptera, Chrysomelidae)

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ABSTRACT

Many insects lay their eggs in masses, and larvae generally hatch from each mass more or less synchronously. The present study investigated the mechanisms behind hatching synchrony and the relationship between hatching synchrony and cannibalism in the leaf beetle, *Gastrophysa atrocyanea*. Eggs kept singly hatched earlier than those kept in groups of four. However, the hatching intervals for groups of four eggs were similar, regardless of whether they were kept singly, in pairs, or in groups. This result suggests the absence of interactions among eggs that affect hatching synchrony. In contrast, the hatching intervals from the first to the last egg increased as group size grew, with an estimated hatching interval of 3.6 hours for an average egg mass of 34 eggs at 25°C. No significant difference in survival rate within 12 hours of hatching was observed between eggs kept singly and those kept in masses. Oophagy (egg cannibalism) by early-hatched larvae was rare when eggs laid on the same day, or one day apart, were placed together on a small leaf disc. Significant egg mortality was detected only when eggs produced 2 or more days apart were placed together, though this was largely mitigated on larger leaves. From these results, it was concluded that cannibalism within the same egg mass is not a driving factor in the evolution of hatching synchrony.

Key words: cannibalism, egg mass, hatching synchrony

Introduction

The larval stage immediately after hatching is one of the most vulnerable stages of an insect's life. Newly hatched larvae are exposed to various physical and biological threats, including extreme temperatures, desiccation, heavy rain, predators, pathogens, starvation, etc. In response to these threats, insects have developed mechanisms to control the hatching time to ensure that it occurs at the 'right' time and that larvae hatch simultaneously in groups.

Hatching time may be influenced by at least three factors. 1) Seasonal adjustment of

hatching time: This may be regulated by embryonic diapause. For example, embryonic diapause is induced by photoperiod and temperature in the migratory locust *Locusta migratoria* (Verdier, 1972; Tanaka, 1994a; Tanaka, 1994b). Females grown under long photoperiods produce non-diapause eggs that hatch within a few weeks, while those grown under short photoperiods produce diapause eggs, which require several months to hatch, typically after overwintering. 2) Daily adjustment of hatching time: In some species, this is controlled by daily light and temperature cycles (Saunders *et al*., 2002). Under daily light/dark or high/low temperature cycles, the desert locust *Schistocerca gregaria* hatches during the dark period or the low-temperature period (Pagham, 1981; Nishide *et al*., 2015a, b), whereas *L. migratoria* and the lubber grasshopper, *Romalea microptera,* hatch during the light period or the high-temperature period (Smith *et al*., 2013; Nishide *et al*. 2015a). In the field, egg hatching is observed between 09:00 and 16:00 in *L. migratoria*, although hatching from each egg pod occurs within a short time frame (Chen, 1999; Nishide *et al*., 2017a). 3) Synchronous hatching in an egg mass: This is facilitated by parental intervention and interactions among the eggs. Synchronous hatching can be triggered by water splashing or vibrations generated by the parent in subsocial insects (Ohba, 2002; Mukai *et al*., 2012). In non-social insects, such as grasshoppers, embryo-to-embryo communication may help coordinate synchronous hatching within the egg mass (Tanaka, 2017, 2021a,b). In these species, the eggs within the mass are typically in contact with one another, allowing vibrational signals to be transmitted from one egg to another.

The dock leaf beetle *Gastrophysa atrocyanea* lays its eggs in masses on the underside of leaves of dock plants such as *Rumex japonicus* and *Rumex obtusifolius* (Lee, 2022; Tanaka, 2024). On average, each egg mass contains 33.4 eggs in central Japan (Tanaka 2023a). The eggs are typically laid in a single layer, adhering to one another and the leaf surface with a sticky substance. The egg stage in this species is relatively short, lasting 6 days at 20 °C (Miyazaki and Naito, 1981), leading to near-simultaneous hatching within each egg mass. Egg mortality is high, ranging from 65% to 81% under field conditions, primarily due to physical factors such as leaf friction and low humidity. However, cannibalism (oophagy) by larvae and adults is also observed under food shortage conditions (Miyazaki and Naito, 1981).

In another dock leaf beetle, *G. viridula*, similar characteristics are observed. Kutcherov (2015) examined whether the synchrony of hatching is merely a by-product of cannibalism or whether an additional synchronizing factor exists in this beetle. He observed that eggs individually isolated in different dishes hatched less synchronously than those kept in cohesive groups, though no significant difference was found in the mean hatching time between the two treatments. Cannibalistic behavior was observed as early as the 4th hour after hatching; however, the median and modal time of the first cannibalistic attacks was the 7th hour, which largely coincided with the complete emergence of all larvae from the egg mass. Furthermore, egg mortality was similar between the two treatments. Kutcherov (2015) suggested that cannibalism alone cannot explain synchronization simply because it occurs after the hatching process has already been synchronized and suggested the existence of a synchronizing factor.

In the present study, I examined the effect of egg group size on hatching time and hatching intervals in *G. atrocyanea* under laboratory conditions. I also investigated how differences in hatching times affected the survival rates of eggs and larvae when exposed to small and large quantities of food. Additionally, I tested the rates of oophagy by larvae at different instar stages, both in the absence and presence of food. The present paper describes the results of these experiments and discusses the significance of hatching synchrony and cannibalism in this beetle.

Materials and Methods

Insects

Gastrophysa atrocyanea adults and eggs were collected from leaves of *Rumex japonicus* and *Rumex obtusifolius* along two unpaved roads (each measuring 4 x 80 m) and adjacent grassland along Hasunuma river in Tsukuba, Ibaraki, Japan (36.1°N, 140.1°E) during March

and April 2023 and 2024. The two dock plant species coexisted in the area and were not distinguished in this study. The average daily air temperature during March and April was 9.7°C (range: 2.7-17.1°C) and 15.6°C (range: 9.5-20.9°C), respectively (Japan Meteorological Agency 2024).

To obtain eggs for experiments, groups of 10 female and 10 male adults collected in March were held in plastic containers with dock plant leaves and incubated at 25±1°C and LD 12:12 h (light on at 08:00 and light off at 20:00) in an incubator (CN-40A; Mitsubishi Electric Engineering Co, Tokyo, Japan) according to the method previously described (Tanaka, 2024). The temperature was monitored hourly with thermorecorders (Ondotori TR-74Ui, T and D Co., Tokyo, Japan). Leaves with eggs were removed from the containers shortly before and after the dark period each day, and fresh leaves were supplied.

Effects of group size on hatching time and interval

It is difficult to accurately count the number of larvae hatching from an egg mass of *G. atrocyanea* without disturbing them at regular intervals, as they usually remain aggregated and often overlap. Therefore, hatching activity was observed for small egg groups consisting of 1, 2, and 4 eggs. In other insects, including grasshoppers and bugs, significant effects of such small group sizes have been observed on hatching activity, such as hatching time and hatching intervals (Tanaka, 2017, 2021a; Tanaka and Kotaki, 2020). In 2023, each of 7 egg masses of *G. atrocyanea* (666 eggs) obtained within 12 h at 25±1°C were divided into batches of 4 eggs, and the eggs of each batch were placed on dock plant leaf discs (diameter, 9.8 mm) either singly or in a group of 2 or 4 individuals. In the latter, eggs were kept in contact with one another. When mortality occurred, the data for those triplets were discarded. The eggs on leaf discs were placed on moist sand held in the cells of a 24-well plastic plate (Costar Co., 24-well cell culture cluster) and incubated at 25±1°C under continuous illumination. In 2024, eggs from 11 egg masses were similarly handled, but the experiments were performed at 20±1°C because *G. atrocyanea* eggs are usually exposed to low temperatures in spring. To record the number of hatchlings, a digital camera installed in an incubator was used to photograph the plates containing eggs every 15 min at 25±1°C or 30 min at 20±1°C until no more hatchlings appeared, and the hatched larvae in the photographs were counted later on a computer.

The hatching time was compared among treated eggs derived from the same egg mass, with the first hatching in each egg mass designated as 1 h. Based on these hatching times, the hatching intervals from the first to the last hatching for 4 eggs kept in different group sizes—1, 2, or 4- were measured to determine whether the egg condition affected the hatching time and interval. Additionally, the hatching interval for eggs kept in pairs was compared with those kept in groups of four. Because hatching activity was recorded at 15- or 30-min intervals, the minimum hatching interval was 15 min at 25°C and 30 min at 20°C.

Gastrophysa atrocyanea eggs in an egg mass are glued to each other and the leaf by a sticky substance. In the present experiments, the eggs were individually removed from the substrate (leaf) and placed on another substrate. This was done by gently touching the eggs with the tip of tweezers, as the sticky eggs easily adhere to them. However, the effect of handling on hatching activity was tested by comparing handled groups of 2 or 4 eggs with intact groups of 4 eggs at 20±1°C. The latter were kept intact by removing the surrounding eggs and cutting the substrate (leaf) to approximately 5 x 5 mm. Eggs from 12 egg masses were divided into 3 batches and assigned to the following treatments: 1) 2 eggs kept together on a leaf disc (diameter 9.8 mm), 2) 4 eggs kept together on a leaf disc, and 3) 4 intact eggs on a leaf piece. These eggs on leaves were held in the cells of 24-well plates as described above and incubated at 20±1°C. The hatching time and hatching intervals were determined as described above.

To determine the relationship between group size and hatching interval, egg masses consisting of 11 to 45 eggs were collected in late March 2023, and each egg mass, along with the attached 2 x 2 cm leaf, was placed on moist tissue paper in 6-cm Petri dishes. They were incubated at 25±1°C under continuous illumination and photographed at 15-min intervals as described above. As mentioned, it was difficult to

accurately determine the hatching times for more than 4 eggs in this study. However, focusing on the white body color at hatching in the photographs made it easy to identify the first and last hatching times for each egg mass. The data for groups of 2 and 4 eggs were derived from the mean values of the previous experiment.

Hatching asynchrony and cannibalism

To determine how hatching asynchrony would affect egg and larval survival, two groups of 10 eggs laid at different times (with hatching time differences ranging from 0 to 3 days at 25±1°C and from 0 to 5 days at 20±1°C) were kept together on leaf discs (diameter: 9.8 mm) in 3-cm Petri dishes. The egg period lasted 4 days at 25°C and 6 days at 20°C. After these periods, mortality was considered due to cannibalism among larvae. The eggs and surviving larvae were counted daily for 3 days after the start of hatching in the early group. After 3 days, the leaf was mostly consumed, and some larvae died, apparently due to starvation. Individuals in the early and late groups were identified based on differences in body size, except in treatments where hatching occurred within 1 or 2 days, as these eggs were laid on the same day (control) or consecutive days. In the latter case, the total number of individuals (initial N= 20) was recorded, and the average value was used to calculate the survival rate for each group. Egg mortality in the late groups was determined by counting the eggs and newly hatched larvae during the 4-day (20°C) and 6-day (25°C) egg periods, assuming no cannibalism occurred among the larvae before the expected date of the last egg hatching.

To determine whether the amount of food (leaf size) would affect egg and larval survival, two groups of 10 eggs with deposition time differences ranging from 2 to 4 days were placed on large leaf discs (diameter, 3 cm) in 3-cm Petri dishes. The eggs and surviving larvae were counted daily for 3 days after the start of hatching at 20±1°C, as described above.

Egg survival rate

Survival rates of eggs in different group sizes, ranging from 1 to 42, were determined. Eggs from each of 8 egg masses deposited within 12 h were divided into three groups of 10-16 eggs and kept singly, in pairs, and groups of 4 eggs, respectively, on leaf discs (diameter, 9.8 mm) placed on moist sand in wells of 24-well plates at 25±1°C, as described above. They were photographed at 15-min intervals, and the number of hatched eggs was later determined based on the photographs. The egg masses containing 23-42 eggs on leaf pieces were placed on moist sand in 6-cm Petri dishes, and the number of hatchlings was recorded on day 4. Egg hatching was checked at 08:00 and 20:00.

Cannibalism by other stages

Five larvae at the second or third instar were kept with 30 newly laid eggs on moist tissue paper in 6-cm Petri dishes at 20±1°C. The eggs were counted the following day. Likewise, adult males were individually kept with 10 eggs on moist tissue paper in 3-cm Petri dishes, and the eggs were counted the following day.

To determine whether larvae consume eggs in the presence of leaves, 5 one-day-old third-instar larvae and 10 newly laid eggs were placed on leaf discs (3 cm in diameter) in 3-cm Petri dishes at 20±1°C. The number of eggs was counted every 12 h until all were eaten. Simultaneously, the leaf area consumed was visually estimated at 10% intervals. The experiment was replicated 10 times.

Statistical analyses

Hatching times of eggs across treatments were analyzed using Tukey's multiple comparison test following a one-way ANOVA. Hatching intervals were analyzed using the Steel-Dwass test after performing a Kruskal-Wallis test. Pearson's correlation coefficient was used to examine the relationship between hatching intervals and group sizes and between the leaf area consumed and the number of eggs. Survival rates of eggs and larvae across different treatments were analyzed using the Kruskal-Wallis or Mann-Whitney tests, with comparisons to the control made using Steel's test. All statistical analyses were conducted using the services available at http://www.gen-info.osaka-u.ac.jp/MEPHAS/kaiseki.html, Excel (Microsoft Office 365), or StatView (SAS Institute Inc., NC, USA). Differences were considered significant when $P < 0.05$.

Results

Effects of group size on hatching time and interval

The relative time of hatching with the first hatching designated as 1 h for eggs from the same egg mass was significantly influenced by group size either at 20°C (Fig. 1A) or 25°C (Fig. 1B) (ANOVA, $P< 0.05$). The mean value was not significantly different between group sizes 1 and 2 (Tukey's multiple comparison test, $P> 0.05$), but a significant difference was observed between groups 1 and 4 at either temperature ($P< 0.05$, Fig. 1A, B).

The hatching interval of 4 eggs, whether kept singly, in pairs, or groups of 4, was similar across the 3 treatments at both 20°C and 25°C (Fig. 2). No significant difference was observed between the treatments (Kruskal-Wallis test, $P= 0.25$ and 0.89 at 20°C and 25°C, respectively), indicating that the hatching interval was not influenced by whether the eggs were kept singly or in groups.

A similar experiment examined whether handling affected hatching time and interval. Figure 3A compares handled eggs' relative hatching time with intact eggs. One-way ANOVA indicated no significant difference among the three treatments, including groups of 2 and 4 handled eggs and 4 intact eggs ($P= 0.81$), which is consistent with the results shown in Fig. 1A. A similar outcome was observed for the hatching interval of 4 eggs (Fig. 3B, Kruskal-Wallis test, $P= 0.32$).

To further confirm the above result, 10 eggs from an egg mass were individually placed approximately 5 mm apart on a 3-cm leaf disc in a 3-cm Petri dish, and their hatching interval from the first to the last hatching time was compared with that of intact 10 eggs from the same egg mass. The results showed no significant difference between the 2 treatments (Fig. 3C, $N= 20$ each, Mann-Whitney test, $P= 0.50$).

In contrast, the hatching interval of 2 eggs in the above experiment was significantly smaller than that of 4 eggs at both 20°C (Fig. 4A, Mann-Whitney test, $P< 0.001$) and 25°C (Fig. 4B, Mann-Whitney test, $P< 0.001$). Figure 4C illustrates the relationship between group size and hatching intervals. A significant positive correlation was observed between the 2 variables ($r= 0.68$, $N= 18$, $P< 0.01$), suggesting that the hatching interval increased as the group size increased.

Hatching synchrony and cannibalism

When two groups of 10 eggs derived from different egg masses but laid on the same day (control) were placed on leaf discs and observed at 25°C, most eggs (91.5%, $N= 200$) hatched 4 days after deposition. In this experiment, the day when the first hatchlings appeared was designated as day 1. By day 2, half of the leaf discs, except for the veins, had been consumed. On day 1, a few control eggs (12%) remained unhatched, and the rest (0.5%) were missing, likely consumed by larvae. The average survival rate, including both larvae and eggs on day 1 ($N= 10$, each consisting of 10 eggs), was 97.5% (Fig. 5A). On day 2, only larvae were found, with a survival rate of 88.0%. By day 3, the survival rate had declined to 78%, with a significant difference in average survival rate detected across the 3 days (Kruskal-Wallis test, $P< 0.001$). Similar results were observed over 3 days for the two groups of eggs combined (initial $N= 20$) with 1-, 2-, and 3-day differences in deposition day (Kruskal-Wallis test, $P< 0.01$, 0.001, and 0.0001, respectively). Steel's test indicated that the survival rate on day 1 showed no significant difference in any treatment compared to the control, but the treatments with 2- and 3-day differences in deposition day were significantly different from the control on days 2 and 3 (Fig. 5A, $P< 0.05$).

Figure 5B illustrates the average survival rates of the early and late groups in each treatment separately on day 3, when some larvae began molting to the second instar. Because the two groups were not distinguished in the control and the treatment with a 1-day difference in deposition time, the average values are shown, which were 78.0% and 79.5%, respectively. Steel's test indicated that, compared with the control, the survival rate of the early group was significantly higher in the treatment with a 3-day difference in deposition day (Fig. 5B, $P< 0.05$). This suggests that the survival rate in the early group increased when kept with eggs laid 3 days later, likely due to oophagy. In contrast, the survival rate in the late

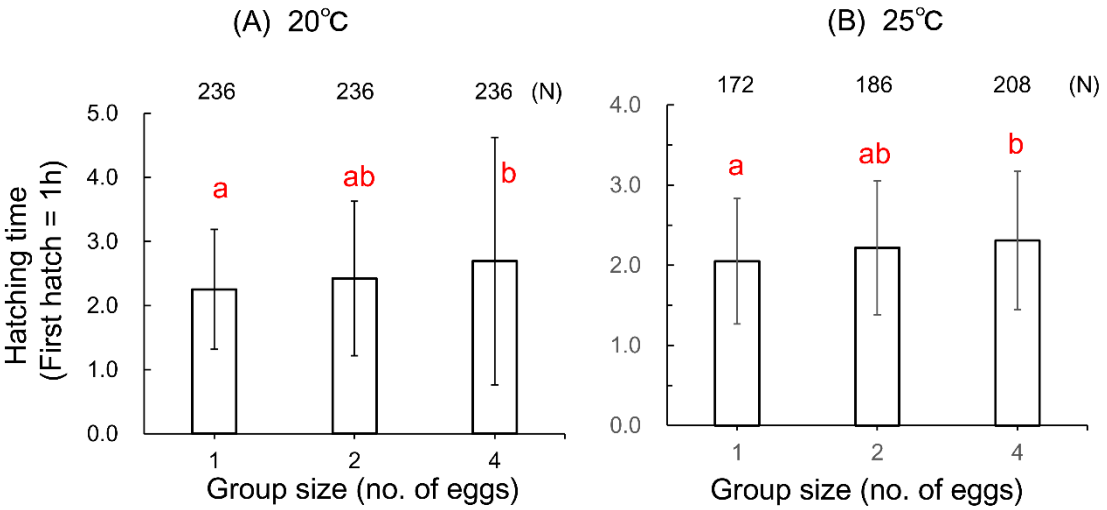

Fig. 1. Mean hatching time (± SD, h) of *G. atrocyanea* eggs that were kept singly, in pairs, and in groups of 4 at 20°C (A) and 25°C (B). The hatching time was calculated by designating the time of the first egg hatching in each egg mass as 1h. Different letters in in (A) and (B) represent significant differences in mean values, according to the Tukey's multiple comparison test at P< 0.05.

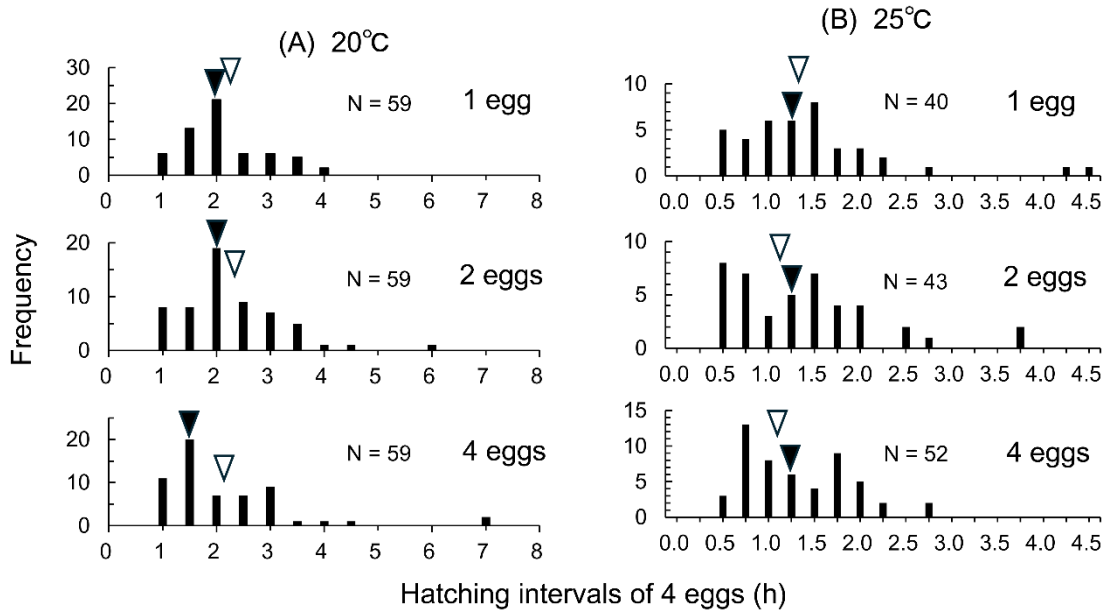

Fig. 2. Frequency distribution of hatching intervals of four *G. atrocyanea* eggs that were kept singly, in pairs, and in groups of four at 20°C (A) and 25°C (B). ▽ represents the mean, and ▼ represents the median. The Kruskal-Wallis test indicated no significant difference among the three treatments at both temperatures (P> 0.05).

group significantly decreased in the treatments with 2- and 3-day differences in deposition day compared with the control (Fig. 5B, P< 0.05), probably due to oophagy by the early group. Egg mortality in the late group was low when kept with the early group produced on the same day or 1 day earlier, but it increased rapidly when the early group was produced 2 or 3 days earlier (Fig. 5C, Steel's test, P< 0.05).

A similar experiment yielding similar results was conducted at 20°C (Fig. 6). A significant difference was observed in the survival rate of the early and late groups combined (initial N= 20 eggs) over 3 days in all treatments with a 0- to 5-day difference in deposition time (Fig. 6A, Kruskal-Wallis test, P<

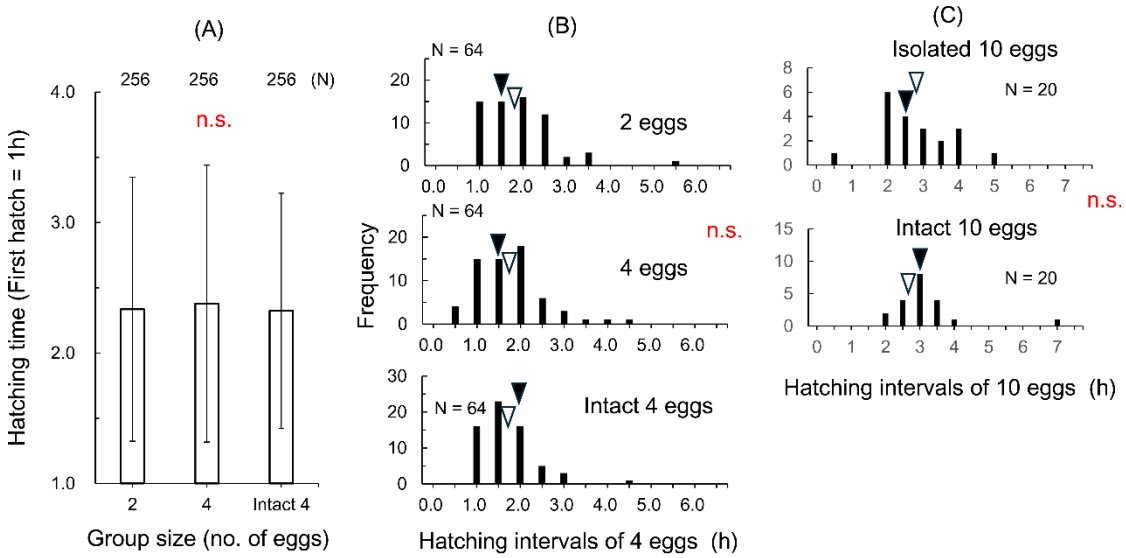

Fig. 3. Effect of handling on hatching time and hatching intervals in *G. atrocyanea* at 20°C. Comparison of mean hatching time (± SD, h) among handled eggs kept in pairs or in groups of four and intact eggs kept in groups of four (A). Frequency distribution of hatching intervals of four handled *G. atrocyanea* eggs that were kept in pairs or in groups of four, and four intact eggs that were kept in groups of four (B). Frequency distribution of hatching intervals of ten *G. atrocyanea* eggs that were either kept separated from one another or kept as intact masses on leaves. 'n.s.' indicates no significant difference in mean values among the treatments, according to one-way ANOVA (A), the Kruskal-Wallis test (B), and the Mann-Whitney test (C) (P> 0.05). In (A), the hatching time was calculated by designating the time of the first egg hatching in each egg mass as 1 h.

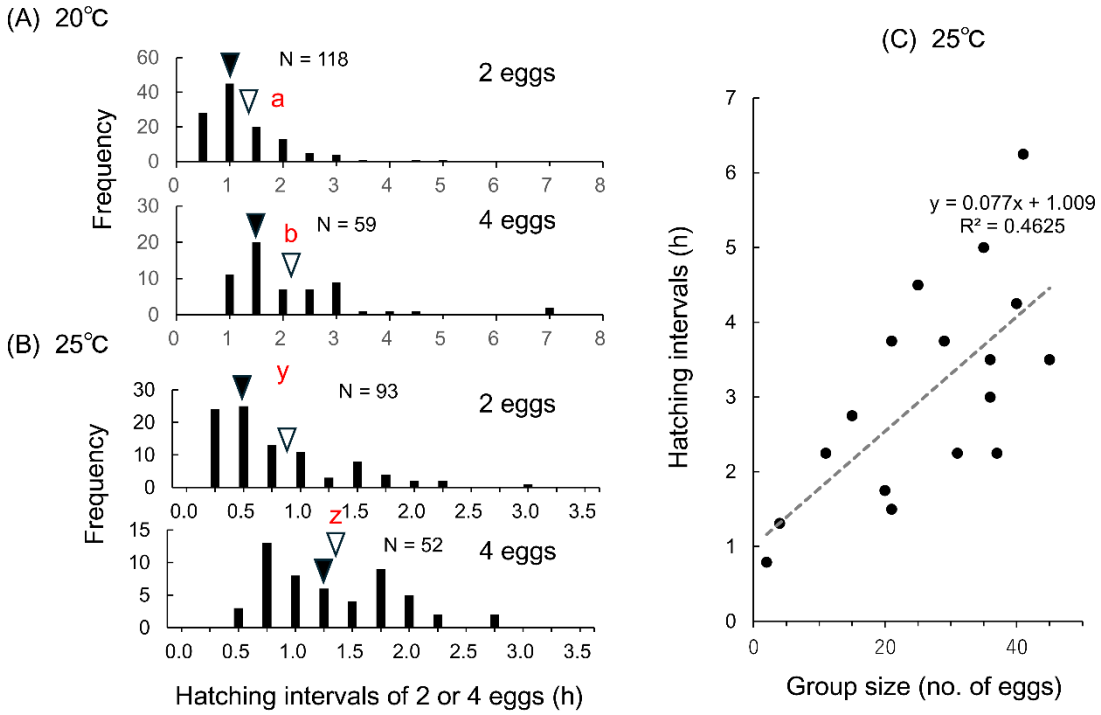

Fig. 4. Hatching intervals of *G. atrocyanea* eggs in different group sizes at 20°C (A) and 25°C (B, C). In (C), each symbol represents one intact egg mass, except for group sizes of 2 and 4, which are mean values derived from (B). Different letters in (A) and (B) represent a significant difference in mean values, according to the Mann-Whitney test at P< 0.05.

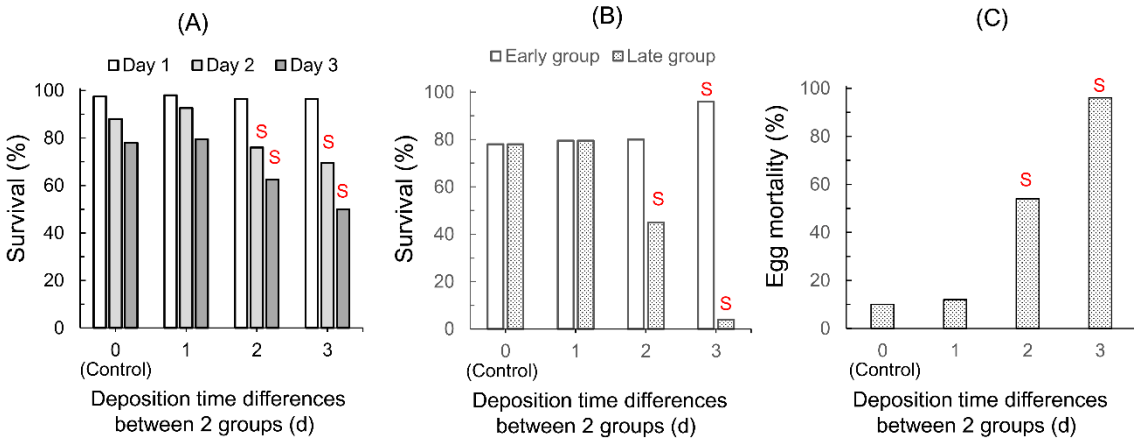

Fig. 5. Combined survival rates of two groups of 10 *G. astrophysa* individuals with different deposition times over three days after the first hatchlings appeared (A), mean survival rates for the two groups on day 3 (B), and egg mortality of the late group (C) at 25°C. Sample size = 10, with each sample consisting of 20 eggs in (A) and 10 eggs in (B) and (C). 's' indicates mean values significantly different from the control, according to the Steel's test at P< 0.05.

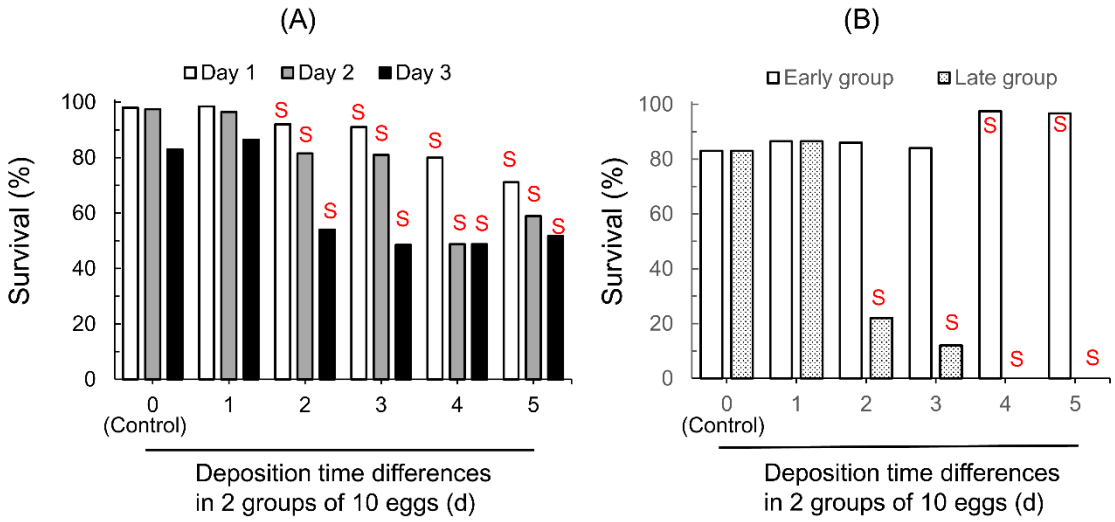

Fig. 6. Combined mean survival rates of two groups of 10 *G. astrophysa* eggs produced 0-5 days apart over three days after the first hatchlings appeared (A), and mean survival rates for the two groups on day 3 (B) at 20°C. Sample sizes range from 8 to 10, with each sample consisting of 20 eggs in (A) and 10 eggs in (B). 's' indicates mean values significantly different from the control, according to the Steel's test at P< 0.05.

0.05 each). At this temperature, larvae hatched from eggs in 6 days and began to molt to the second instar 3 days later. Leaf discs were mostly consumed by day 3. Steel's test indicated that the survival rate on each day was significantly different from the control in all treatments except for the treatment with a 1-day difference in deposition time (P< 0.05 each).

Figure 6B shows the survival rates of the early and late groups separately on day 3. As mentioned earlier, the average value of the two groups was calculated for the control and the treatment with a 1-day difference in deposition time. Steel's test indicated that the survival rate of the early group on day 3 was significantly different from the control in the treatments with a 4- and 5-day difference in deposition time (P< 0.05 each). This suggests that, compared with the control, the survival rate in the early group increased when kept with eggs laid 4 or 5 days later, likely due to oophagy. In the late group, the survival rate was high (> 80%) when the two groups were produced on the same day (control) or 1 day apart, but it was significantly different from the control when the early group hatched 2 or more days earlier. This suggests cannibalism

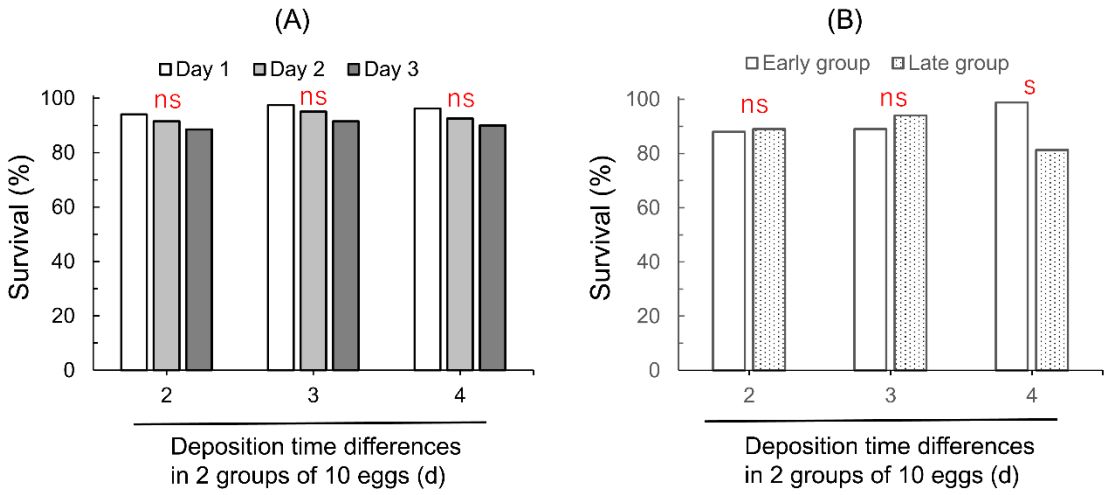

Fig. 7. Total mean survival rates of two groups of 10 *G. astrophysa* eggs produced 2-4 days apart over three days after the first hatchlings appeared (A), and mean survival rates for the two groups on day 3 at 20°C (B). Sample sizes range from 8 to 10, with each consisting of 10 early-deposited eggs and 10 late-deposited eggs. 'n.s.' indicates no significant difference in mean values among the treatments, according to the Kruskal-Wallis test at P< 0.05.

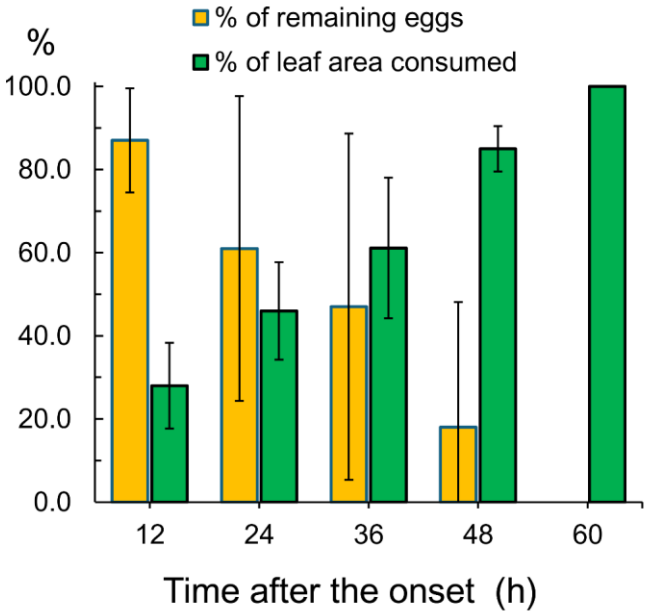

Fig. 8. Relationship between egg survival rates and leaf area consumed by larvae at the third instar stage in *G. astrophysa* at 20°C. Five third-instar larvae were kept with 10 newly deposited eggs on a 3-cm leaf disc for 60 hours. Histograms and bars represent mean values and SD from 10 replicates.

likely occurred when larvae were kept with eggs produced 2 or more days earlier.

In the observations at 20°C described above, 9.8-mm leaf discs were mostly consumed by day 3, except for the veins. In the next experiment, the hypothesis that cannibalism would similarly occur even on larger leaf discs was tested. Ten eggs (early group) were placed on large leaf discs (30 mm in diameter), together with eggs (late group) laid 2–4 days later at 20°C. The number of eggs and larvae was observed over 3 days after the first hatchling appeared. The leaf discs were heavily consumed by day 3, but there was no case in which most or all leaf areas were consumed. The combined survival rate of both the early and late groups remained high (> 80%) in all treatments, with no significant difference observed over the 3 days in any of the three

Table 1. The hatchability of *G. atrocyanea* eggs in different group sizes at 25°C. Each of the 8 egg masses was divided into 3 batches, which were then kept singly, in pairs, and in groups of 4 eggs, respectively. Ten egg masses consisting of 23-42 eggs were separately kept in Petri dishes, and the mean hatchability was determined.

	Group size (No. of eggs)				Kruskal-Wallis test
	1	2	4	23-42	P
Mean %	95.6	96.4	97.9	96.3	0.67
Range (%)	80.0 - 100.0	75.0 - 100.0	83.3 - 100.0	88.1 - 100.0	
No. of egg masses	8	8	8	10	
Initial no. of test eggs	104	112	116	3510	

treatments (Fig. 7A, Kruskal-Wallis test, P= 0.24). The survival rates of the two groups on day 3 are shown separately in Fig. 7B, which indicated no significant difference between the two groups in any treatment (Mann-Whitney test, P> 0.05) except for the treatment with a 4-day difference in deposition time (P< 0.01). This suggests that cannibalism is alleviated on larger leaf discs.

Egg survival rate

Hatchability at 20°C was generally high, ranging from 95.6% to 97.9%, across all egg group sizes tested (Table 1). No significant difference was observed among the four egg group sizes—1, 2, 4, and 23–42 eggs (Kruskal-Wallis test, df= 3, P= 0.67). This result suggests that the low rates of egg mortality in all treatments were primarily due to unfertilization or developmental failure, as similarly low mortality was also observed in the singly kept eggs.

Oophagy by large larvae and adults

When 30 eggs were kept with 5 second- or third-instar larvae on moist tissue paper in 6-cm Petri dishes at room temperature, the eggs were completely consumed within 1 day (data not shown). An additional 30 and 100 eggs were then provided to the second- and third-instar larvae, and these eggs were again consumed completely by the following day. This result suggests that larger larvae voraciously consume eggs when no leaves are available.

Similarly, each of 5 male adults was housed with 10 eggs in a 3-cm Petri dish. After one day, 4 to 10 intact eggs were found, with the mean percentage of remaining intact eggs being 62% (N= 5), suggesting that adults also consume eggs when no leaves are available.

In preliminary observations, female adults were also observed eating eggs, but precise data were not collected because some females produced eggs, making it difficult to accurately count the number of test eggs consumed.

Gstrophysa atrocyanea eggs are usually deposited on leaves. The next experiment was designed to test the hypothesis that third-instar larvae would not consume eggs in the presence of sufficient food (leaf). When 10 eggs and 5 third-instar larvae were placed on a leaf disc (3 cm in diameter) in a 3-cm Petri dish at 20°C, the mean number of eggs remaining on the leaves decreased over time, reaching 0 by 60 h after the experiment began. In contrast, the mean leaf area consumed increased from 28% at 12 h to 100% at 60 h (Fig. 8). No larval mortality was observed (N= 50). Although a significant negative relationship was found between the number of remaining eggs and the leaf area eaten (r= -0.69, N= 37, P< 0.05), all eggs could still be consumed while substantial amounts of leaf remained available. On average, it took 43.2 h (range: 24–60 h) for all eggs to be eaten by the larvae (N= 10). The result does not support the above hypothesis.

Discussion

The deposition of eggs in masses is common among insects. For example, grasshoppers typically lay their eggs in masses or pods in the

soil (Uvarov, 1977). In the ten species examined, highly synchronous hatching within an egg pod occurs when the eggs are kept together, compared to those separated individually (Tanaka, 2021a,b, Tanaka. 2023b). Vibrational stimuli transmitted between neighboring eggs are thought to induce synchronous hatching in these species. Many bugs also lay eggs in masses on plants and other substrates, and simultaneous hatching from an egg mass is often observed (Tomokuni *et al*., 1993; Kiritani, 1964). In eight species of Pentatomidae, hatching synchrony—when comparing eggs kept in contact with one another versus those individually separated—can be promoted, suppressed, or unaffected, depending on the species (Endo and Numata, 2017). Thus, densely deposited egg masses may or may not be associated with synchronous hatching.

As observed in other leaf beetles (Oliveria and Solomon, 2004; Eaton, 2016; Gibb and Sadof, 2017), *G. atrocyanea* deposits eggs as an egg mass in which the eggs are held together by a sticky substance. Because the eggs in each mass are laid within a short period and have a short embryonic stage, it seems reasonable that they are often observed hatching more or less synchronously. The present study examined whether this synchrony is caused by some egg interaction. In another beetle, *G. viridula*, eggs that were separated and kept in different dishes hatched less synchronously than those kept intact in masses. Kutcherov (2015) suggested the presence of a synchronizing factor in this beetle. However, in *G. atrocyanea*, the condition of the eggs—whether kept together or separated—did not affect the hatching interval; the hatching interval of four eggs was similar regardless of whether they were kept singly, in pairs, or groups (Fig. 2). This result also excludes the possible involvement of vibrational stimuli transmitted through the substrate, as demonstrated in a stink bug, *Halyomorpha halys*, in which synchronous hatching was observed when the eggs were glued to a piece of paper separately (Tanaka and Kotaki, 2020). It was initially thought that handling the eggs might affect the hatching time, but no significant difference was observed between intact and handled eggs. Therefore, it is concluded that there is no interaction between eggs influencing hatching synchrony in *G. atrocyanea*.

In eight species of grasshoppers, two species of bugs, and one species of moth, there is a tendency for eggs to hatch earlier on average in larger egg masses, and the hatching interval is smaller for eggs in masses than for those kept singly (Tanaka, 2021b, 2023b). However, no such phenomena were observed in *G. atrocyanea*, where no interaction between eggs was detected. Instead, the opposite tendency occurred: the mean hatching time was larger in the eggs kept in a group of 4 than in those kept singly (Fig. 1), and the hatching interval increased as the group size increased, with an estimated hatching time of 3.6 h for an average egg mass of 34 eggs at 25°C (Fig. 4C).

The significance of hatching synchrony has been suggested in various animals: it may reduce cannibalism, mitigate predation by diluting individual risk, overwhelm predators upon emergence, help hatchlings form aggregations, and enable them to start feeding as a group (Ghent, 1960; Morimoto and Sato, 1962; Arnold and Wassersug, 1978; Dehn, 1990). Cannibalism is known to occur in leaf beetles (Miyazaki and Naito, 1981; Collie *et al*., 2013; Kutcherov, 2015). However, the relationship between cannibalism and hatching synchrony has not been studied in *G. atrocyanea*. The present study examined this issue by carrying out two observations: (1) Comparison in survival rates between eggs in masses and those kept individually, and (2) comparison in survival rates between early-deposited and late-deposited eggs kept together in the same dishes.

In the first observation, survival rates were equally high regardless of whether the eggs were kept in masses or individually. The few eggs that either failed to hatch or were consumed by newly hatched larvae were likely unfertilized. This result provides no evidence to suggest the possibility that cannibalism within the same egg mass is a factor for the evolution of hatching synchrony in this beetle, as reported for *G. viridula* (Kutcherov, 2015).

In the second observation, eggs from two different egg masses were kept together. Survival in the late-deposited group was high if the eggs of the two groups were produced on the same day or just one day apart and placed together on a small leaf disc (Figs. 5, 6). This

result suggests that a hatching asynchrony of up to 24 hours does not affect oophagy rates by early-hatched larvae within the same egg mass. The longest hatching interval observed among intact egg masses was approximately 6 h under the same temperature conditions (Fig. 4C).

In contrast, egg survival declined significantly when eggs were placed together with those deposited 2 or more days earlier (Figs. 5, 6). At 20°C, egg mortality reached nearly 100% when eggs were kept with those produced 4 or 5 days earlier (Fig. 6B). In these cases, larvae from the early group showed significantly higher survival rates compared to the control, suggesting that oophagy increases larval survival. A similar phenomenon was observed when larger leaf discs were used as the substrate and food. However, in this scenario, the total survival rates of both groups remained generally high, with no significant changes observed during the 3 days following the onset of hatching. These results suggest that oophagy is largely mitigated on larger leaves.

In this study, all experiments were conducted under artificial conditions. Differences between artificial and natural conditions that may affect cannibalism in *G. atrocyanea* include the quality and quantity of host leaves, the space available for larval and adult movement, egg, larval, and adult densities on leaves, and the presence or absence of predators, as well as physical factors such as temperature, light, and humidity. These differences should be considered when discussing the significance of cannibalism in this beetle.

Additionally, another observation revealed that egg survival was inversely proportional to the amount of leaf consumed by the larvae. One factor contributing to mortality may be the amount of available food (leaves), while another could be the size of the leaf, which affects the likelihood of larvae encountering eggs. These findings are consistent with the outdoor observations by Miyazaki and Naito (1981), who noted that egg mortality due to cannibalism by larvae was high when larvae were abundant near the eggs, leading to increased consumption of both leaves and eggs.

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蓼藍齒脛金花蟲（*Gastrophysa atrocyanea*，鞘翅目：金花蟲科）的孵化同步性與同類相食

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摘　　要

許多昆蟲會將卵集中產下，通常每個卵塊中的幼蟲會或多或少地同步孵化。本研究探討了蓼藍齒脛金花蟲（*Gastrophysa atrocyanea*）的孵化同步性機制，以及孵化同步性（hatching synchrony）與同類相食（cannibalism）之間的關係。單獨放置的卵比四個一組放置的卵更早孵化。然而，無論是單獨放置、成對放置，還是四個一組放置，四個卵的孵化間隔相似。這一結果表明，卵之間不存在影響孵化同步性的相互作用。相對地，從第一顆卵到最後一顆卵的孵化間隔隨著組別大小的增加而延長，估計在 25°C 的條件下，平均每個卵塊（34 顆卵）的孵化間隔為 3.6 小時。在孵化後 12 小時內，單獨放置的卵與成塊放置的卵的存活率之間無顯著差異。當在同一天或相隔一天產下的卵被放置在一個小葉片上時，早期孵化的幼蟲很少出現卵食現象。當相隔 2 天或以上產下的卵被一起放置時，顯著的卵死亡率才被觀察到，儘管在較大的葉片上這種現象大大減少。基於這些結果，得出結論：同一卵塊中的同類相食並不是驅動孵化同步性進化的主要因素。

關鍵詞：同類相食、卵塊、孵化同步性