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Characteristics of Eggs and Egg Masses of *Gastrophysa atrocyanea* (Motschulsky) (Coleoptera, Chrysomelidae)

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ABSTRACT

Overwintered leaf beetles (*Gastrophysa atrocyanea*) laid eggs on Polygonaceae plants in central Japan. To understand the oviposition strategy, the pattern of egg mass distribution among dock plants and leaves of individual plants was investigated. Egg masses consisted of 33.4 eggs on average. There was a positive correlation between egg length and egg mass size. More egg masses were observed on larger host plants; however, neither the total number of egg masses nor the proportion of leaves with eggs on each plant showed a correlation with plant size. The distances between adjacent egg masses on a leaf were positively correlated with the leaf lengths and negatively correlated with the number of egg masses per leaf. These results suggest that adults did not have a preference for a particular size of host plant or leaf as the oviposition site, although they showed a preference for the underside of leaves in the field. Adults sometimes laid egg masses close to one another but seldom deposited them over existing ones.

Key words: egg mass, oviposition behavior, host plant size

Introduction

The leaf beetle (*Gastrophysa atrocyanea*) is distributed over a wide area, including Vietnam, China, Taiwan, Korea, Japan, and Russia (Wen, 1991; Lee, 2022) and feeds on various species of the Polygonaceae family (Chûjô and Kimoto, 1961). In central Japan, the Japanese dock (*Rumex japonicus*) and the bitter dock (*R. obtusifolius*) are widespread in spring, and overwintered adults actively lay eggs on the leaves of these plants. Subsequently, their larvae hatch and feed intensively on the leaves, causing severe defoliation in some dock plant individuals (Lee, 2022). Because of this phenomenon, this beetle has been considered a candidate for biological control against dock plants, which are known as troublesome plants in farmland, disturbed ground, short-term leys, and poorly managed grassland (Tsuyuzaki *et al.,* 2017; Miyazaki and Naito, 1981).

Various studies, including the seasonal life cycle (Kikuchi *et al.,* 1982; Wen, 1991), resource utilization (Suzuki, 1985, 1986; Ohsaki *et al.,* 2022), host specificity (Miyazaki, 1979; Naito *et al.,* 1979a), oviposition and mating behavior (Sugeno and Matsuda, 2002; Sugeno *et al.,* 2006), feeding behavior (Matsuda, 1981), defensive behavior (Sugawara *et al.,* 1979), adult diapause (Ojima *et al.,* 2015), cold tolerance (Hoshikawa *et al.,* 1988), morphology (Lee, 2022), and biological control (Miyazaki and Naito, 1974; Naito *et al.,* 1979b), have been conducted to understand the biology of this beetle.

I have been interested in studying the mechanisms and significance of hatching synchrony in insects and noticed that eggs of *G. atrocyanea* hatch simultaneously from the egg mass. They are usually kept in contact with one another as a coherent mass. In some grasshoppers, eggs are also laid together as an egg pod and hatch in synchrony from the egg pod, but only sporadically if they are individually separated several days before hatching (Nishide and Tanaka, 2016; Tanaka, 2021, 2023). In another dock plant beetle (*G. viridula* De Geer), Kutcherov (2015) observed that eggs within the same egg mass exhibited relatively synchronized hatching, whereas those individually kept in different dishes hatched less synchronously. To understand the hatching behavior and its significance in *G. atrocyanea*, some basic information regarding the oviposition behavior and the pattern of egg mass deposition on host plants is important because it is likely related to the mechanism controlling the hatching behavior and related phenomena, such as cannibalism at and after hatching. Therefore, in the present study, the patterns of egg masses of this beetle between and within host plants and the variation in the number of egg masses per leaf were investigated to understand the oviposition strategy of *G. atrocyanea*.

Materials and Methods

Insects

G. atrocyanea adults and eggs were collected from leaves of *R. japonicus* and *R. obtusifolius* along two unpaved roads (each measuring 4 x 80m) and adjacent grassland along Hasunuma river in Tsukuba, Ibaraki, Japan (36.1°N, 140.1°E) during March and April 2023. The two dock plant species coexisted in the area and were not distinguished in this study. The mean daily air temperature during March and April 2023 was 13.2°C (range: 5.5-20.8°C), with daily minimum and maximum temperatures recorded at -2.4 and 27.3°C, respectively (Japan Meteorological Agency 2023). Except for a few rainy days, I visited the site and observed beetle behavior for 0.5-1 h daily.

Distribution of egg masses on host plant and leaf

The oviposition pattern of *G. atrocyanea* was studied by observing the egg masses deposited on dock plant leaves during March 27-28, 2023. I observed a significant variation in the number of egg masses on each leaf. I investigated this variation in the initial observation by examining the abundance of egg masses per leaf and plant. I haphazardly selected 23 dock plants at the study sites. I counted the number of leaves on each plant and collected the leaves with eggs to determine the number of egg masses per leaf. Two plants out of the 23 examined, which had 58 and 98 leaves, respectively, showed no presence of beetles or eggs and were excluded from the analyses. The spatial distribution of plants and the distance between plants were not determined for this study. An egg mass was defined as a group of 5 or more eggs deposited in direct contact with one another, while single or small groups of eggs numbering less than 5 were ignored. Plant size was determined by the number of leaves, and the length and width of each leaf were measured using a ruler with an accuracy of 1 mm. Most egg masses were observed on the undersides of leaves located in the lower part of the plant. On March 15, I examined a single dock plant and found 36 leaves with eggs and 38 without eggs. Among the leaves with eggs, only 1 leaf had eggs deposited on the front side.

The number of eggs per egg mass was counted under a binocular microscope. Beetles laid the eggs at 25 ± 1°C and a light-dark cycle of 12:12 h in an incubator (CN-40A; Mitsubishi Electric Engineering Co., Tokyo, Japan) from April 1-9. Eight groups of 10 female and 10 male adults collected from the study sites on April 1 were placed in plastic containers (14 cm diameter, 7 cm height) with 2 pieces of dock plant leaves. Each container had a vinyl lid with a small mesh-covered window (3 cm diameter). Leaves with eggs were replaced with fresh leaves at 08:00 and 20:00 each day. The size of the egg mass and/or the number of eggs in each egg mass was recorded. The lengths of 3 haphazardly chosen eggs from each of 53 egg masses were measured using an ocular micrometer (1 unit = 0.067 mm) installed in a binocular microscope.

In another observation, I collected leaves with two or more egg masses on March 31 and April 1, 2023, to examine the variation in the distance between egg masses, which might affect the survival rate of eggs and hatched larvae. The shortest distance between two adjacent egg masses on leaves was measured using digital calipers with an accuracy of 0.1 mm (Digipa pro; Mitsutoyo Co., Kanagawa, Japan) under a binocular microscope. The shortest distance was defined as the distance between the nearest two eggs of two adjacent egg masses. Leaf length was also measured as described above to investigate its potential association with the shortest distance between egg masses.

Statistical analyses

The relationships between egg lengths and egg mass size and various factors related to egg mass distribution and plant size were analyzed using Pearson's correlation coefficient and linear regression. These statistical methods were employed to examine the correlations between different variables. The Descriptive Statistics tool in Excel, Microsoft Office 365, was utilized for conducting these analyses. Statistical significance was determined at a significance level of $P < 0.05$.

Results

Biological notes on the active season in 2023

At the start of the study on March 13, a significant number of adults and eggs were observed. On March 16, out of the more than 200 dock plants observed, larvae were only found on a single plant, indicating that hatching had recently begun. A week later, newly hatched larvae were commonly observed, coinciding with a mean air temperature of 11.5°C between March 16-22 (Japan Meteorological Agency, 2023). In late March, numerous larvae were observed and caused serious defoliation of some dock plants by April. Mature larvae presumably sought shelter in the soil for pupation as they disappeared from the defoliated host plants and became scarce by the end of April.

During the early stages of the study, the number of adults observed on March 14 ranged from 9 to 19 on 6 dock plants. These adults were actively engaged in feeding, mating, and oviposition but were not observed flying. Dissection of adults collected on March 28 revealed thin, whitish flight muscles in all individuals (5 females, 5 males), indicating the occurrence of histolysis. As April progressed, the abundance of adults declined at the study sites, with only one female adult observed during April 12-15. Adults from the subsequent generation were first observed on dock plants at the study sites on April 19. These individuals exhibited soft bodies and did not fly when tossed in the air (N= 20). The first flying adults were observed on April 21, and both male and female adults (N= 5 each) collected on that day had pinkish flight muscles. However, no adults were observed on dock plants on June 1, suggesting they entered a dormancy state underground.

Characteristics of eggs and egg masses

An analysis of 1159 egg masses from 493 leaves of 21 dock plants showed that leaves with single egg masses were most frequently observed (43.4%, 214 leaves), and the frequency rapidly decreased with the number of egg masses per leaf (Fig. 1A). The percentage of leaves with eggs per plant was 23.5% on average (range, 2-56%) and the number of egg masses per plant was 55.2 on average (range, 2-157). In this observation, the largest number of egg masses per leaf was 10. However, in another observation in which 2 or more egg masses per leaf were examined on March 23 and 24, a maximum of 13 egg masses per leaf were observed (data not shown). The egg mass size was determined by counting the eggs in each mass for those deposited by field-collected adults at 25°C. It varied greatly, with a mean value of 33.8 (SD= 12.1, N= 275; Fig. 1B). The distribution was left-skewed, and the median and mode were 37 and 44, respectively.

The egg color at deposition was yellow or golden yellow in most egg masses (Fig. 2A, D, G), but it was greenish (B), light yellow (C), whitish (E), or brownish (F) in a few egg masses. In most cases, egg color was considerably homogeneous within an egg mass, but a few egg masses consisted of a mixture of eggs with different colors (F, G). Eggs were usually deposited as a

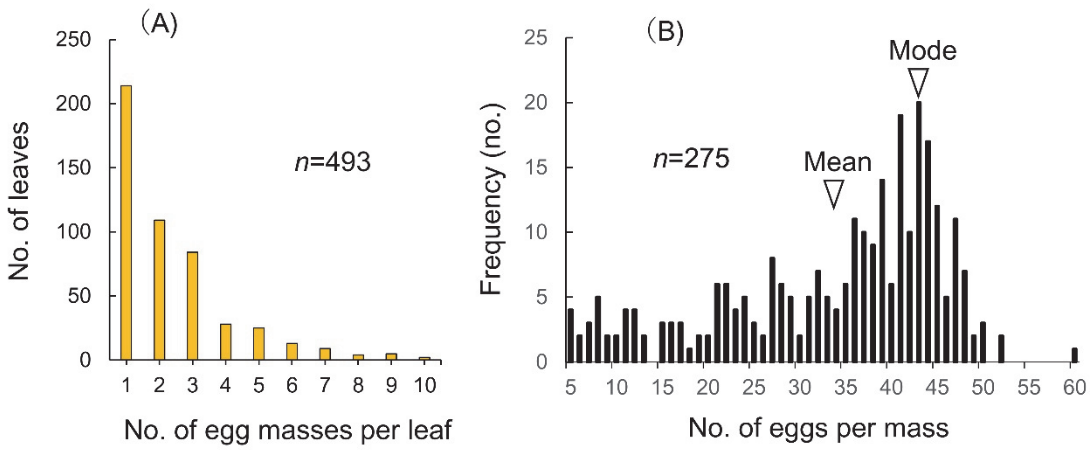

Fig. 1. The numbers of leaves with different numbers of egg masses on 21 dock plants observed during March 27-30 (A) and the frequency distribution of egg mass sizes (no. of eggs per egg mass)(B). Triangles in (B) indicate the mean (33.8 eggs) and mode (44 eggs).

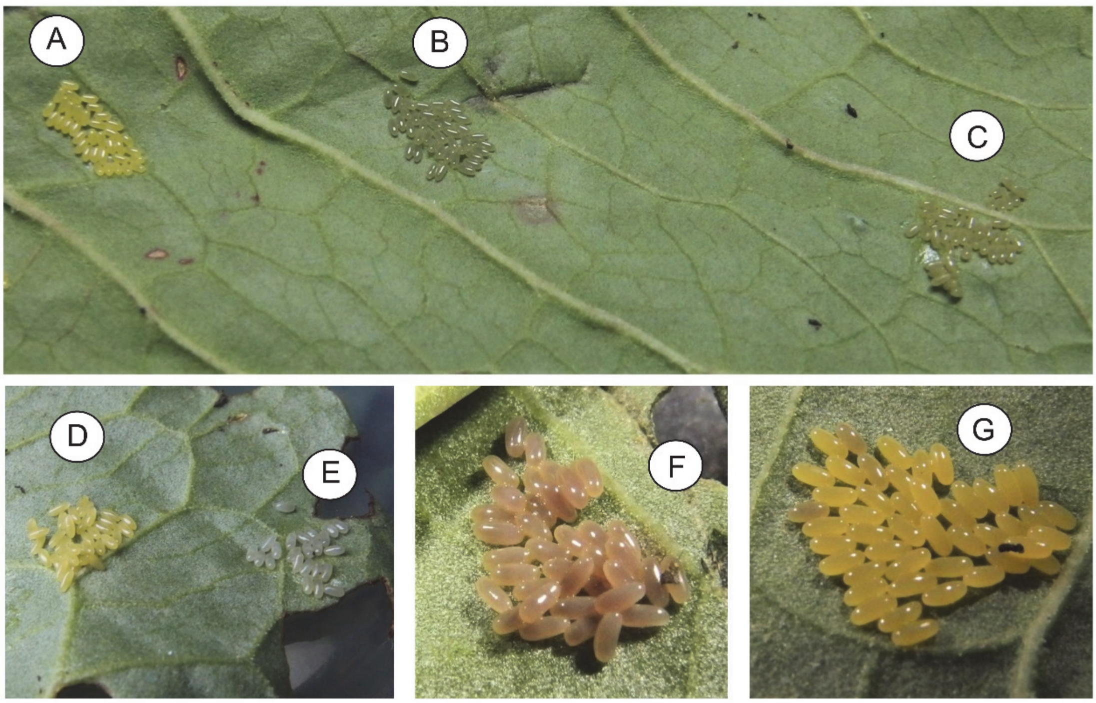

Fig. 2. Photographs showing various colors of eggs laid by *G. atrocyaneae* on dock plant leaves. Yellow (A, D), golden yellow (G), greenish (B, C), whitish (E), and brown and dark brown (F).

single egg layer on the leaf surface (A, B, C), but in rare cases, they were piled up as shown in Fig. 2D and F.

The mean egg length varied slightly, with a mean of 1.15 mm (range, 1.28-0.97 mm, N= 53). A weak but significant positive correlation was observed between the egg lengths and the number of eggs per egg mass (r= 0.32, N= 53, P< 0.05; Fig. 3).

Distribution of egg masses on host plants

The number of leaves with eggs or egg masses tended to increase with increasing plant size (r= 0.48, N= 21, P< 0.05, Fig. 4A) based on observations of 21 dock plants in the field on March 27 and 28. However, neither the proportion of leaves with eggs (r= 0.03, N= 21, p= 0.90, Fig. 4B) nor the total number of egg masses on each plant (r= 0.31, N= 21, p= 0.18, Fig. 4C) exhibited a significant correlation with plant size. These findings suggest that the oviposition behavior occurred independently of plant size.

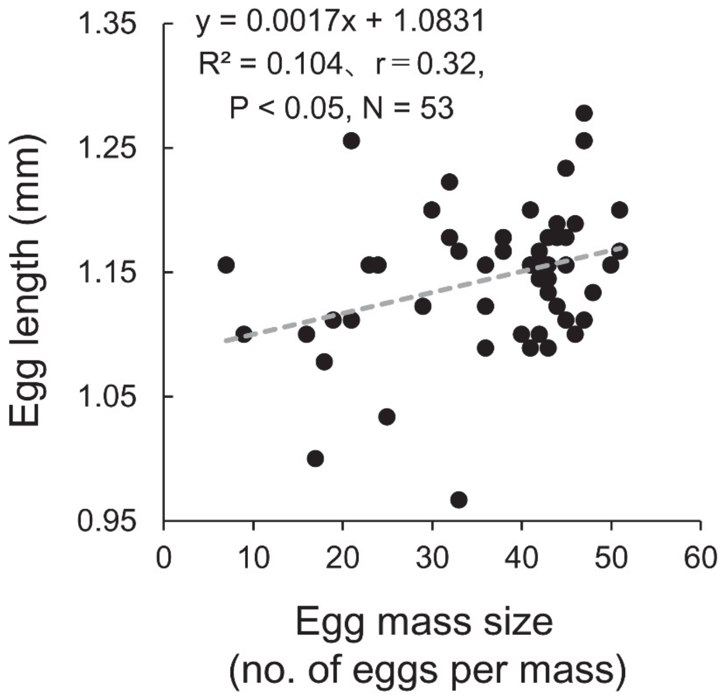

Fig. 3. Association between egg lengths and egg mass sizes in *G. atrocyaneae*. Egg length is the mean of 3 eggs haphazardly chosen from each egg mass. N = 53.

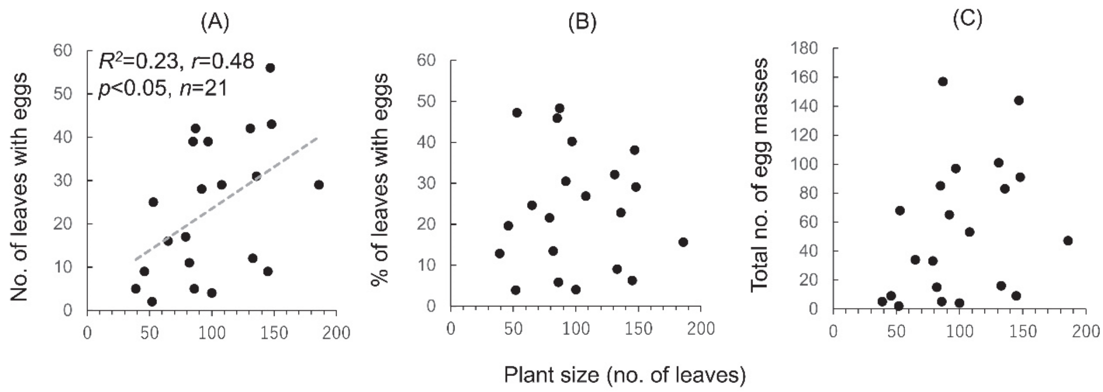

Fig. 4. Plots depicting the numbers of leaves with eggs (A), proportions of leaves with eggs (B), and total number of egg masses (C) in relation to plant sizes (number of leaves) in 21 dock plants observed on March 27 and 28.

Oviposition and leaf size

This analysis was conducted on leaves with two or more egg masses, and changes in leaf size that could have resulted from the period between the deposition of egg masses and the collection dates (March 31 or April 1) were not considered. The length of dock plant leaves ranged from 15.5 to 36.8 cm, and their maximum width varied from 4.7 to 16.3 cm. There was a highly significant positive correlation between leaf length and width ($r = 0.77$, $N = 78$, $p < 0.001$) (Fig. 5A). The number of egg masses per leaf did not exhibit a significant correlation with leaf length ($r = -0.10$, $N = 78$, $P = 0.367$) (Fig. 5B) or leaf width ($r = -0.05$, $N = 78$, $p = 0.664$, not shown), suggesting that the number of egg masses per leaf was independent of leaf size. In the subsequent analysis, leaf length will be used as a representative measure of leaf size.

Distance between adjacent egg masses

The shortest distance between adjacent egg masses on leaves ranged from 0.7 to 145.0 mm (Fig. 6A). As mentioned earlier, some eggs were piled up or overlapped within an egg mass (Fig. 2D, E). However, in this observation, no

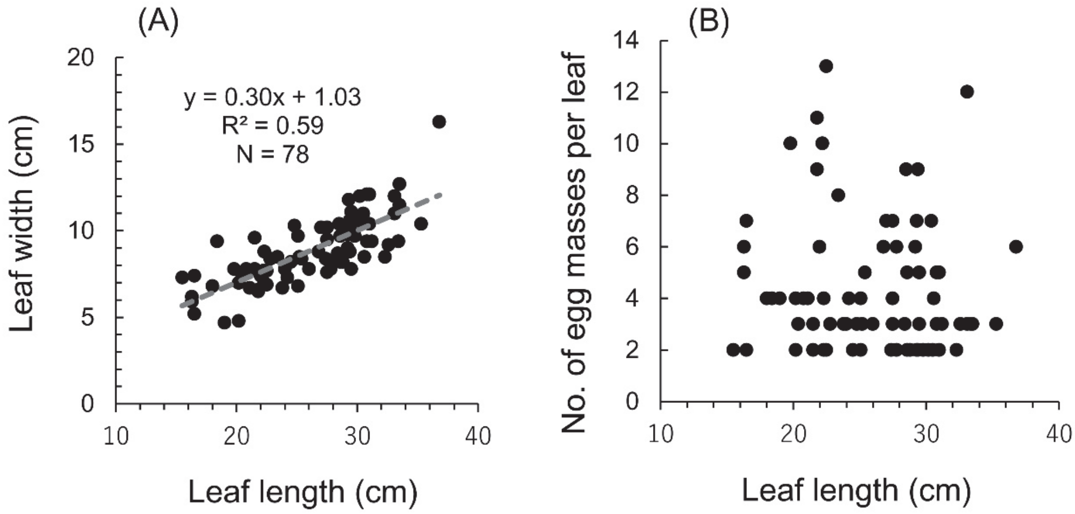

Fig. 5. Plots illustrating the association between leaf length and width of dock plants (A) and between the number of egg masses per leaf and leaf length (B). N = 78.

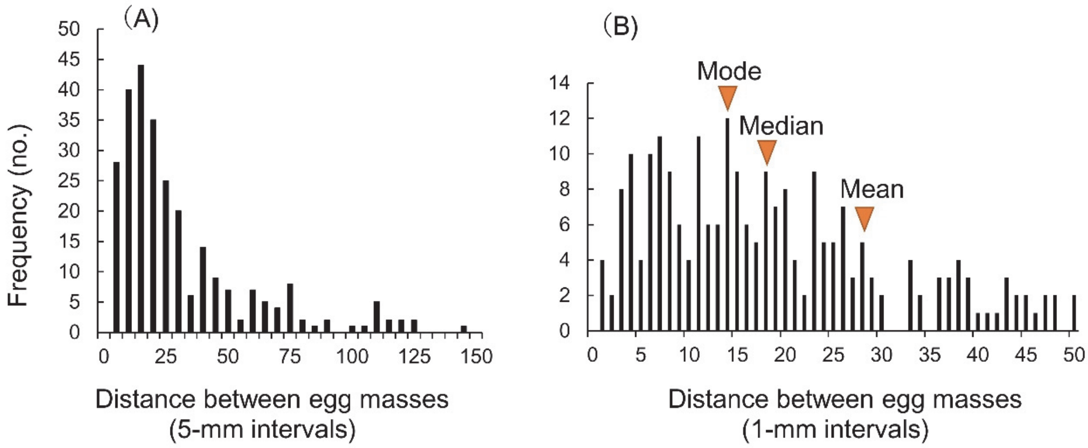

Fig. 6. Frequency distribution of shortest distances between adjacent egg masses plotted at 5mm- (A) and 1mm-intervals (B). In (B) the horizontal axis represents distances up to 50mm. N = 273.

overlapping of egg masses was observed, suggesting that female beetles frequently deposited egg masses close to one another but did not lay eggs directly on top of another egg mass. The frequency distribution of the shortest distances, plotted at 1-mm intervals within a range of less than 50 cm using the same dataset (N= 273), is shown in Fig. 6B. The mode, median, and mean distances were 14, 19, and 28.2 mm, respectively.

The distance between adjacent egg masses was positively correlated with leaf length (r= 0.37, N= 78, P< 0.001, Fig. 7A) and negatively correlated with the number of egg masses per leaf (r= -0.38, N= 78, P< 0.001, Fig. 7B). It was observed that when 2 egg masses were deposited on a single leaf, they were separated by various distances ranging from 5.4 to 145.0 mm, with a mean of 51.4 mm (N= 20). These observations provide no evidence suggesting that a female adult actively controls the distance between her eggs and other eggs on the same leaf. However, they appear to avoid overlapping their eggs with those laid by other females.

Discussion

As shown in Figure 18 of Lee (2022), most *G. atrocyanea* eggs observed in the present study were yellow or golden yellow in color. However, some variations were noticed in egg color, with a few egg masses appearing whitish, greenish, or

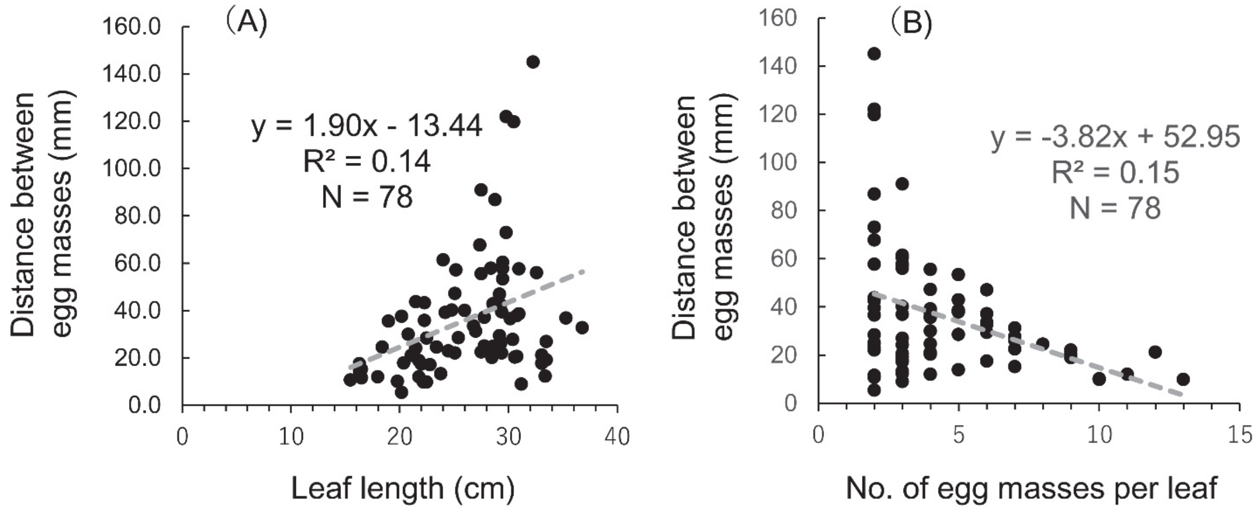

Fig. 7. Plots depicting the shortest distances between adjacent egg masses in relation to leaf lengths (A) and the number of egg masses per leaf (B). N = 78.

brownish. To understand this variation, biochemical analysis is necessary. Although no data were collected, it was observed that eggs of different colors gave rise to healthy-looking larvae that turned black shortly after hatching, similar to the yellow eggs (Tanaka, S., unpublished observation). The significance of the variation in egg color remains unknown.

Egg length exhibited slight variation and positively correlated with egg mass size in *G. atrocyanea*, which was an unexpected finding. One possible explanation for this positive correlation is that female adults may have started producing smaller eggs and egg masses when kept under crowded conditions. Another factor that could have influenced the results is aging. The beetles used in the study were collected on April 1 and allowed to lay eggs at 25°C until April 9. By mid-April, adults became scarce in the field, indicating that the insects used in this observation were older individuals. Therefore, further investigation is required to understand the association between variations in egg and egg mass sizes and maternal aging. It is worth noting that a decrease in egg size with maternal age has been observed in other insect species (Murai and Kiritani, 1970; Fox, 1993; Yanagi and Miyatake, 2002).

The present study focused on the distribution pattern of egg masses among individual dock plants and their leaves. In *G. atrocyanea*, adult beetles typically deposited egg masses consisting of an average of 33.8 eggs, a value similar to that reported for a Chinese population (34 eggs; Wen, 1991). The frequency distribution of egg mass sizes exhibited a left-skewed pattern, with a median of 37 and a mode of 44. One possible explanation for this distribution pattern is the crowded conditions under which the eggs were deposited (20 adults per container). Increased physical interactions among individuals under such conditions might have disrupted normal oviposition behavior, leading to the deposition of relatively smaller egg masses. This study determined the number of eggs per egg mass by examining laboratory-laid egg masses under a binocular microscope. Further investigation is needed to determine the egg mass sizes for egg masses collected in the field.

The results of this study also demonstrated that *G. atrocyanea* females did not exhibit a clear preference for a particular plant size or leaf size as their oviposition site; however, they did show a preference for the underside of leaves. The distances between adjacent egg masses on a leaf were found to have a positive correlation with leaf lengths and a negative correlation with the number of egg masses per leaf, which suggests that both leaf size and the number of egg masses on a leaf may establish an upper limit for the distance between egg masses (Fig. 7). These findings suggest that the beetles may lay egg masses indiscriminately, regardless of the presence of existing egg masses on the leaves. This behavior may contribute to overcrowding in the new generation, leading to severe defoliation of the host plants.

This study also revealed that *G. atrocyanea* females did not exhibit preferred distances between egg masses; however, they rarely laid eggs over previously deposited egg masses on the same leaf. The mechanisms by which females avoid depositing their egg masses on top of existing ones on the same leaf require further investigation. This behavior may be significant in terms of mitigating cannibalism of eggs by larvae emerging from adjacent egg masses. Understanding the hatching behavior of the egg mass and the rates of intra- and inter-egg mass cannibalism could provide valuable insights into the oviposition strategy of this beetle and the pattern of egg mass distribution on leaves.

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研究蓼藍齒脛金花蟲 ***Gastrophysa atrocyanea* (Motschulsky)** (鞘翅目，金花蟲科）卵和卵塊的一些特性

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摘　　要

在日本中部，越冬後的蓼藍齒脛金花蟲（*Gastrophysa atrocyanea*）會在蓼科植物上產卵。為了瞭解其產卵策略，針對蓼科植物和個別植物的葉片上卵塊的分布模式進行研究。卵塊平均由 33.4 顆卵組成。卵的長度和卵塊的大小呈正相關。較大的寄主植物上可觀察到更多的卵塊；然而，無論是卵塊的總數還是每個植物上帶有卵的葉片比例都與植物大小無明顯相關。葉片上相鄰卵塊之間的距離與葉片的長度呈正相關，與每葉的卵塊數量呈逆相關。這些結果表明，雖然成蟲傾向在葉片背面產卵、但在產卵場所上並未對特定大小的寄主植物或葉片有偏好。成蟲有時會在卵塊附近產卵，但很少在既有的卵塊上產卵。

關鍵詞：卵塊、產卵行為、寄主植物大小