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The recognition of the leaf size determines the egg cluster size while leaf abundance is correlated to the laying frequency for *Luehdorfia puziloi* (Lepidoptera: Papilionidae) oviposition

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Abstract

The life cycle of butterflies is closely related to the growth of food plants and, through a prolonged coevolutionary process, has undergone an ecological adaptation. So, it is important that control the egg-laying number and place to secure enough food plant by female adult to guarantee the survival of the larvae. To study whether oviposition control of the *Luehdorfia puziloi* takes into greater consideration food plant leaf biomass or leaf abundance, correlation among the egg cluster size, the leaf size, and the number of leaves around egg clusters was investigated. According to the results, the egg clusters size exhibited positive correlation with the leaf size of food plants on which eggs had been laid but did not do so with the number of surrounding leaves. In addition, the number of egg clusters laid exhibited positive correlation with the number of surrounding food plant leaves but not with the leaf size on which eggs had been laid. Consequently, for the *Luehdorfia puziloi*, the females' recognition of the leaf size seems to be the most important factor in the egg cluster size, and the number of egg clusters had positive correlation with food plant density per unit area.

Keywords: *Luehdorfia puziloi*, Oviposition, Egg clusters, Leaf size, butterfly

INTRODUCTION

In herbivorous insects, food plant's growth and larval growth are known to have synchronized through a prolonged co-evolutionary process and it mainly achieved by oviposition behavior of adult (Ehrlich and Raven 1964). Most butterfly larvae feed mainly on certain plants and long-distance travel is difficult in their early larval stage, so, selection of egg-laying location and egg production number considerably affects food securement for larvae and survival during larval period (Rausher and Papaj 1983, Banno 1984, Ohsaki and Sato 1994, Hunter and Mcneil 1997, Benrey and Denno 1997). Consequently, in the oviposition process, butterflies select egg-laying locations according to the types and the degree of growth of food plants (Porter 1992, Watanabe et al. 1986, Thomas 1983), and some species prefer locations with a high density of food plants while others prefer those with a low density (Hirose et al. 1976, Rausher 1983, Root and Kareiva 1984). In addition, some select egg-laying locations in consideration of the avoidance of parasitoids or natural enemies (Fox and Eisenbach 1992), and others select open or sunny spaces according to the scenery (Warren 1987).

Egg-laying behavior of butterflies can be classified two categories. First, laying one egg at a time and second, laying many at one time as egg cluster. The latter case, egg

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production number control is required more importantly than in the former case (Stamp 1980). Butterflies have been reported to be capable of recognizing food plant leaf size and growth (Alonso and Herrera 1996), and in an experiment related to butterflies egg cluster size, the larvae of groups with large egg cluster sizes exhibit relatively high survival rates and low predation ratios (Lawrence 1990). The egg cluster size can be affected by several factors. For example, it can be changed according to the food plant species, and large egg clusters appear with certain food plants (Kagata and Ohgushi 2001) and it exhibits positive correlation with food plant growth rate (Reed 2003) as well as the number of leaves (Vasconcellos-Neto and Monteiro 1993). It also can be affected according to food plant density (Tsubali 1995) and the ages of the female usually smaller for an aged (Begon and Parker 1986).

Butterflies in the genus *Luehdorfia* are widely distributed in East Asia, and the *Luehdorfia japonica* has been designated as a second degree threatened species and is protected in Japan (Yata 2007). Intensive studies have been carried out for this species including oviposition behavior, egg cluster size and the survival rate of larvae (Matsumura 2004). In early study, Ito et al (1982) reported that the larval survival rate was decreased when the egg cluster size increases. In another study, however, highest survival has achieved when the egg production number approximates the average egg production number regardless of the egg cluster size (Matsumoto 1990). In addition, it has been evaluated that, in terms of larval survival rate, new leaves are slightly higher than are old leaves in larval growth rate but do not affect larval survival rate (Hatada 2007). In a study on butterflies and food plant quantity, the average size of egg clusters has been reported to increase in areas with a large food plant quantity per unit area (Matsumoto et al. 1993). However, Hatada (2008) has stated that in areas distributed with shrubs, the laying frequency is high at the edge of the forest regardless of food plant leaf abundance. In addition, according to the results of a 5-year study, egg production number decreases as food plants decrease (Matsumoto 2003), which implies that female butterflies recognize at least the distributed quantity and locations of food plants. These studies including research on the copula success rates have been attempted in relation to the preservation of butterflies in the genus *Luehdorfia* (Tsubaki and Matsumoto 1998). However, despite the results of such diverse ecological studies, there still remains a question of whether, with food plants, the quantity, leaf size, and leaf abundance are the direct factors that determine the egg cluster size and the laying frequency of egg cluster-laying butterflies.

The *L. puziloi* develops from April to May and oviposits on the plant *Asarum* (Hatada 2007). A perennial plant that grows single leaves, the genus *Asarum* is characterized by the fact that it no longer produces new leaves after budding in early spring (Lee 2003). Because the larvae of the *L. puziloi* exhibit oligophagy and eat only the *Asarum*, the egg production number control becomes an important factor in determining food securement during the larval period and guarantees successful growth. Consequently, because of the ease of observing the egg clusters laid and of making quantitative measurements of food plant, using the *L. puziloi* as a model of the egg production number control through the recognition of food plant leaf size and leaf abundance can be of great help to the understanding the oviposition behavior of egg cluster-laying butterflies.

In the present study, through the examination and analysis of the size of leaves on which the *L. puziloi* had laid eggs, the egg cluster size, surrounding food plant density, and egg cluster frequency in a colony of the *Asarum sieboldii* in the central Korean Peninsula, which boasts an outstanding habitational environment for the *L. puziloi*, it was inferred that the egg production number of the *L. puziloi* had strong correlation with leaf size of food plant where eggs were laid.

MATERIALS AND METHODS

Study Butterflies and Site

Matsumura(1919) reported this species as *L. puziloi* Erschoff (Loc. Korea) for the first time in Korea. This species is occurred through Korean Peninsula proper, but there is no record of collection in the adjacent islands so far (Lee 1982). Outside Korea, it is distributed in Northern China, southeast Russia, and Japan.

The butterfly is univoltine with adult emergence in early spring, and the females lay eggs in clutches on leaves of the larval host-plant, which is any available species of the genus *Asarum* (Aristolochiaceae), and in most cases is an evergreen species. (Fig. 1). The hatched larvae feed on the leaf in groups during the early instar stages. After the leaves of original host-plants are entirely eaten up, the larvae disperse in search of the neat host-plant, splitting up into continuously smaller groups, until they become solitary in the last (fifth) instar stage. In early summer, the larvae pupate near the ground, and the pupal stage lasts until the next spring (Hatada 2007).

The area studied was on Mt. Noseong (350-400 m above the sea level) in Hwabuk-myun, Boeun-gun, Chun-

Fig. 1. Photograph of the *A. sieboldii* (a) and larvae (b), a pupae (c) and a female adult (d) of the *L. puziloi*.

gcheongbuk-do, South Korea. On Mt. Noseong, afforested areas and trees such as pine trees, chinese cork oak trees (*Quercus variabilis*), and chestnut trees form a tree layer. The *A. sieboldii* colonies were developed around the valley and the stocking density of the *L. puziloi* was high, thus making it a location for easy data collection.

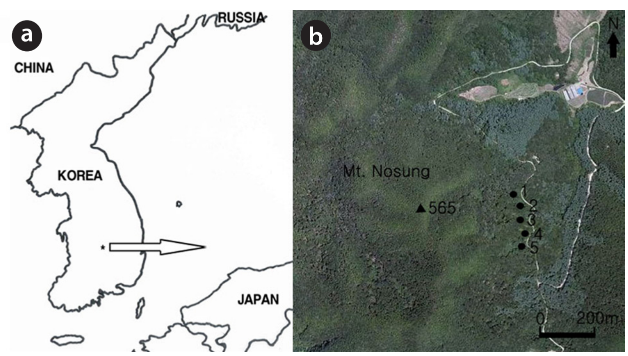

Fig. 2. The studied sites located on Mt. Noseong (350-400 m above the sea level) in the central Korean peninsula (A). Five sites (1-5) were represented where *A. sieboldii* colonies were developed around the valley.

Field Data

Butterflies were studied during the entire 1999 field season (late April-early May). To study the correlation between the egg cluster size of the *L. puziloi*, leaf size of food plants, and the number of surrounding leaves, lattices were created at intervals of 2 m × 2 m centered with the food plants on which eggs had been laid, then the length of the long axis of the leaves on which eggs had been laid was measured and the number and locations of surrounding leaves were marked. The lattices were 2 m × 2 m ~ 8 m × 8 m sizes at sites 1 and 2 because most of the oviposition intervals were 3 m or greater while it were 6 m × 12 m, 10 m × 10 m, and 10 m × 25 m at sites 3, 4, and 5 where had plenty food plant colonies and showed frequent laid eggs (Fig. 2). The length of the long axis of the food plant leaves on which eggs had been laid and the egg cluster sizes were measured using squared paper and a ruler. The number and locations of surrounding leaves were recorded together on a field note. As for the measurement of the number of leaves around egg clusters, using the field notes thus completed, concentric circles with radii of 1 m, 2 m, and 3 m, respectively, were drawn around each egg cluster and the number of leaves within the intervals was counted.

Statistical Analysis

As for statistical analysis, SAS (StatView 5.0.1) was used to conduct regression (simple and multiple) analysis and an x^2 test of goodness of fit and a variance-mean ratio test were conducted for an analysis of the spatial distribution of egg clusters (Blackman 1942). To calculate food plant leaf area which is difficult directly to calculate, the long axis length was measured then regression equations for the leaf size and the long axis length were calculated in the laboratory using a Computer-Coordinating Area Curve meter. According to the results, the leaf size and the long axis length exhibited correlation, and it was demonstrated that estimating the leaf size according to the long axis length could be an appropriate means to calculate leaf area (Fig. 3).

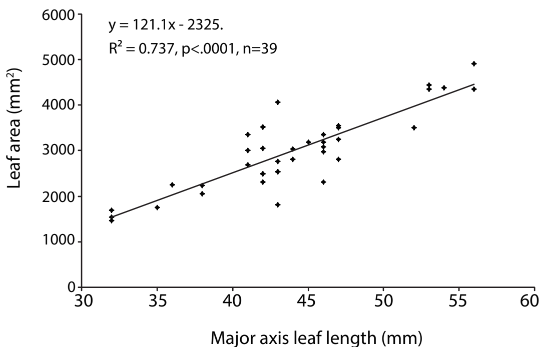

Fig. 3. Relationship between major axis leaf length and leaf area of host plants. Regression equations for the leaf size and the long axis length were calculated in the laboratory using a Computer-Coordinating Area Curve meter.

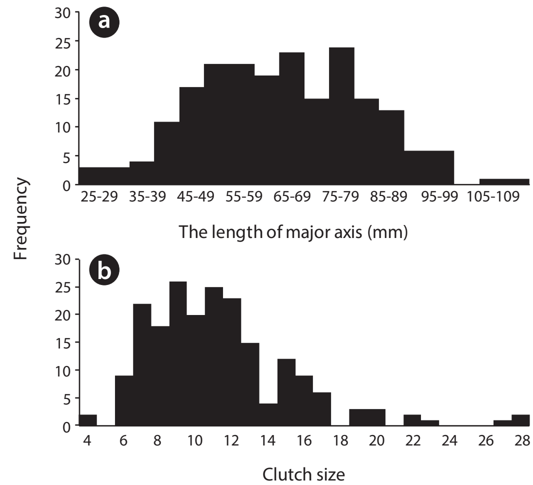

Fig. 4. Leaf size distributions of the *A. sieboldii* represented with the major axis length (a) and clutch size distributions of *L. puzilio* laid on the leaf of *A. sieboldii* (b).

RESULTS

The Size of Food Plant Leaves and the *Luehdorfia puziloi*

The egg clusters of the *L. puziloi* were distributed on the food plant leaves which had long axis lengths from 25 mm to 109 mm. The Poisson distribution analysis for the spatial distribution of the egg clusters was unclear to be seen as random ($X^2 = 310.93$, df = 201, $x^2_{(0.01,\ 120)} = 140.16$) however, a uniform pattern was deducted when this was subjected to variance-mean ratio test (t = 49.36. df = 202, $t_{(0.01,\ 120)} = 2.617$). Consequently, egg clusters were not concentrated on leaves of particular sizes but were distributed comparatively evenly, thus exhibiting no preference for particular leaf sizes (Fig. 4A) for oviposition. The number of eggs for an egg cluster were 4~28 in studied area with an average 11.1 (SE = 1.34, n = 203) and 81% of the egg clusters were sized with 7~15 eggs (Fig. 4B).

The Size and Number of Food Plant Leaves and the *L. puziloi*

To understand whether the size of the egg clusters was controlled by the size and number of food plant leaves, major axis length of food plant where egg were laid, number of leaves, and egg cluster size were subjected to regression analysis. The result showed a positive correlation (F = 48.54, p < 0.0001, df = 202) between the leaf size and the egg cluster size, however, the size of the egg clusters and the number of surrounding (within a radius of 3 m) food plants did not (F = 0.318, p < 0.812. df = 53). In addition, the results of regression analysis conducted with the number of food plants around the egg clusters divided per radii of 1~3 m also did not exhibit correlation (Fig. 5A and B). Consequently, the females of the *L. puziloi* seem to recognize the sizes of the food plant leaves on which they lay eggs but do not seem to be able to distinguish the number of surrounding food plant leaves.

The Number of Food Plant Leaves and the Laying Frequency of the *L. puziloi*

The number of food plant leaves and the laying frequency of the *L. puziloi* exhibited high significance (F = 771.79, p < 0.0001, df = 440) when analyzed the correlation between the number of egg clusters of the *L. puziloi* and the number of food plant leaves within 3 m (Fig. 6A). In addition, all the analysis results exhibited high reliability when analysis was conducted per radii of 1 m, 2 m, and 3 m and as the radius increased, so did the correlation coefficient (Table 1). However, there was no significance (F = 2.66, p < 0.058, df = 50) between the number of egg clusters and the sizes of the leaves when the number of egg clusters and the leaf size were subjected to multiple regression analysis per radii of 1~3 m. We also could not

Table 1. ANOVA table between the number of clutches and the number of host-plants

Range (m)	DF	F	p	Regression equation
1 m radius	202	111.99	0.0001	y = 0.059x + 1.384 R^2=0.360
2 m radius	187	193.90	0.0001	y = 0.065x + 1.655 R^2=0.510
3 m radius	53	70.28	0.0001	y = 0.057x + 2.081 R^2=0.574

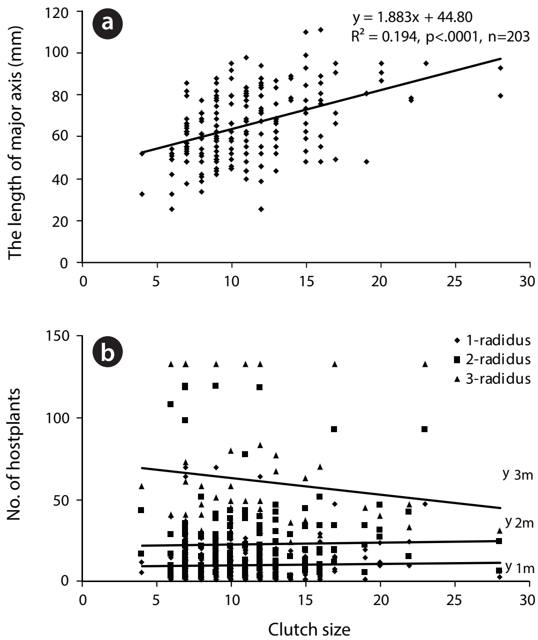

Fig. 5. Relationship between the clutch size of *L. puzilio* and the major axis length of *A. sieboldii* where eggs were laid (A) and between the clutch size and the number of surroinding host-plant leaves (B). Equations for regression lines are as follows: y 1m=0.078x+9.144, R^2=0.000; y 2m=0.113x+21.43, R^2=0.000; y 3m=-1.012x+73.16, R^2=0.018.

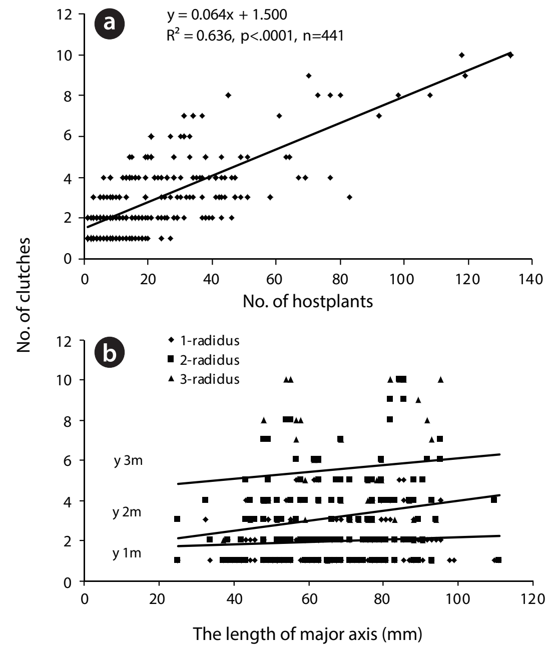

Fig. 6. Relationship between the number of clutches and the number of host-plants (A) and each relationship was also examined based on radius size (1 to 3 m) (B). Equations for regression lines are as follows: y 1m=0.0057x+1.599, R^2=0.007; y 2m=0.0249x+1.5035, R^2=0.0414; y 3m=0.0172x+4.3729, R^2=0.0091.

find significance when they were subjected to simple regression analysis per radii of radius 1 m, 2 m, and 3 m and the correlation coefficients were very low (Fig. 5B). Consequently, the number of egg clusters of the *L. puziloi* was increased when there were many surrounding food plant leaves; however, there was no correlation between the sizes of the food plant leaves and the number of egg clusters.

DISCUSSION

In this study, we suggested that the size of the egg clusters of the *L. puziloi* had strong correlation with the leaf size of the food plants on which eggs had been laid and that food plant density was closely related to the laying frequency (Fig. 5A) through the analysis of field data which collected to obtain clearer conclusions how do a egg cluster-laying butterfly recognize the surrounding environment to control the egg production number and the laying frequency. Such results imply that the female adult of the *L. puziloi* recognize the sizes of the leaves where they landed for oviposition and control the egg laying number, thus guarantee a stable growth at the initial larval stage. Approximately at their 3rd instar, the larvae of the *L. puziloi* move in search for other food (Matsumoto 1990) after completely consuming the leaves where they were laid, and their survival probability increases as do surrounding food plants. However, the females of the *L. puziloi* do not demonstrate cognitive preference for leaves of particular sizes in the selection process for oviposition (Fig. 4A). Matsumoto et al. (1993) have suggested that the mean egg cluster size of the *L. puziloi* changes according to the fresh weight of leaves per unit area so that as leaf biomass increases, so does the mean egg cluster size . However, in calculating leaf biomass, they have determined that the dry weight of food plants per unit area, with the egg clusters laid at the center, and disregarded whether butterflies recognize the size of each leaf on which eggs have been laid, thus exhibiting disparity from the results of the present study which exhibit the greatest link between the size of the egg clusters and the leaf size of the food plants on which eggs had been laid.

In this study, the average egg production number of the *L. puziloi* was 11, identical to the previous study accomplished in Japan (Matsumoto 1990). It suggested that the survival rate of 3rd-instar larvae is the highest when the average egg production number is 11 ± 1, at 66-70%. However, the survival rate estimated by applying the share of

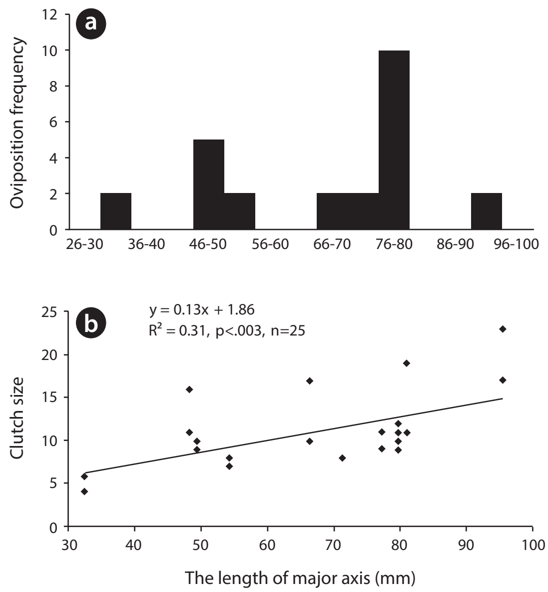

Fig. 7. Oviposition frequency of *L. puzilio* where many egg clusters had been laid on one food plant leaf (a) and relationship between the clutch size and the major axis length of the *A. sieboldii* (b).

the average egg production number in the size of the egg clusters collected for the present study was 40%, lower than 66~70% for Japan. Such differences seem to be because the size distribution of the egg clusters of Korean *L. puziloi* is more diverse than that in Japan, thus leading to differences in the survival rate according to the egg cluster size (Fig. 4B).

The egg production number of the *L. puziloi* is also expected to change according to the habitation environment. For example, analysis was conducted in Yongchon-li, Annae-Myun (food plant distribution area: 2 m × 2 m) where is one of the habitats of the *L. puziloi* and a nearby from the studied area with little distribution of food plants. In this area, the egg cluster size and the food plant leaf size exhibited correlation ($F = 75.55$, $p < 0.000$, $df = 4$) while the number of oviposition and the number of food plant leaves exhibited inverse correlation. This seems to be a result of intensive oviposition in one location because there was no distribution of other food plants nearby. Consequently, in areas with few food plants, the correlation between the oviposition number and food plant density does not seem to be significant.

In the present investigation, a case where many egg clusters had been laid on one food plant leaf was observed, but whether a single butterfly had laid these egg clusters could not be directly confirmed. Judging from changes in the colors of the eggs, the oviposition periods seemed to be disparate. There were 25 cases (12.3% of the total) which found two egg clusters or more on one leaf, 9 cases for two egg clusters, 1 case for three egg clusters, and 1 case for 4 egg clusters (Fig. 7A). The correlation analysis between the size of egg clusters and the leaf size also exhibited positive correlation and there was no preference regarding the leaf size in selecting the leaf for oviposition, identical to the results for the total egg clusters (Fig. 7B). However, even though it was not determined whether such ovipositional habits were due to preference regarding considerations of the locations of food plants, they do not seem to be efficient for larvae's food distribution.

Through the present study, it was possible to obtain advanced knowledge of the factors for female butterflies' recognition of food plants for control the egg production number of the *L. puziloi*, and it was demonstrated that the areas of the leaves on which eggs had been laid had close correlation with the egg cluster size and that food plant density per unit area was related to the laying frequency. If related studies are expanded to other butterflies as well, it will be possible to obtain clearer conclusions on recognition patterns of butterflies for food plants and egg production control, and, through the process, to use as important data for the protection and restoration of butterflies' habitats.

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